ABSTRACT

Title of Dissertation: **KINESIN MOTOR PROTEINS ARE ESSENTIAL FOR MALE GAMETOPHYTE DEVELOPMENT IN *MARSILEA VESTITA***

Erika Jean Tomei, Doctor of Philosophy, 2016

Dissertation directed by: Professor Stephen M. Wolniak, Department of Cell Biology and Molecular Genetics

The male gametophyte of the semi-aquatic fern, *Marsilea vestita*, produces multiciliated spermatozoids in a rapid developmental sequence that is controlled post-transcriptionally when dry microspores are placed in water. Development can be divided into two phases, mitosis and differentiation. During the mitotic phase, a series of nine successive division cycles produce 7 sterile cells and 32 spermatids in 4.5-5 hours. During the next 5-6 hours, each spermatid differentiates into a corkscrew-shaped motile spermatozoid with ~140 cilia. This document focuses on the role of motor proteins in the regulation of male gametophyte development and during ciliogenesis. In order to study the mechanisms that regulate spermatogenesis, RNAseq was used to generate a reference transcriptome that allowed us to assess the abundance of transcripts at different stages of development. Over 120 kinesin-like sequences were identified in the transcriptome that represent 56 unique kinesin

transcripts. Members of the kinesin-2, -4, -5, -7, -8, -9, -12, -13, and -14 families, in addition to several plant specific and 'orphan' kinesins are present. Most (91%) of these kinesin transcripts change in abundance throughout gametophyte development, with 52% of kinesin mRNAs enriched during the mitotic phase and 39% enriched during differentiation. Functional analyses show that the temporal regulation of kinesin transcripts during gametogenesis directly correlates with kinesin protein function. Specifically, *Marsilea* makes one kinesin-2 (MvKinesin-2) and two kinesin-9 (MvKinesin-9A and MvKinesin-9B) transcripts, which are present during spermatid differentiation and ciliogenesis. Silencing experiments showed that MvKinesin-2 and MvKinesin-9A are required for ciliogenesis and motility in the *Marsilea* male gametophyte; however, these kinesins display atypical roles during these processes. In contrast, spermatozoids produced after the silencing of MvKinesin-9B exhibit normal morphology. MvKinesin-2 is necessary for cytokinesis as well as for regulating ciliary length and MvKinesin-9A is needed for the correct orientation of basal bodies, events not typically associated with these proteins. In addition, *Marsilea* makes motile, ciliated gametophytes without the help of IFT dynein, outer arm dynein, or the BBsome. These results are the first to investigate the kinesin-linked mechanisms that regulate ciliogenesis in a land plant.

KINESIN MOTOR PROTEINS ARE ESSENTIAL FOR MALE GAMETOPHYTE DEVELOPMENT IN *MARSILEA VESTITA*

by

Erika Jean Tomei

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2016

Advisory Committee:
Professor Stephen M. Wolniak, Chair
Professor Kan Cao
Professor Todd Cooke
Professor Antony M. Jose
Professor Lisa A. Taneyhill, Dean's Representative

Dedication

This dissertation is dedicated to my family. To my parents for their endless love and understanding, my sister for forever being my best friend and ultimate advisor, and my husband for his constant support and encouragement throughout this process. I would especially like to dedicate the writing of this dissertation to my mother, without her enormous strength and courage these past months the writing of this work would not have been possible.

Acknowledgements

I would like to acknowledge my advisor, Dr. Stephen Wolniak. I thank him for encouraging me to continue with my PhD four years ago and for his continual support and guidance. I would also like to acknowledge Dr. Corine Van der Weele. Her years of experience provided me with invaluable insights and her discussions of my research were extremely helpful. I want to extend a special thanks to Alisha Barnes for teaching me how to navigate a new lab, providing a listening ear, and above all, entertaining me during incubations. To Richard Zipper, thank you for getting up at 4am and helping me start long incubations. I am forever thankful for your enjoyment of mornings, excel spreadsheets, and cameras. Thanks to all current and former inhabitants of the Wolniak lab and all that you have done for my work.

I would also like to acknowledge my committee members, Dr. Kan Cao, Dr. Todd Cooke, Dr. Antony Jose, and Dr. Lisa Taneyhill. Your helpful comments and advice have made me a better researcher and I'm thankful for the challenges you have given me. I also thank Dr. Steve Mount for first encouraging me to make phylogenetic trees, and then teaching me the best way to construct them. I would like to acknowledge the National Science Foundation for providing the funding for this research and for their continual support of basic science. Finally, thank you to all the friends I've meet during my graduate studies. We've helped each other greatly through the years and I'm proud to call such accomplished individuals my friends.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ARK	Armadillo repeat kinesin
ARM	Armadillo repeat domain
At	*Arabidopsis thaliana*
ATK1	*Arabidopsis thaliana* kinesin 1—member of the kinesin-14 I family
ATK2	*Arabidopsis thaliana* kinesin 2—member of the kinesin-14 I family
ATK3	*Arabidopsis thaliana* kinesin 3—member of the kinesin-14 I family
ATK4	*Arabidopsis thaliana* kinesin 4—member of the kinesin-14 II family
ATK5	*Arabidopsis thaliana* kinesin 5 member of the kinesin-14 I family
AtKP1	*Arabidopsis thaliana* kinesin-like protein 1—member of the kinesin-14 II family
ATP	Adenosine triphosphate
b	Blepharoplast
bb	Basal bodies
BBsome	Bardet–Biedl syndrome proteins
BLAST	Basic local alignment search tool
BLAST2GO	BLAST to gene ontology
C-terminus	Carboxyl-terminus
CB	Ciliary band
cDNA	Complementary DNA
CDS	Coding sequence
CDZ	Cortical division zone
Ce	*Caenorhabditis elegans*
CEM	Cephalic male cilia
CENP-E	Centrosome protein-E--a member of the kinesin-7 family
CH	Calponin homology domain
Cr	*Chlamydomonas reinhardtii*
DAPI	4', 6-diamidino-2-phenylindole
DIC	Differential interference contrast microscopy
Dm	Drosophila melanogaster
DNA	Deoxyribonucleic acid
dsRNA	Double stranded RNA
EJC	Exon junction complex
f	Flagella
F-actin	Filamentous actin
FDR	False discovery rate
FERM	4.1, ezrin, radixin, and moesin protein module domain
FLA10	Flagellar assembly 10—member of the kinesin-2 family in *Chlamydomonas*
FLA3	Flagellar assembly 3—the kinesin-2 adaptor protein from *Chlamydomonas*

FLA8	Flagellar assembly 8—member of the kinesin-2 family in Chlamydomonas
FPKM	Fragments Per Kilobase Million
FRA1	Fragile fiber 1--a member of the kinesin-4 I family from *Arabidopsis*
GAP1	GTPase-activating protein 1
GO	Gene ontology
GTP	Guanosine triphosphate
h	Hours
HC	Dynein heavy chain
Hs	Homo sapiens
IAD	Inner arm dynein
IBBR	Institute for Bioscience and Biotechnology Research
IC	Dynein intermediate chain
IFT	Intraflagellar transport
j	Jacket cells
jc	Jacket cells
KAP	Kinesin associated protein
KCA1	A member of the kinesin-14 V family in *Arabidopsis*
KCA2	A member of the kinesin-14 V family in *Arabidopsis*
KCBP	Kinesin calmodulin binding protein—member of the kinesin-14 VI family from plants
KCH	Kinesin with calponin homology domain—member of the kinesin-14 II family in plants
KIF17	Kinesin family 17—member of the kinesin-2 family from mammals
KIF2	Kinesin family 2—member of the kinesin-13 family
KIF24	Kinesin family 24—member of the kinesin-13 family
KIF5	Kinesin family 5—member of the kineisn-1 family from mammals
KIFC1	Kinesin family c-terminal 1—member of the kinesin-14 I family from mammals
KINUA	Kinesin ungrouped A—member of the ARK family from *Arabidopsis*
KLP1	Kinesin-like protein 1, flagellar associated—member of the kinesin-9A family from *Chlamydomonas*
KRP125	Kinesin related protein 125—member of the kinesin-5 family from *Arabidopsis*
LC	Dynein light chain
LIC	Dynein light intermediate chain
logFC	Log fold change
m	Mitochondria
MAP	Mitogen activated protein
MIRO	Mitochondrial Rho
MKRP	Mitochondrial kinesin-related protein--a member of the kinesin-7 I family from *Arabidopsis*

MLS	Multilayered structure
mRNA	Messenger RNA
MSA	Multiple sequence alignment
mt	Microtubule ribbon
Mv	*Marsilea vestita*
MyTH4	Myosin tail domains
n	Nucleus
N-terminus	Amino-terminus
NACK	NPK1-activating kinesin-like protein—member of the kinesin-7 II family from *Arabidopsis*
ne	Nuclear envelope
NPK1	A MAP kinase kinase kinase
OAD	Outer arm dynein
OSM-3	Osmotic avoidance abnormal protein 3, homodimeric kinesin-2
p	Prothallial cell
p	Plastid
PAKRP	Phragmoplast-associated kinesin-related protein—member of the kinesin-12 II family from *Arabidopsis*
PAKRP2	Phragmoplast-associated kinesin-related protein 2—member of the kinesin-orphan II family from *Arabidopsis*
PAP	Polyadenylate polymerase
PFA	Paraformaldehyde
PFR	paraflagellar rod
POK	Phragmoplast orientating kinesin—member of the kinesin-12 I family from *Arabidopsis*
Poly(A)	Polyadenylated
Poly(A)+RNA	Polyadenylated RNA
Pp	Physcomitrella patens
PPB	Pre-prophase band
PRP19	A spliceosomal factor
Ran	Ras-related nuclear protein
RING	Really interesting new gene
RNA	Ribonucleic acid
RNAi	RNA interference
RNAseq	RNA sequencing
RT-PCR	Reverse transcriptase polymerase chain reaction
SAM	Sterile alpha motif
sp	Spermatid
spi	Primary spermatogenous cells
st	Starch filled plastids
TAN	Tangled
TBO	Toluidine blue-O
VDAC3	Voltage dependent anion channel 3

Chapter 1: Introduction

Marsilea vestita

Marsilea vestita is a heterosporous semi-aquatic fern that grows in shallow ponds with sporophytes that resembles a four-leaf clover (Figure 1-1A). Like all ferns, *Marsilea* undergoes an alternation of generations, with the sporophyte being the diploid product and the gametophyte is haploid. In California, *Marsilea vesita* is found in vernal pools and shallow ponds. During the dry spring and summer months, the ponds become dry and the sporophyte dies and forms sporocarps (Figure 1-1B). Sporocarps are modified leaf structures that hold meiotic products called megaspores and microspores within sporangia (Figure 1-1C). Female gametophytes are produced from the megaspores and male gametophytes are produced from the microspores. The spores undergo a natural process of desiccation within the slowly drying sporocarp where they can remain quiescent and viable in a dormant state for over 100 years (Moran, 2004). Gametophyte development begins upon sporocarp hydration. After fracture, decay or scarification of the sporocarp wall, the entry of water causes the hydration and expansion of a fertile frond, revealing the clusters of sporangia that contain the mega and microspores (Figure 1-1D). The dry spores readily absorb water and initiate complex, but highly ordered developmental programs leading to the production of egg cells (from megaspores) and motile spermatozoids (from microspores (Figure 1-1E). These gametes fuse to form zygotes for the next sporophyte generation.

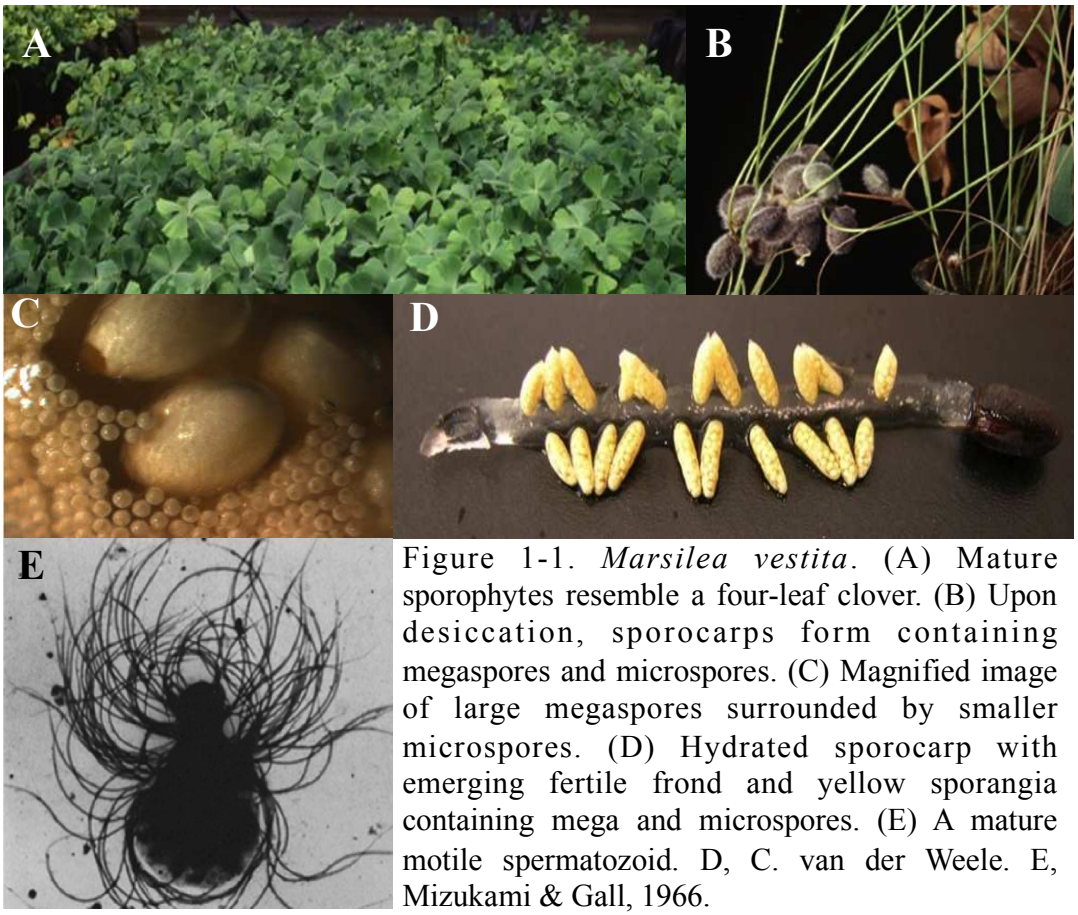


Figure 1-1. *Marsilea vestita*. (A) Mature sporophytes resemble a four-leaf clover. (B) Upon desiccation, sporocarps form containing megaspores and microspores. (C) Magnified image of large megaspores surrounded by smaller microspores. (D) Hydrated sporocarp with emerging fertile frond and yellow sporangia containing mega and microspores. (E) A mature motile spermatozoid. D, C. van der Weele. E, Mizukami & Gall, 1966.

Male Gametophyte Development in *Marsilea*

The process of hydration initiates a series of events that leads to a dramatic transformation in the *Marsilea* male gametophyte. Shortly after hydration, the gametophyte begins a rapid developmental program that culminates with the production of 32-corkscrewed shaped spermatozoids, each with about 140 cilia (Figure 1-1E) from a single undifferentiated cell (Sharp, 1914; Mizukami and Gall, 1966; Myles and Bell, 1975; Myles and Hepler, 1977). Spermatogenesis takes place synchronously in populations of spores hydrated at the same time. At 20°C, motile spermatozoids are released 11 hours after hydration (Hepler, 1976) whereas culturing gametophytes at 30°C results in the release of mature spermatozoids in about 6 hours

(Pennell et al., 1986, 1988). Development can be divided into two distinct phases. The first phase consists of a series of nine mitotic division cycles that produce 32 spermatids, six sterile jacket cells, and one prothallial cell that takes place during the first four and a half to five hours of development. After the division cycles are complete, the second phase of gametophyte development occurs and each spermatid differentiates into a corkscrew-shaped motile spermatozoid with about 140 cilia.

Mitotic divisions

Before hydration, the gametophyte consists of a single, undifferentiated cell with a large central nucleus surrounded by starch containing plastids (Figure 1-2A). Shortly after hydration, the nucleus moves to one side of the cell and the plastids migrate to the opposite side (Figure 1-2B). Within 30 minutes, the first division occurs. This division is asymmetric and produces a small prothallial cell and a much larger germ cell (Figure1-2C). The germ cell then divides symmetrically to produce two antheridial initials (Figure1-2D). Each antheridial initial divides asymmetrically three times to yield six jacket cells and two primary spermatogenous cells (Figure1-2E-G). The primary spermatogenous cells serve as the initials for spermatid production. Unlike the spermatogenous cells, the jacket cells are unable to divide, they contain starch filled plastids, and they surround the much larger primary spermatogenous cells. The divisions that distinguish primary spermatogenous cells from the sterile jacket cells are completed two hours after hydration and are responsible for establishing cell fate early in development.

Four more symmetric divisions then occur in each primary spermatogenous cell to produce a total of 32 spermatids (Figure 1-2H-J) (Sharp, 1914; Hepler, 1976).

The spermatids will ultimately differentiate into ciliated gametes. A blepharoplast forms *de novo* during these divisions and functions like a centrosome (though it lacks a centriole) during the 9th division to organize spindle microtubules (Hepler, 1976; Hoffman and Vaughn, 1994). All nine of the division cycles are completed by four and a half to five hours after spore hydration.

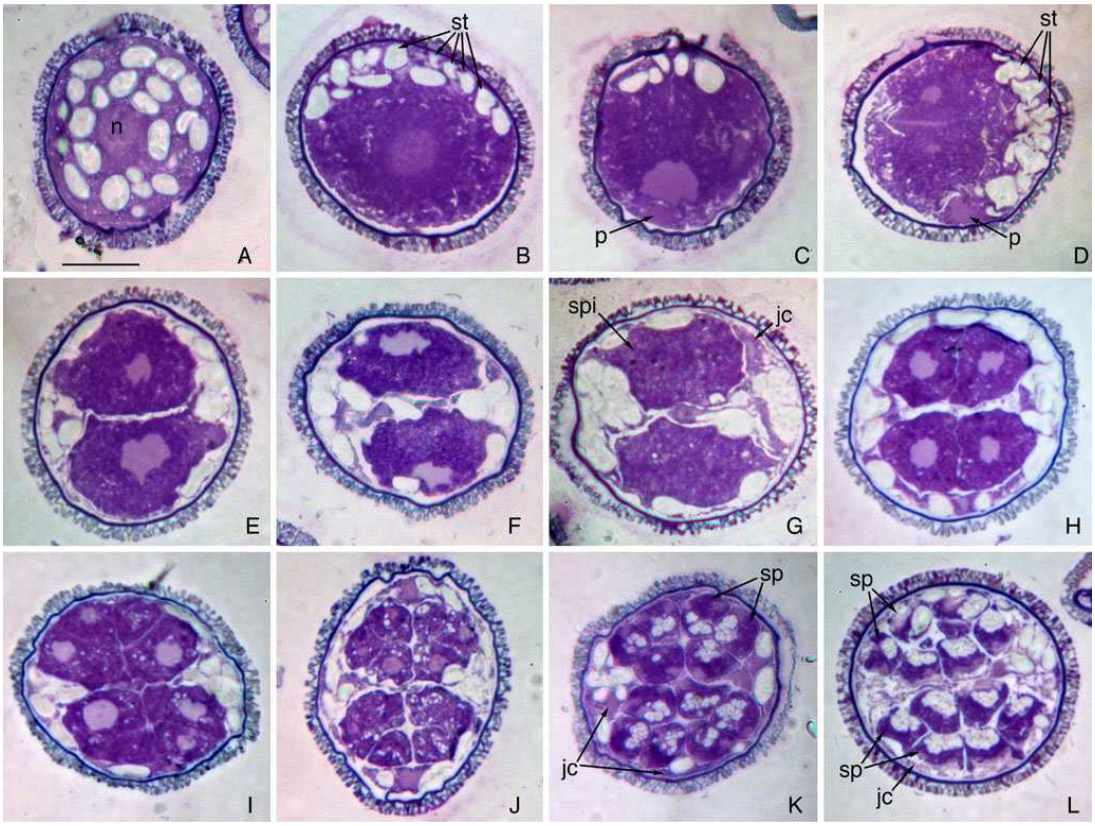


Figure 1-2. Spermatogenesis in *M. vestita.* Fixed cross-sections of microspores stained with Toluidine blue-O (TBO). (A) Before hydration the centrally positioned nucleus (n) is surrounded by plastids. (B) After hydration the spore rearranges the plastids (st). (C) The first division is asymmetric and produces a prothalial (p) cell. (D) The second division is symmetric and produces two antheridial initials. (E-G) Three asymmetric divisions follow producing six jacket cells (jc) and two primary spermatogenous cells (spi). These divisions are responsible for establishing cell fate during gametogenesis. (H-J) Four symmetric divisions in each primary spermatogenous cell produce 32 spermatids (sp) surrounded by seven sterile jacket cells (jc). (K,L) Once divisions are complete, each spermatid then differentiates into a motile spermatozoids. Bar = 25 μm. (Wolniak et. al., 2011).

Both the asymmetric and symmetric divisions not only occur at predictable times during development, but also along pre-determined planes. Like all other plants, the cells of the developing gametophyte cannot move. Thus, the position and size of each cell is constant and determines cell fate (Sharp, 1914; van der Weele et. al. 2007; Wolniak et. al, 2011). This precise spatial and temporal patterning of the divisions simplifies the detection of abnormalities during development.

Differentiation and ciliogenesis

Sterile cells are quite different from spermatogenous cells, yet they both arise from a single progenitor. Jacket cells are much smaller than spermatogenous cells and contain many starch-filed plastids. These cells cannot divide and as the gametophyte matures they become less obvious within the spore wall (Figure 1-2L, K). At five hours following hydration, the initial division phase of development is complete and a second phase of differentiation begins. During this phase, each spermatid develops into a helically coiled gamete with about 140 motile cilia.

This unusual shaping of the gamete is achieved through the elongation and coiling of the nucleus and mitochondria along a coiled microtubule ribbon (Figure 1-3A). The nucleus coils until it makes four to five gyres, while the microtubule ribbon and mitochondria continue to coil for nine gyres (Figure 1-3B). Basal bodies, already formed *de novo,* from the maturation and enlargement of the blepharoplast (Figure 1-3C, D) are placed in two rows at regular intervals along the dorsal face of the microtubule ribbon to become the sites of ciliogenesis (Figure 1-3E, F) (Sharp, 1914; Myles and Hepler, 1977). The coiling becomes apparent when an organelle called the multilayered structure (MLS) forms *de novo*. The exact function of the MLS is

unknown, but it seems to play a role in regulating the coiling process and the placement of basal bodies along the microtubule ribbon (Figure1 -3D) (Myles and Hepler, 1977; Marc and Gunning, 1986; Deeb et. al., 2010, Wolniak et. al., 2011).

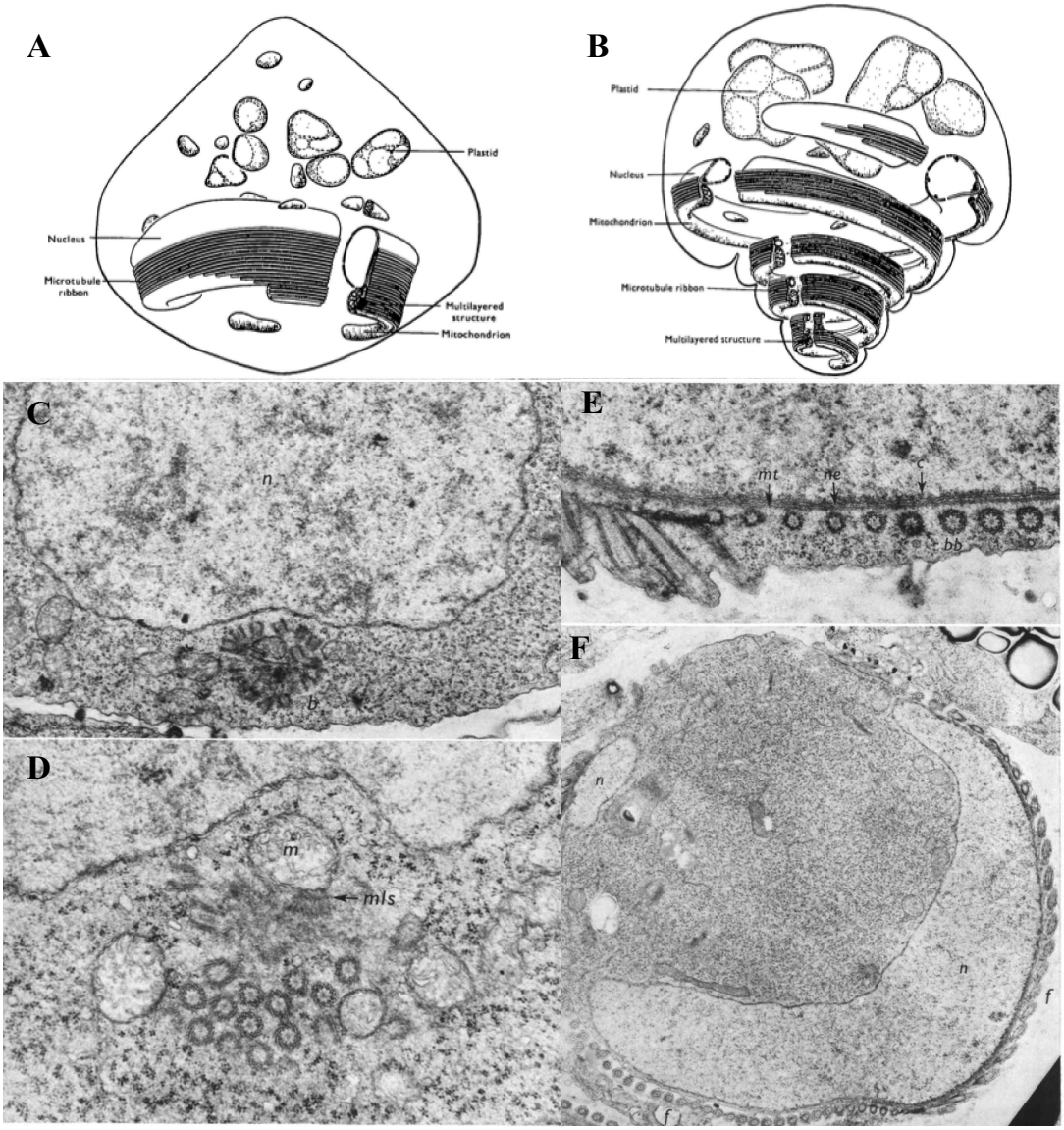


Figure1-3. Differentiation. (A) Cartoon image of the nucleus and mitochondria beginning to coil with the microtubule ribbon along the outside edge. (B) Coiling later in development. (C) Electron micrograph of a cross-section through the blepharoplast (b) and nucleus (n) at 5 h. (D) The blepharoplast then enlarges and transforms into basal bodies. Also in this region is the multilayered structure (mls) in close association with the mitochondria (m). (E) By 7 h basal bodies (bb) are placed at regular intervals along the nuclear envelope (ne) and microtubule ribbon (mt). Condensing chromatin (c) is seen along the nuclear envelope. (F) Flagella (f) grow out of the cell. (Myles and Hepler, 1977).

At first, basal bodies are oriented so cilia diverge away from each other and are parallel to the plasma membrane of each spermatid (Figure 1-4A). Near the end of spermiogenesis, the basal bodies rotate 90° so that the ciliary axonemes protrude vertically from the microtubule ribbon and nuclear coil in two regular rows (Figure 1-4B) (Myles et al., 1978; Myles and Hepler, 1982). At nine and a half hours, an extension of cytoplasm begins to grow around the anterior end of each spermatid and eventually fuses together to surround each cell. This creates an internal, but extracellular space that contains the microtubule ribbon and organelle coil plus all of the cilia (Figure 1-4C). Upon release from the microspore, each spermatozoid breaks free from the surrounding cytoplasmic space and leaves behind a thin vesicle-like structure (Myles and Hepler, 1977). The ciliary axonemes have the typical 9+2 architecture (Figure 1-4D) (Myles et al., 1978) found in motile organisms and spermatozoids are able to swim towards the megaspore for fertilization.

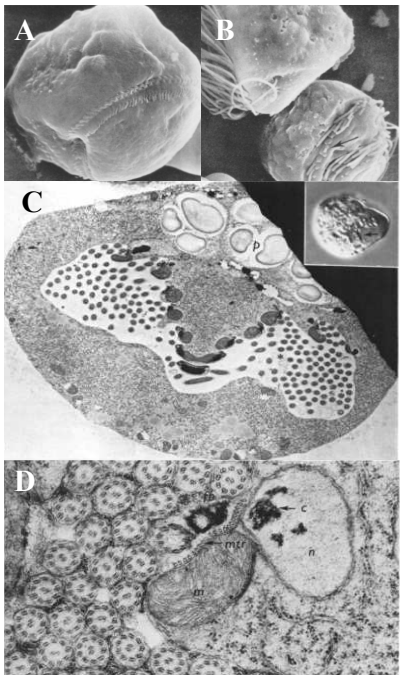


Figure 1-4. Ciliogenesis. (A-B) Scanning electron micrograph showing the orientation of the forming cilia to the spermatid plasma membrane. (A) Cilia first begin to appear as two diverging rows that are parallel to the plasma membrane. (B) Cilia then rearrange to be parallel to each other and extend vertically from the spermatid. (Myles et al., 1978). (D) Electron micrograph showing the extension of cytoplasm that forms around the anterior end of each spermatid. Cilia are enclosed in an internal, but extracellular spare (*). Insert: phase-contrast image at same developmental stage. (E) At 10 h, the cilia can be seen with a typical 9+2 architecture. One gyre of the organelle coli is pictured here with the nucleus (n), microtubule ribbon (mtr), and the mitochondria (m) close to their final shapes. (Myles and Hepler, 1977).

Gametophyte development is regulated post-transcriptionally

Similar to other rapidly developing systems, spermatogenesis in *Marsilea* is post-transcriptionally controlled. Virtually all of the RNA required for development is present in dry microspores, having been transcribed during the desiccation process prior to spore quiescence, and almost no additional transcription is required for gametogenesis. At most, a small amount of new transcription might be required for late stages of development when mature spermatozoids emerge from the spore wall (Hart and Wolniak, 1998; Klink and Wolniak, 2003). The RNA is stored in the dry spore within nuclear speckles as partially processed pre-mRNAs (Boothby and Wolniak, 2011). During gametophyte development, the stored pre-mRNAs become unmasked, spliced, polyadenylated, and translated in a precise spatio-temporal relationship to form motile spermatozoids (Tsai et al., 2004; van der Weele et al., 2007; Deeb et al., 2010; Boothby and Wolniak, 2011; Boothby et al., 2013).

Marsilea vestita as an Experimental Model for Rapid Development

Over the past twenty years, our lab has focused on the processes that regulate rapid development in the male gametophyte of *M. vestita.* Although this may seem like an unfamiliar model for developmental biology, there are many useful and interesting aspects of gametophyte development that make this system biologically relevant. *Marsilea* has proved to be excellent and extremely useful for studies on cell fate determination, spatial organization (Tsai et al., 2004; van der Weele et al., 2007; Deeb et al., 2010), cellular morphogenesis (Sharp, 1914; Myles and Hepler, 1977,

1983), the *de novo* formation of basal bodies (Mizukami and Gall, 1966; Hepler, 1976; Hart and Wolniak, 1998, Klink and Wolniak, 2001), ciliogenesis, (Myles and Hepler, 1977), and post-transcriptional regulation (Boothby and Wolniak, 2011; Boothby et al., 2013).

One of the main advantages in the *Marsilea* male gametophyte is the combination of rapid development with developmental synchrony. Development occurs in only eleven hours and does not begin until dry microspores are submerged in water. During the first two hours of development, cell fate is established within the gametophyte. This involves only two cell types, sterile cells and spermatogenous cells, greatly simplifying the study and identification of these cells. The seven sterile cells in the gametophyte are formed through asymmetric divisions and this type of cell division is not used at any other time during development. During the following three hours of development, spermatogenous cells undergo four rounds of symmetric divisions to produce 32 spermatids. Both the asymmetric and symmetric divisions are temporally and spatially precise, meaning that they occur at predictable times and in pre-determined planes. This makes the construction of a fate map very easy and the detection of abnormalities during development is obvious. Extreme morphological changes in each spermatid then occur during the next five to six hours of development. During this time, spermatids transform from stationary, rounded cells, to coiled cells with about 140 motile cilia that are capable of swimming in shallow ponds and are chemically attracted to the female gametophyte.

Another enormous advantage is the ease at which studies in on male gametophyte development in *Marsilea* are conducted and their translatability to

general cell and developmental biology. Microspores are easily harvested from mature plants. To do this, the shallow ponds that make the ideal habitat for *Marsilea* sporophytes are neglected (much to the chagrin of our horticulturalist neighbors in the greenhouse facilities) and allowed to dry. The sporophytes form sporocarps as they mature, and the microspores and megaspores contained within the sporangia undergo their maturation process as the ponds dry out (Boothby and Wolniak, 2011). The drying process must be slow in order for the spores to mature properly. Spore maturation involves the production of stored mRNAs and proteins essential for rapid gametophyte development. After the plants have dried completely, the sporocarps are then simply pulled off the plant and placed in plastic bags stored at room temperature until use. Dry microspores retain their developmental potential for over 100 years (Moran et al., 2004), although we typically use them within a year or two in the lab. To study development, we are able to fix microspores and use standard sectioning, labeling, and microscopy techniques for visualization. In addition, we have found great success using reverse genetic approaches to study development. RNAi is simply conducted in *Marsilea* as microspores absorb dsRNA, and other small compounds like drugs or dyes, upon hydration. This straightforward approach to deliver dsRNA greatly simplifies RNAi and makes knockdown analyses easy to perform. One major disadvantage with this approach is that we have very little control over how much dsRNA is absorbed by a single microspore we frequently observe a range of effects from the addition of one type of dsRNA. At about six hours of development, small molecules like dsRNA, dyes, and drugs, are generally excluded from being absorbed by the microspore as the rehydrated cells reseal and the gametophyte becomes largely

impermeable from the surrounding environment.

Transcriptome assembly and analysis

In the past, our lab used a cDNA library to find transcripts encoding proteins necessary for gametophyte development. The cDNA library was sufficiently large to provide many diverse cDNAs and allow for numerous productive and informative studies. However, the development of next generation RNAseq technology gave us the opportunity to survey all the transcripts present during development, and not just a selection of the most prevalent or abundant. Two of the major advantages to using the developing male gametophyte of *Marsilea* for RNAseq analysis is that when rehydrated at 20°C, development proceeds synchronously within the microspore and that the gametophyte is transcriptionally quiescent. Therefore, at specific times after spore hydration, we know which developmental processes are occurring within the spore wall, and that changes in RNA abundance during specific time intervals are due to post-transcriptional regulation (unmasking, polyadenylation, splicing, degradation) and not the consequence of changes in gene expression.

In order to assemble a *de novo* transcriptome we used next generation RNAseq by isolating poly(A+)-RNA from developing gametophytes at 1-2 hours, 3-5 hours, and 6-8 hours after spore hydration. Our isolated RNA was sequenced using Illumina technology and we assembled into a *de novo* transcriptome using the computer application Trinity (Grabherr et al., 2011). The entire isolation and sequencing process was performed in three replicate runs. Our >590 million reads enabled us to assemble a combined reference transcriptome containing over 150,000 transcripts. Individual transcripts were then combined into unigenes. This left us with

about 90,000 unique isoforms for analysis. RNAseq reads were mapped to unigenes to calculate fragments per kilobase million (FPKM) values using the Tuxedo suite (Tophat, Bowtie and Cufflinks) (Trapnell et al., 2009; 2010) for each transcript. The transcriptome was annotated using Trinotate (http://trinotate.github.io), which compiles sequences for multiple BLAST analyses. We used EdgeR (Robinson et al., 2010) to compare RNA isoform abundance between samples obtained at different times of development, and gene ontology enrichment analysis (Blast2GO) (Ashburner et al., 2010) to cluster transcripts together based on function, so we could assess patterns of transcript enrichment during different phases of development. The construction of this transcriptome allows us to determine not only the identity, but also the relative abundance of every transcript present during three distinct stages, 1-2h, 3-5h, and 6-8h, of gametophyte development.

By combining our FPKM values and gene ontology (GO) analysis we found that transcripts that are enriched early in development (during the 1-2 hour time point) are involved in mitosis, cell cycle regulation, DNA replication, and splicing. Late in development (6-8 hours post hydration), transcripts that are enriched include GO terms for microtubule and cytoskeletal associated proteins, ciliogenesis, centrosome, and axonemal components. The middle time point of development (3-5 hours post hydration) appears to be a transition time between the early stage of development, marked by cell division, and the later portion of development, which is dedicated to spermatid differentiation and ciliogenesis. GO terms that are enriched during this transition time include the proteasome and ubiquitin ligase components. It is therefore likely that significant amounts of protein and/or transcript degradation are

required during the 3-5h time point to transition the gametophyte from cell division to ciliogenesis (Boothby, 2013; Wolniak et al., 2015).

Spatial organization and rapid development

I am interested in the cytoskeletal dynamics that underlie the establishment of polarity and the morphological changes associated with differentiation and ciliogenesis during rapid development in the male gametophyte of *Marsilea*. Previous work points to the importance of division planes in establishing cell fate and polarity during gametogenesis. In the presence of cell division inhibitors, translation is able to continue normally (Tsai and Wolniak, 2001); however, cell fate is distorted in the gametophyte and spermatogenous cells are far less different compositionally from sterile jacket cells. Typically, sterile jacket cells and spermatogenous cells are morphologically distinct and, not surprisingly, they display different protein expression patterns. Visualizing spores with anti-centrin (which labels the blepharoplast and MLS) and anti-β-tubulin (which labels the microtubule ribbon) antibodies shows that these proteins are only found in the spermatogenous cells (Klink and Wolniak, 2001). Proteins that are important for formation of the cytoskeleton and ciliary apparatus also become exclusively localized in spermatogenous cells in normal gametophytes (Klink and Wolniak, 2003). The localization of these proteins in spermatogenous cells is what allows them to differentiate into motile spermatids. If division cycles are arrested, many of the mRNAs that encode these proteins are not localized to regions of the gametophyte that would become the spermatogenous cells, and these transcripts can be found throughout the entire gametophyte (Tsai et. al., 2004). Moreover, factors that control

the processing and translation of stored mRNA appear to be responsible for the differences in protein expression observed between jacket and spermatogenous cells (Tsai et. al., 2004; van der Weele et al., 2007; Wolniak et. al, 2011).

RNA processing mechanisms, namely splicing and polyadenylation, are essential processes that affect translation. Pre-mRNA splicing affects the timing for transcript export from the nucleus and this affects its ultimate destination in the cytoplasm (St Johnston, 2005; Besse and Ephrussi, 2008; Martin and Ephrussi, 2009). The association of the exon junction complex (EJC) with spliced mRNA mediates polysome association with mRNA (Nott et. al., 2004). Unlike other mRNA transcripts that show an equal distribution throughout the gametophyte, PRP19, a spliceosomal factor, is only found in the spermatogenous cells (Tsai et. al., 2004). Another mRNA that is similarly localized in the *Marsilea* gametophyte is RNA helicase (Tsai et. al., 2004), which attaches the EJC to mRNA (Anderson and Kedersha, 2006) and regulates splicing. In general, polyadenylation occurs in the nucleus; however, once transcripts reach the cytoplasm, lengthening or shortening of the poly(A) tail can occur (Villalba et. al., 2011). Longer poly(A+) tails have a higher level of translational activity, while transcripts with shorter poly(A+) tails show lower levels of translation (Gorgoni and Gray, 2004). Cytoplasmic polyadenylate polymerase (PAP) is responsible for lengthening poly(A+) tails after nuclear export. Interestingly, spermatogenous cells contain large amounts of polyadenylated mRNA, while jacket cells have very little. This difference may be due to the observed high levels of cytoplasmic PAP in spermatogenous cells (Tsai et. al., 2004). It is possible that the

localization of these RNA processing activities drives the differences in protein expression that underlie cell fate determination.

Upon desiccation in *Marsilea*, pre-mRNAs necessary for gamete development are stored as masked transcripts in large nuclear aggregates containing many nuclear speckles (Boothby and Wolniak, 2011). Most of these transcripts have retained introns and the splicing of these transcripts is necessary for their translation (Boothby et. al., 2013). The nuclear speckles move into the cytosol of the antheridial mother cell during the first mitotic division and then separate during the second mitotic division so that each antheridial mother cell has an equal amount of stored mRNA essential for gamete formation (Boothby and Wolniak, 2011). It is unclear how this occurs and how the transcripts are later dispersed among the spermatogenous cells. It is likely that nuclear speckles are dispersed into areas that become spermatogenous cells prior to the second division, though rises in the amount of the polyamine, spermidine, play a key role in the unmasking of stored pre-mRNAs in the gametophyte (Deeb et al., 2009; Boothby and Wolniak, 2011).

From these experiments, we know that cell division planes are required to establish cell fate (Tsai and Wolniak, 2001), that sterile jacket cells and spermatogenous cells contain significantly different sets of proteins that allow for the differentiation of spermatids into motile cells (Klink and Wolniak, 2001; Klink and Wolniak, 2003), and that these proteins become sequestered in spermatogenous cells through localized transcript unmasking, polyadenylation, splicing, and translation (Tsai et. al., 2004; van der Weele et al., 2007; Deeb et al., 2010; Boothby and Wolniak, 2011; Boothby et al., 2013). However, we do not know how division planes

are patterned during development, how mRNA processing components become localized to spermatogenous cells, or how differentiation is controlled in the gametophyte. Preliminary evidence from our lab suggests that the cytoskeleton and motor proteins are required to control the localization of many of these molecules (Molk and Wolniak, 2001; Deeb, 2009). Kinesins are molecular motors that are important for a variety of microtubule-orchestrated events that underlie cell biology. Kinesins use the energy from ATP hydrolysis to transport organelles, vesicles, and chromosomes along microtubules and to organize microtubule arrays during mitosis, cell morphogenesis, and ciliogenesis (Inoué and Salmon, 1995; Hirokawa, 1998; Sharp et al., 2000). Therefore, these proteins are likely to be important for the processes that regulate rapid development during male gametophyte development in *Marsilea*.

Kinesin Motor Proteins in Plants

The kinesin superfamily of motor proteins is marked by the presence of a highly conserved motor domain responsible for ATP hydrolysis and microtubule binding. Most kinesins function as homodimers, although some exist as monomers, heterodimers, or heterotrimers, and oligomerize through coil-coil regions of the proteins. Based on alignments of the conserved motor domain, kinesins are separated into fourteen families, kinesin-1 through kinesin-14 (Lawrence et al., 2004). Of these, kinesin-1 through kinesin-12 have N-terminal motors, kinesin-14s have C-terminal motors, and kinesin-13s have a centrally located motor domain (Endow, 1999). In general, this nomenclature has successfully been applied and expanded to include a wide range of eukaryotic organisms (Reddy and Day, 2001; Reddy and Day, 2011;

Shen et al., 2012); however, a more 'holistic' overview of the kinesin superfamily in eukaryotes questioned some aspects of this naming system (Wickstead and Gull, 2006). In this analysis, kinesin-4 and -10 are grouped together, and two subgroups within the kinesin-12 family were separated into new families termed kinesin-15 and kinesin-16. In addition, some previously orphaned kinesins were found conserved across a variety of taxa and, in accordance, were given new kinesin family names including kinesin-17, -18, -19, and -20 (Table 1-1). Although this new naming system is useful when describing and comparing the kinesin superfamily in diverse eukaryotes, it has not been universally adopted and the traditional kinesin-1 through -14 naming system prevails. For the purpose of consistency, I will be using the kinesin naming system that most frequently appears the literature when discussing plants in the upcoming chapters of this dissertation; however, references to 'holistic' kinesin families will be used when appropriate.

Table 1-1. Making sense of the kinesin nomenclature and family members

	Traditional Nomenclature	**'Holistic' Nomenclature**	**Plant Nomenclature**
Kinesin-1	HsKIF5	Kinesin-1A	Kinesin-1
Kinesin-2	HsKIF3A, Ce_klp20, DmKlp64D	Kinesin-2A	
	HsKIF3B, HsKIF3C, Ce_klp11, DmKlp68D	Kinesin-2B	
	HsKIF17, Ce_osm3	Kinesin-2C	
		Kinesin-2X	Kinesin-2
Kinesin-3	Ce_klp6	Kinesin-3A	
	HsKIF16B	Kinesin-3B	

	HsKIF1, Ce_unc104	Kinesin-3C	
	HsKIF13, Ce_klp4	Kinesin-3D	
	HsKIF14, DmNebbish	Kinesin-3E	
Kinesin-4	HsKIF21, Ce_klp12, DmKlp31E	Kinesin-4/10A	
	HsKIF4, Ce_klp3, DmKlp3A	Kinesin-4/10B	Kinesin-4 I
	HsKIF7, HsKIF27, DmKlp61F	Kinesin-4/10X	Kinesin-4 II
Kineisn-5	HsKIF11, Ce_bmk1	Kinesin-5	Kinesin-5
Kinesin-6	HsKIF23, Ce_zen4, DmPavarotti	Kinesin-6A	
	HsKIF20, DmSubito	Kinesin-6B	
Kinesin-7	HsCENP_E	Kinesin-7	Kinesin-7 I, -7 II, -7 III, -7 IV
Kinesin-8	Dm_Klp67A	Kinesin-8A	
	HsKIF19, Ce_klp13	Kinesin-8B	
		Kinesin-8X	Kinesin-8 I, -8 II
Kinesin-9	HsKIF9	Kinesin-9A	Kinesin-9
	HsKIF6	Kinesin-9B	Kinesin-9
Kinesin-10	HsKid	Same as kinesin-4	
Kinesin-11	KIF26	No evidence	
Kinesin-12	HsKIF12, HsKIF15	Split to kinesin-15 and kinesin-16	
Kinesin-13	HsKIF24	Kinesin-13A	Kinesin-13
	HsKIF2, Ce_klp7, DsKlp10A, DsKlp59	Kinesin-13B	
Kinesin-14	HsKIFC1, Ce_klp15, Ce_klp17, DmNcd	Kinesin-14A	Kinesin-14 I
	HsKIFC2, HsKIFC3	Kinesin-14B	Kinesin-14 II

		Kinesin-14B	Kinesin-14 III
		Kinesin-14C	Kinesin-14 VI
	HsKIF25	Kinesin-14D	Orphan II
		Kinesin-14X	Kinesin-14 IV
		Kinesin-14X	Kinesin-14 V
New Kinesin Families from 'Holistic' Analysis	HsKIF15, Ce_klp18	Kinesin-15	Kinesin-12 I, -12 II
	HsKIF12, DmKlp54D	Kinesin-16A	Orphan III
		Kinesin-16B	
		Kinesin-17	Kinesin-17
	HsKIF22	Kinesin-18	Kinesin-10
		Kinesin-19	ARK, ARK-LIKE
		Kinesin-20	
Orphaned Kinesins			Orphan I
			Orphan IV

In addition to providing a useful tool for the separation of kinesins into families, the location of the motor domain is also important for kinesin motility. Most kinesins have amino-terminus motors and are responsible for plus end directed microtubule transport, also known as anterograde transport. The kinesin-13 and kinesin-14 families are unique with motor domains located in the middle and at the carboxyl-terminus of the protein, respectively. Kinesin-13s are known for their microtubule depolymerizing activity while the c-terminus motor found in many members of the kinesin-14 family is responsible for retrograde, minus end directed, microtubule transport (Endow, 1999).

Outside the conserved motor domain there is limited homology between kinesins. Even kinesins within the same family and in the same organisms show little

similarity outside of the conserved motor domain. These non-motor regions are necessary for cargo binding and are responsible for directing many of the functions of individual kinesins. It is therefore difficult to infer kinesin protein function based on family (Dagenbach and Endow, 2004) and plant and animal kinesins of the same family do not frequently have conserved functions (Lee and Liu, 2004; Lee et al., 2015). This is especially troublesome for plant biologists, where compared with animals and fungi, less is known about the function of individual kinesins in orchestrating microtubule dynamics.

Compounding on this general lack of knowledge is the fact that plant cells have significantly more kinesin protein types than animal cells. In *Arabidopsis thaliana*, a dicot flowering plant and a common model system in plant cell biology, 61 kinesin genes have been identified (Reddy and Day, 2001). Rice, *Oryza sativa*, another angiosperm, has 52 kinesin genes (Richardson et al., 2006). Even in a relatively simple plant, such as the moss *Physcomitrella patens*, a large kinesin family of 78 genes has been discovered (Shen et al., 2012; Miki et al., 2014). In contrast, mammals have 45-50 kinesins in the genome (Miki et al., 2001). The reason why there are so many more kinesins in plant cells than animal cells is generally attributed to the fact that plants lack genes that encode for cytoplasmic dynein. Genes for intraflagellar transport (IFT) dynein and axonemal dynein are found in plants that make ciliated cells, such as the green algae *Chlamydomonas* and the lycophyte *Selaginella moellendorffii*, but cytoplasmic dynein-1, the dynein responsible for retrograde cytoplasmic transport outside of cilia, is absent in the plant kingdom (Wickstead and Gull, 2007). To date only a few plant kinesins have been implicated

in retrograde transport (Jonsson et al., 2015; Walter et al., 2015) and the biological significance of this transport is not yet fully understood.

Comparative analyses of the kinesin family among eukaryotes have revealed a number of interesting characteristics that distinguish the kinesin family in plant cells from animal cells. In addition to a larger total number of kinesins, unique, plant-specific, kinesins with an armadillo repeat (ARM), a myosin tail (MyTH4), a malectin, and actin binding calponin homology (CH) domains, plus a number of 'orphan' kinesins have been identified. Some of these 'orphan' kinesins are homologous to animal kinesins, but others have a more restricted distribution. For example, the kinesin-'orphan' III motor in plants (Shen et al., 2012) is homologous to human Kif12 and *Drosophila* KLP54D (Wickstead and Gull, 2006; Wickstead et al., 2010b) (Table 1-1). However, members of the kinesin-'orphan' I and IV families are true orphans and have no animal homologs (Table 1-1). In contrast, many kinesin families can be found in animals that are not present in plants. The kinesin-3, -6, and -11 families are excellent examples and members of these families are absent in plants.

Of particular interest is the kinesin-14 family. In plants, this family is very large, with 21 members in *Arabidopsis* (Richardson et al., 2006) and fifteen in *Physcomitrella* (Shen et al., 2012) that can be grouped into six subfamilies, kinesin-14 I through VI (Shen et al., 2012). In general, kinesin-14s are known for their c-terminus motors and are implicated in minus-end directed transport in animal cells (Endow, 1999). Plant kinesin-14s not only have this typical c-terminus motor (kinesin-14 I and VI), but members of this family also have motors domains that are at the n-terminus (kinesin-14 IV and V) and in the middle of the protein (kinesin-14 II

and III) (Reddy and Day, 2001; Lee and Liu, 2004; Shen et al., 2012). Unique domains not associated with animal kinesins are found in the non-motor regions of many of these proteins. Kinesin-14 II contains an actin binding CH domain that is important for crosslinking actin and microtubule arrays (Tamura et al., 1999; Preuss et al., 2004; Frey et al., 2009; Xu et al., 2009; Umezu et al., 2011), a function not associated with any animal kinesin. Kinesin-14 III has a glucose-binding malectin domain (Schallus et al., 2008) of unknown biological significance for the kinesin motor. As intriguing as these kinesins appear, perhaps the most bizarre is kinesin-14 VI, also known as KCBP. This minus-end directed (Song et al., 1997) plant-specific (Abdel-Ghany et al., 2005) motor has a calmodulin-binding domain that negatively regulates its ability to bind microtubules (Narasimhulu et al., 1997; Deavours et al., 1998). It is also considered to be 'half myosin' with MyTH4 and FERM domains located in the n-terminus, which are typically associated with myosin motors (Abdel-Ghany et al., 2005). These domains are important for crosslinking microtubules, membrane material, and F-actin arrays (Narasimhulu and Reddy, 1998; Oliver et. al., 1999; Tian, et al., 2015).

Although many differences exist between plant and animal kinesin families, in all organisms these motors are important for regulating microtubule based transport and cytoskeletal dynamics during mitosis, cell morphogenesis, and ciliogenesis. In general, kinesins can be separated into two main groups based on function; kinesins that have roles during mitosis or cytokinesis, and those with functions outside of cell division, although some overlap between these groups does exist.

Plant cell division and mitotic kinesins

Plants must overcome a number of obstacles that are absent in animals in order to complete cell division. Firstly, plant cell mitosis occurs without the involvement of a focused centrosome to organize microtubule spindles. Secondly, plant cells lack cytoplasmic dynein, which is important for spindle alignment (McGrail and Hays, 1997; Busson et al., 1998; Sharp et al., 2000), microtubule focusing (Merdes et al., 2000; Quintyne et al., 2005), and kinetochore-spindle interactions (Howell et al., 2001; Wojcik et al., 2001; Varma et al., 2008; Howell et al., 2001). Thirdly, most vegetative plant cells are immotile and are surrounded by a rigid cell wall. The wall places special constraints upon cytokinesis and makes the selection of a division plane is especially important for cell morphology, cell fate determination, and the regulation of subsequent differentiation (Jürgens, 2000). In order to surmount these challenges, plant cells utilize two specialized microtubule arrays to guide the placement of the division plane and to form the cell plate that is responsible for partitioning of the forming daughter cells. These arrays are called the pre-prophase band (PPB) and the phragmoplast, respectively. The PPB is a belt-like array of microtubules that forms during the G_2/M transition and encircles the cell in the area where the cell plate will later fuse with the plasma membrane (Dhonukshe et. al., 2003; Müller et. al., 2009; Rasmussen et. al., 2011). Upon nuclear envelope breakdown, the PPB disappears and the area is 'remembered' by positive and negative identification markers localized in an area known as the cortical division zone (CDZ) (Lipka and Müller, 2012). The phragmoplast is a non-overlapping array of antiparallel microtubules that forms in late anaphase/early telophase and functions

to allow vesicle fusion for cell plate formation, and to then align the plate in the CDZ during cytokinesis (Rasmussen et. al., 2011).

Kinesin motor proteins regulate the localization and dynamics of the PPB and phragmoplast microtubule arrays, and other kinesins are important for chromosome alignment and for organizing the mitotic spindle. Most studies on the role of kinesins during mitosis in plant cells have been performed with *Arabidopsis* and *Physcomitrella* vegetative cells. In *Arabidopsis* 23 of the 61 kinesins (37.7%), are upregulated during mitosis (Vanstraelen et al., 2006). In a landmark study in *Physcomitrella* caulonema cells (Miki et al., 2014*)* the endogenous localization of all 78 kinesins were tracked during cell division. It was found that 43 kinesins (55.1%) were associated with mitotic structures such as chromosomes, the kinetochore, spindle microtubules, and the phragmoplast (Table 1-2). Some of these kinesins appear to have conserved functions with their animal counterparts, though most do not, and the mechanism of action for many of these proteins remains unknown. Compared to what is known about animal kinesins, the current state of research on plant kinesins is in its infancy.

Table 1-2. Mitotic kinesins in *Arabidopsis* and *Physcomitrella*

Phragmoplast associated kinesins		
Kinesin-4	AtFRA1	Transports Golgi-derived vesicles containing cell wall material along cortical microtubules (Zhong et al., 2002, Zhu et al., 2015).
	PpKinesin-4Ia	Localizes to the phragmoplast equator during cytokinesis (Miki et al., 2014).
Kinesin-7 II	AtNACK	Localizes to the phragmoplast equator and activates signaling required for cell plate formation by mediating phragmoplast microtubule based transportation (Ishikawa et al., 2002; Nishihama et al., 2002; Strompen et al., 2002; Soyano et al., 2003; Takahashi et al., 2010; Sasabe et al., 2012).
	PpKinesin-7 II	Important for chromosome alignment, interdigitation of phragmoplast microtubules, and for phragmoplast expansion (Natio and Goshima, 2015).
Kinesin-12 II	AtPAKRP	Localize to the plus end of phragmoplast microtubules during phragmoplast development and are needed to organize microtubules (Lee and Liu, 2000; Pan et al., 2004; Lee et al., 2007; Oh et al., 2012).
	PpKinesin12-IIa, -IIc, -IId	Localize to the phragmoplast equator during cytokinesis (Miki et al., 2014).
Orphan II	AtPAKRP2	Contributes to vesicle transport in the phragmoplast (Lee et al., 2007).

	PpOrphan-IIa, -IIb	Essential for the generation of interdigitated antiparallel microtubules in the phragmoplast (Hiwatashi et al., 2008).
Spindle Assembly Kinesins		
Kinesin-5	AtKRP125	Required for organized spindle microtubules. Mutants show defects in the stabilization of anti-parallel microtubules (Bannigan et al., 2007).
	PpKinesin-5	Required for chromosome segregation and organized spindles (Miki et al., 2014).
Kinesin-8 II	PpKinesin-8 II	Located at the midzone from prometaphse to cytokinesis (Miki et al., 2014).
Kinesin-13	PpKinesin-13b and c	Localize to microtubule midzone during metaphase and anaphase and to the phragmoplast equator during cytokinesis (Miki et al., 2014).
Kinesin-14 I	ATK1, ATK5	Required for spindle bipolarity, but not chromosome segregation. ATK5 participates in the capture of antiparallel microtubules to generate the force to align microtubules—spindle length, width, and integrity (Marcus et al., 2003; Ambrose and Cyr, 2007).
	PpKinesin-14 I	Localizes to the midzone and intra-spindle region during metaphase, the spindle during anaphase, and phragmoplast during cytokinesis (Miki et al., 2014).

Kinetochore Associated Kinesins		
Kinesin-4 I	PpKinesin-4 Ic	Localizes to the nucleus, chromosomes, and midzone (Miki et al., 2014).
Kinesin-7 III	PpKinesin-7 III	Localized to the kinetochore from prophase to anaphase (Miki et al., 2014).
Kinesins Important for Establishing and Maintaining Positional Information of the Pre-Prophase Band		
Kinesin-12 I	AtPOK	Recruited to the PPB and preserves the positional information (Lipka et al., 2014).
Kinesin-14 VI	AtKCBP	KCBP interacts with AIR9, which is important for establishing the memory of the PPB (Buschmann et al., 2015).
Kinesins Associated with Nuclear Positioning and Asymmetric Divisions		
Kinesin-14 II	ATK4	Interacts with microtubules and actin filaments and is required for nuclear positioning (Frey et al., 2010).
ARK	AtARK3	Accumulates at the PPB in a cell cycle dependent manner, but does not remain at the PPB, important for asymmetric division planes (Malcos and Cyr, 2011).
	PpARK	Drives nuclear migration during mitosis in moss. In mutants the nucleus fails to reach the center of the cell and there are no microtubule bundles around the nucleus (Miki et al., 2015).
Kinesins with Potential Functions During Mitosis		
Kinesin-1	AtKinesin-1	Required for female gametogenesis (Zhou et al., 2011).

Kinesin-7 IV	PpKinesin-7 IV	Localizes to spindle and phragmoplast microtubules (Miki et al., 2014).
Kinesin-8 I	PpKinesin-8 Ia, b	Weakly detected at the midzone during anaphase and cytokinesis (Miki et al., 2014).
Kinesin-10	AtKinesin-10	Up-regulated during mitosis (Vanstraelen et al., 2006).
Kinesin-13	AtKinesin-13A	Depolymerizes microtubules and influences Golgi motility and distribution; alters cell wall structure (Lu et al., 2005; Wei et al., 2009; Fujikura et al., 2014).
Kinesin-14 II	PpKinesin-14 IIa, c	Localizes to spindle poles during anaphase and cytokinesis (Miki et al., 2014).
Kinesin-14 III	PpKinesin-14 IIIa, b	Localize to microtubules during all stages to mitosis (Miki et al., 2014).
ARK	PpARKa, b, c	Localize to microtubules during all stages to mitosis (Miki et al., 2014).
Orphan II	PpOrphan-IIa, b	Localized to the microtubule midzone during all stages of mitosis and regulate the turnover, directionality, and growth of microtubule bundles in the expansion zone (Hiwatashi et al., 2014; Miki et al., 2014).

The first step in plant cell division is the organization of the PPB. The PPB not only selects the division site, but also preserves the positional information of this area through the establishment of the CDZ. Kinesins are involved in various aspects of PPB formation and CDZ maintenance. In *Arabidopsis* two members of the kinesin-12 I family, more commonly referred to as phragmoplast-orienting kinesin 1 and 2 (POK1/2), are recruited to the PPB during prophase. POK1/2 remain in the CDZ area upon disassembly of the PPB and are required to retain TAN and RanGAP1, important markers of the CDZ, in that region of the cortical cytoplasm (Lipka et al., 2014). *Arabidopsis* Kinesin-14 VI (KCBP) also localizes to the PPB where it interacts with AIR9, another important CDZ marker, and it too remains localized in the area throughout mitosis (Buschmann et. al., 2015). Surprisingly, mutations in the *Arabidopsis* KCBP gene only cause a minor defect in trichome branching and do not show any problems with cell division (Oppenheimer et al., 1997). Similarly, in *Physcomitrella* kinesin-14 VI is only weakly expressed near the nuclear envelope during cytokinesis and is not considered to play a role in mitosis (Miki et al., 2014). Due to the large number of kinesin-14s, functional redundancy could explain a seemingly confusing mixture of phenotypes. It remains unclear if additional kinesins are important for assembling the PPB or for facilitating the localization of CDZ markers through microtubule-based transport after the PPB microtubules disperse.

In plant cells, asymmetric divisions are responsible for establishing cell fate and patterns of differentiation during development. Since the PPB is vitally important for marking the site of cytokinesis, the mechanisms that regulate the position of this microtubule array are very important. One PPB localized kinesin in particular is

necessary for ensuring the fidelity of asymmetric divisions. A member of the ARK family in *Arabidopsis*, ARK3/KINUA, localizes to the PPB, but does not remain there to mark the CDZ later in mitosis. Mutations in this kinesin show defects in stomatal development, which relies on asymmetric divisions for the formation of the guard cells in the epidermis (Malcos and Cyr, 2011). In *Physcomitrella,* ARK is required to drive nuclear migration. Mutations in this kinesin produce cells where the nucleus fails to reach the cell center and no microtubule bundles are visible around the nucleus during prophase (Miki et al., 2015). The *Arabidopsis* kinesin-14 II, ATK4, is also implicated in nuclear positioning during mitosis (Frey et al., 2010), although this motor is not associated with the formation of the PPB. The location of the nucleus is important for establishing polarity and typically the asymmetric localization of the nucleus precedes asymmetric divisions (De Rybel et al., 2010). Since little is known about the mechanisms that regulate nuclear positioning and asymmetric divisions, additional research is needed to determine the exact role of these kinesins in nuclear positioning, the establishment of the PPB, and in the guiding asymmetric divisions that underlie cell fate determination in plants.

Once a division plane has been defined, a barrel-shaped spindle forms to align chromosomes at the metaphase plate and to ensure the fidelity of sister chromosome segregation. Like animal cells, the plant mitotic spindle consists of a bipolar antiparallel microtubule array. Microtubule plus ends are directed towards the center of the cell where they undergo rapid growth and shrinkage. However, unlike animal cells, plant cells lack a centrosome and therefore microtubules must 'self-organize' into a bipolar spindle instead of relying on centrosome-mediated organization. This is

thought to arise through a Ran-based mechanism, where spindle microtubules are assembled in areas high in RanGTP and stabilized with spindle assembly factors that are localized in the spindle pole regions (Zhang and Dawe, 2011). Kineins-5 has been suggested as a likely component involved in the assembly of bipolar spindles, but recent evidence from *Physcomitrella* brings this assumption into question. In *Arabidopsis* kinesin-5 is required for the establishment of organized spindle microtubules. Without this kinesin, monopolar spindles, that are reminiscent of kinesin-5 mutants in animal cells, are present (Bannigan et al., 2007). Although *Physcomitrella* kinesin-5s are found at the spindle midzone, they are required for chromosome segregation and post-anaphase spindle assembly, rather than for the establishment of a bipolar spindle (Miki et al., 2014). Kinesin-14 I is also important for spindle assembly. In *Arabidopsis*, two members of the kinesin-14 I family are implicated in this process. ATK1 is necessary for spindle bipolarity (Marcus et al., 2003) and ATK5 participates in the capture of antiparallel microtubules and is required for regulating spindle size and integrity (Ambrose et al., 2005; Ambrose and Cyr, 2007). Similarly, in *Physcomitrella,* kinesin-14 I localizes to the spindle microtubules (Miki et al., 2014). These functions are reminiscent of kinesin-14A, the animal homolog of kinesin-14 I, which is known as KIFC1 in mammals. This minus-end directed motor is found at the spindle pole and midzone and functions in bipolar spindle organization (Cross and McAinsh, 2014).

In animal cells, kinesin-4, -7, -8, -10, -13, and cytoplasmic dynein are all required for regulating microtubule-chromosome interactions (Cross and McAinsh, 2014). These interactions guide chromosome congression during prometaphase,

orientation on the metaphase plate, and sister chromosome separation (Anaphase A) and spindle elongation (Anaphase B) during anaphase. Kinesin-4, -7, and -10 are chromokinesins, so called for their motor activity and ability to directly bind chromosomes either at the kinetochore (kinesin-7) (Yen et al., 1991; Schaar et al., 1997; Wood et al., 1997; Yao et al., 2000; Yardimci et al., 2007; Kim et al., 2008; Cai et al., 2009; Akera et al., 2015) or chromosome arms (kinesin-4 and -10) (Tokai et al., 1996; Tokai-Nishizumi et al., 2005; Wu and Chen, 2008; Bieling et al., 2010). Kinesin-8 and -13 depolymerize microtubules and use this depolymerizing activity to aid in chromosome congression and segregation, respectively (Walczak et al., 1996; Maney et al., 1998; Desai et al., 1999; Kline-Smith et al., 2004; Rogers et al., 2004; Mayr et al., 2007; Varga et al., 2009; Wickstead et al., 2010a; Weaver et al., 2011). In plants, the kinesins that regulate mitotic chromosome movement remain enigmatic. Only one plant kinesin, kinesin-7 III, localizes to the kinetochore. One member of the kinesin-4 I family co-localizes with chromosomes throughout mitosis (Miki et al., 2014). Plant kinesin-13 also depolymerizes microtubules (Lu et al., 2005; Wei et al., 2009) and in *Physcomitrella* kinesin-13 and kinesin-8 II localize to the midzone throughout mitosis (Miki et al., 2014). These observations suggest conserved functions for particular kinesins, but additional functional assays are required to determine whether these localization patterns are linked to specific mitotic mechanisms; for example, *Arabidopsis* kinesin-13 has been shown to be important for Golgi localization and for cell wall structure, and not mitosis *per se* (Lu et al., 2005; Wei et al., 2009; Fujikura et al., 2014).

The vast majority of animal cells undergo cytokinesis using a cleavage furrow that constricts the isthmus between the separating daughter cells. However, in plants, there is no constriction of an isthmus between daughters. Instead plant cells assemble a structure known as a cell plate. As anaphase nears completion in plant cells, the phragmoplast forms to facilitate the movement and fusion of vesicles containing wall material near the site of the former metaphase plate. As the vesicles fuse together, they form a large flattened vesicle that will ultimately fuse with the plasma membrane (at the CDZ) to create a partition between the daughter cells. The mechanisms responsible for cytokinesis differ in plant and animal cells and not surprisingly, the main kinesin associated with cytokinesis in animal cells, kinesin-6 (Vale et al., 2009; Janisch et al., 2013; Janisch and Dwyer, 2016), is absent in plants (Wickstead and Gull, 2006; Wickstead et al., 2010b; Shen et al., 2012). Many kinesins are found at the phragmoplast during cytokinesis, including members of the kinesin-4, -5, -7, -8, -12, -13, -14, orphan-II, and orphan-IV families (Miki et al., 2014). The functions of only a few of these kinesins in phragmoplast assembly, expansion, and transport are currently known. Members of the kinesin-12 II family localize to microtubule plus-ends during phragmoplast development in *Physcomitrella* (Miki et al., 2014) and *Arabidopsis* (Lee and Liu, 2000; Pan et al., 2004) and these kinesins are needed for organized phragmoplast expansion during cytokinesis (Lee et al., 2007; Oh et al., 2012). *Arabidopsis* kinesin-7 II, NACK, is found at the phragmoplast equator where it activates a MAP kinase signaling cascade required for cell plate formation and phragmoplast expansion (Nishihama et al., 2002; Strompen et al., 2002; Soyano et al., 2003; Takahashi et al., 2010; Sasabe et al., 2012). Members of the kinesin-7 II and

orphan-II families are also implicated in phragmoplast microtubule based transport. Kinesin-7 II is a processive, plus-end directed motor required for NPK1 transport to the phragmoplast equator (Ishikawa et al., 2002; Nishihama et al., 2002; Naito and Goshima, 2015). Kinesin-orphan II contributes the organization of the phragmoplast during cytokinesis and is essential for the generation of interdigitated antiparallel phragmoplast microtubules (Lee et al., 2007; Hiwatashi et al., 2008; 2014).

Although information about the function of these mitotic kinesins in other plant systems is important for comparative analysis, it is important to note that *Marsilea* male gametophytes are very different than *Arabidopsis* and *Physcomitrella* vegetative cells. The main difference is that *Marsilea* male gametes are capable of producing motile cilia, while vegetative cells of these plants are non-motile and do not make cilia. Also, the mechanisms of cell division are different. *Arabidopsis* uses a PPB and the phragmoplst to position the plane of cell division and to complete cytokinesis, respectively. *Physcomitrella* caulonemal cells do not use a PPB for division plane selection. Instead these cells use self-organized processes for division plane selection, spindle alignment, and phragmoplast assembly; many of which may be absent in plant cells that use the PPB (Lloyd and Chan, 2006; Bannigan et al., 2008; Müller et al., 2009; Goshima and Kimura, 2010). It is therefore difficult to extrapolate the function of mitotic kinesins from *Arabidopsis* to *Physcomitrella* and to other plants.

Plant kinesins function in intracellular transport and microtubule organization

Both animal and plant kinesins are required for intracellular transport, organelle localization, and general microtubule organization. Recently, the

information on processive kinesins in plants has exploded. During the past few years, the number of plant kinesins with predicted roles in intracellular transport has grown from only one, to at least four, and one other non-processive motor has also been implicated in this process. FRA1, a member of the kinesin-4 I family, was the first motor discovered in plants with the potential ability to transport cargo. Mutations in this kinesin resulted in disorganized cellulose microfibrils and a fragile cell wall. This phenotype provided evidence that FRA1 was involved in the transport of vesicles containing cell wall material (Zhong et al., 2002; Zhang et al., 2010). Evidence now suggests that FRA1 is functions as a plus-end directed processive motor that actively transports Golgi-derived vesicles containing cell wall polysaccharides along cortical microtubules for secretion (Zhu and Dixit, 2011; Zhu et al., 2015). Kinesin-7 IIb and ARK also exhibit plus-end directed motility. *In vitro*, FRA1 and kinesin-7 IIb move at about 400 nm/s (Zhu and Dixit, 2011; Natio and Goshima, 2015). This is a velocity similar to what has been recorded for other motile kinesins (Woehlke and Schliwa, 2000). ARK is slower and moves along microtubules at about 200nm/s (Miki et al., 2015). Kinesin-7 IIb is necessary for the transport of cargo along phragmoplast microtubules (Ishikawa et al., 2002; Nishihama et al., 2002). ARK is implicated in nuclear positioning in *Physcomitrella* (Miki et al., 2015), but in *Arabidopsis* the role of this motor is limited to in general microtubule organization (Jones et al., 2006; Yang et al., 2007; Sakai et al., 2008; Eng and Wasteneys, 2014). A clear role for ARK in intracellular transport has yet to be established.

Adding to the growing list of plant kinesins with the potential to drive long distance transport, two kinesins with microtubule minus-end directed motility have

recently been identified. In *Physcomitrella,* kinesin-14 VIb is able to drive processive retrograde transport after oligomerization, potentially replacing the function of cytoplasmic dynein in plant cells. *In vitro,* the oligomerized kinesin-14 VIb directs very fast transport, at about 600nm/s, and is sufficiently powerful to move experimentally introduced liposomes. However, without oligomerization, kinesin-14 VIb was unable to direct microtubule-based transport, potentially questioning the *in vivo* transport capabilities of this kinesin (Jonsson et al., 2015). In rice, another member of the kinesin-14 family, KCH, also known as kinesin-14 II, was shown to transport actin filaments along microtubules (Walter et al., 2015). In *Arabidopsis,* kinesin-14 II is important for nuclear positioning (Frey et al., 2010). The ability for kinesin-14 II to transport actin along microtubules is likely to be important for this interaction and provides clues for the mechanisms that regulate nuclear localization.

In plants, organelle transport and localization is mostly dependent on attachments between myosin motor proteins and actin (Sparkes, 2010); however, the actions of a few kinesin motors are known to be required. *Arabidopsis* kinesin-13A influences Golgi motility and distribution (Lu et al., 2005, Wei et al., 2009) and reduced activity of kinesin-13A leads to defects in cell wall structure (Fujikura et al., 2014). This occurs through the preferential localization of kinesin-13A to depolymerized cortical microtubules to prevent cell wall deposition (Oda and Fukuda, 2013a; 2013b). Exactly how Golgi morphology is linked to the construction of the cell wall by this kinesins remains unclear. Plant specific kinesin-14 V mediates chloroplast movement in response to light through the stabilization of cortical actin filaments in *Physcomitrella* (Shen et al., 2015). In most plants, chloroplast movement

is dependent on actin dynamics. Since kinesin-14 V links microtubules to actin filaments in *Arabidopsis* and tobacco BY-2 cells (Klotz and Nick, 2012; Schneider and Nick, 2015), a conserved role for kinesin-14 V in chloroplast movement might exist, although it has only been definitively observed in *Physcomitrella*.

In animal cells, kinesin and dynein motors mediate mitochondrial localization. MIRO, a RhoGTPase, binds to mitochondria and facilitates mitochondrial movement by kinesin-1 (KIF5) and dynein through interactions with the adaptor protein Milton/TRAK (Macaskill et al., 2009; Wang and Schwarz, 2009; Morlino et al., 2014). *Arabidopsis* has three MIRO homologs that all localize to mitochondria and these proteins are involved in regulating mitochondrial morphology; however, no obvious homolog for Milton has been identified (Yamaoka and Leaver, 2008; Yamaoka et al., 2011). Therefore, although MIRO is conserved, this mechanism of mitochondrial movement may not exist in plants. *Arabidopsis* MKRP, a member of the kinesin-7 I family, is expressed in mitochondria via an n-terminus mitochondrial targeting signal (Itoh et al., 2001). A member of the kinesin-14 II family, AtKP1, also localizes to the mitochondria via a c-terminus domain that specifically interacts with the mitochondrial outer membrane protein, VDAC3 (Ni et al., 2005). Neither MKRP nor AtKP1 is currently implicated in mitochondrial localization. Instead AtKP1 and VDAC3 work together within the mitochondrion to regulate aerobic respiration during seed germination (Yang et al., 2011). The consequence of the MKRP/mitochondrial interaction is not yet known.

Kinesins and ciliogenesis

The most well studied non-mitotic kinesins in plants are those involved in ciliogenesis. Cilia consist of microtubule projections, called axonemes, which extend from a basal body situated in the cortical cytoplasm, and they extend outward from the cell surface where they are surrounded by a specialized membrane. The typical axoneme of motile cilia consists of nine parallel outer microtubule doublets in a cylinderical array that surround a central pair of single microtubules. The microtubule plus ends are oriented distally. Axoneme microtubules are nucleated from the basal body. This arrangement, known as the 9 + 2 arrangement, was first observed in the cilia of a fern sperm cell (Manton and Clarke, 1951) and is conserved in almost all organisms that possess motile cilia (Phillips, 1969; Ross and Robinson, 1969). Dynein arms attach to the outer microtubules and power motility through sliding interactions between adjacent doublets (Satir and Christensen, 2007). Ciliogenesis is the conserved process responsible for building both motile and sensory cilia.

The green alga *Chlamydomonas reinhardtii* is a common model system used for studies on ciliogenesis and motility (Silflow and Lefebvre, 2001). The motility of this unicellular organism is powered by the action of two flagella (though they actually beat like cilia as the cell swims forward). Proteins important for the assembly and function of cilia and flagella are transported by the intraflagellar transport (IFT) complex to the distal ends of forming axonemes by members of the kinesin-2 family and transported proximally back to the cell body by IFT dynein, which is also referred to as dynein-1b or cytoplasmic dynein-2 (Kozminski et al., 1993; Rosenbaum and Witman, 2002). Kinesin-2 is a heterotrimeric protein consisting of kinesin-2α, -

2β, and a kinesin associated protein (KAP) important for cargo binding (Cole et al. 1993, Wedaman et al. 1996; Takeda et al., 2000; Jimbo et al., 2002). Without FLA10, which is a member of the heterotrimeric kinesin-2 complex in *Chlamydomonas*, IFT is blocked and ciliary assembly is disrupted (Huang et al., 1977; Walther et al., 1994; Kozminski et al., 1995; Cole et al., 1998). Kinesin-2 also exists as a homodimer of two kinesin-2γ subunits (Signor et al., 1999). Kinesin-2γ, also known as OSM-3 or KIF17, has a distinct role in assembling cilia that is separate from heterotrimeric kinesin-2 and both types of kinesin-2 motors must work together for the biogenesis of sensory cilia (Perkins et al., 1986; Setou et al., 2000; Snow et al., 2004; Evans et al., 2006; Imanishi et al., 2006; Pan et al., 2006).

The first step in IFT is the recruitment and assembly of kinesin-2, IFT dynein, IFT particles, and the loading of cargo to the region where the basal body meets with the ciliary base (Deane et al., 2001). This region is known as the transition fiber. Next, kinesin-2 transports IFT particles containing cargo and IFT dynein towards the distal tip of the cilium. IFT particles are classified into two subcomplexes, IFT-A and IFT-B (Piperno and Mead, 1997; Cole et al., 1998; Ou et al., 2005). Both IFT-A and IFT-B consist of multiple IFT proteins and form large scaffolding complexes that bind the cargo necessary for ciliary assembly and function (Cole, 2003; Jekely and Arendt, 2006). The IFT-A subcomplex is necessary for returning IFT proteins to the cell body and the IFT-B subcomplex is indispensible for ciliogenesis (Piperno et al., 1998; Iomini et al., 2001; Tsao and Gorovsky, 2008; Absalon et al., 2008). IFT46 and IFT27, which are components of the IFT-B subcomplex, are required for the transport of outer-arm dynein and for flagellar assembly in *Chlamydomonas*, respectively (Hou

et al., 2007; Ahmed et al., 2008). Once kinesin-2 and its multitude of cargo reach the tip of the cilium, a series of events termed 'turnaround' occur (Pedersen et al., 2008). During 'turnaround' kinesin-2 is inactivated and all its cargo is unloaded. IFT dynein then replaces kinesin-2 as the motor for retrograde transport and uptakes any cargo destined for the cell body. IFT dynein moves this cargo towards the base of the cilium where the IFT machinery is dissembled and recycled.

In addition to kinesin-2, several other kinesins participate in ciliogenesis, although considerably less is known about their exact roles. In *Chlamydomonas* kinesin-like protein 1 (KLP1), a member of the kinesin-9A family, localizes to the central pair of microtubules in ciliary axonemes (Bernstein et al., 1994) where it is regulates the activity of flagellar dynein through interactions with Hydin (Yokoyama et al., 2004; Lechtreck and Witman, 2007). Hydin is a conserved protein that positions central pair microtubules and is necessary for motility in eukaryotes (Dawe et al., 2007; Lechtreck et al., 2008). Members of the kinesin-13 family regulate ciliary length during ciliogenesis. Mutations in kinesin-13 produce cilia that are either too long or too short, depending on the type of mutation and the organism used for analysis (Blaineau et al., 2007; Dawson et al., 2007; Piao et al., 2009; Chan and Ersfeld, 2010; Kobayashi et al., 2011; Delgehyr et al., 2012; Wang et al., 2013). These opposing phenotypes were reconciled in a recent study where kinesin-13 was found to function as an assembly-promoting factor in the cilia (Vasudevan et al., 2014). The plant-specific kinesin-14 VI, KCBP, concentrates at basal bodies in *Chlamydomonas*. This kinesin is also enriched on cytoplasmic microtubules and in the ciliary membrane (Dymek et al., 2006). It is tempting to speculate that the

recently discovered ability of kinesin-14 VI to direct retrograde transport (Jonsson et al., 2015) might explain these localization patterns and define a role for KCBP during ciliogenesis.

Although the mechanisms and motors involved in ciliogenesis are highly conserved in eukaryotes, the presence of motile cilia across land plants is not as uniformly distributed. In the transition from water-based to land-based life, many groups of organisms lost the ability to make cilia, including higher plants like conifers and angiosperms. The non-vascular bryophytes (mosses, liverworts, hornworts) are thought to be among the early land plants and their only ciliated cells are male gametes (Brown et al., 2015). Sporophytes of these plants do not produce ciliated cells. Similarly, seedless vascular plants such as ferns produce cilia only in their male gametes (Raven et al., 1999; Pryer et al., 2004). Selected members of the gymnosperms, namely, *Ginkgo biloba*, and the cycads produce ciliated male gametophytes. In these organisms pollen grains, which contains the sperm, are shed and carried by the wind to the ovule. Once attracted to the ovule, the pollen begins to develop and releases spermatozoids that transport themselves through the pollen tube to fertilize the ovule. It is ironic that these gametes may possess thousands of cilia, yet they swim extremely short distances in order to fuse with an egg cell (Wolniak et al., 2011). Kinesin-2, kinesin-9, and kinesin-'orphan' III are conspicuously absent in *Arabidopsis* but present in *Physcomitrella*, *Chlamydomonas*, and almost all other ciliated eukaryotes (Wickstead and Gull, 2006; Wickstead et al., 2010b). It is likely that *Arabidopsis* has lost these kinesins, plus other components of the IFT apparatus, along with the ability to make motile cilia.

The Purpose and Significance of this Dissertation

With this dissertation, my goal is to provide evidence to show that kinesin motor proteins are essential for establishing cell fate and in regulating spermatid differentiation and ciliogenesis in the male gametophyte of *Marsilea vestita*. In Chapter 2, I begin by using transcriptome analysis and bioinformatics to identify and classify each of the kinesins present in the *Marsilea* male gametophyte. The kinesin superfamily has never been identified in a fern and although this analysis is limited to the transcriptome from the male gametophyte, it has proved to be a useful tool for comparative studies and evolutionary biology. Chapter 3 investigates how these kinesins motor proteins are involved in male gametophyte development in *Marsilea*. Transcriptome analysis shows that kinesin transcripts change in abundance during development and that transcripts that encode mitotic kinesins are abundant early in development, while transcripts that encode kinesins associated with ciliogenesis are abundant later, as the spermatids mature. Reverse genetic approaches confirmed this and demonstrated that the temporal regulation of kinesin transcript abundance changes directly correlates with protein function and with overarching cellular processes that occur during successive phases of gametophyte development. In Chapter 3, I provide evidence that kinesins are likely to play key roles in limiting the rate and extent of rapid development during both the mitotic and differentiation stages in the gametophyte. Chapter 4 focuses on how specific kinesins are important for spermatid differentiation. Particularly, I address the roles of kinesin-2 and kinesin-9 during ciliogenesis. I found that kinesin-2 and kinesin-9 are important for distinct

aspects ciliogenesis and that their roles in forming the ciliary axonemes in *Marsilea* differ from those observed in *Chlamydomonas*. This is the first time the mechanisms that regulate ciliogenesis have been studied in a land plant. Chapter 5 continues this analysis by examining the presence of other important mediators of ciliogenesis and motility such as dynein and the IFT machinery. Transcriptome analysis shows that functional cilia are constructed without the help of IFT dynein, outer arm dynein, or the BBsome; although some of the IFT machinery is present. The *Marsilea* male gametophyte makes what appears to be the basic complement of proteins required for the construction of motile cilia through IFT.

Chapter 2: Identification of 56 Kinesin-like Transcripts Present During Male Gametophyte Development in *Marsilea vestita*

Note: The following has been partially adapted from Tomei and Wolniak, Cytoskeleton, 2016. DOI 10.1002/cm.21285.

Introduction

Plant cells contain a large and highly diverse variety of kinesin motor proteins

The kinesin family of motor proteins consists of a large superfamily of microtubule binding proteins that use the energy from ATP hydrolysis to power the transport of cargo along microtubules and to organize large cytoskeletal arrays. Kinesin proteins are implicated in the processes that regulate microtubule dynamics during mitosis, intracellular transport, and ciliogenesis in all eukaryotic cells (Miki et al., 2005). The kinesin motor, or head, domain is highly conserved among members of this superfamily and contains both ATP and microtubule binding sites.

The kinesin superfamily has been separated into fourteen distinct protein families, kinesin-1 through kinesin-14, based on alignments of the motor domains (Lawrence et al., 2004). The fourteen consistently accepted kinesin families come in three main flavors, those with N-terminal motor domains, those with C-terminal motors, and those with motor domains located in the middle of the protein. Typically, the direction of microtubule based movement correlates with the location of the motor domain in the protein. Members of the kinesin-1 through kinesin-12 families have N-

terminal motor and, when processive, they generally move towards the plus end of microtubules. Traditional kinesin-14s have C-terminal motors and exhibit minus end directed motility along microtubules (Endow, 1999). Kinesin-13s are known for their centrally located motor domain. Kinesin-13s are not processive and instead these kinesins display microtubule-depolymerizing activity (Desai et al., 1999). Kinesin-8 is also able to depolymerize microtubules, even though it has an N-terminal motor (Varga et al., 2006; Mayer et al., 2007; Varga et al., 2009). Most kinesins have a neck region adjacent to the motor that contains family specific features, a coiled coil region necessary for dimerization, and a tail region that is important for binding to cargo and accessory proteins. Unlike the motor and neck region, the tail region is highly divergent and contains a wide range of, often species-specific (Wickstead et al., 2010b), domains necessary for protein-protein interactions. These regions direct the specific functions of individual kinesin motor proteins.

In plants, the kinesin family is much larger than that in animal cells. For example, *Arabidopsis thaliana*, a flowering plant that is frequently used a model system in plant cell biology, has 61 kinesins (Reddy and Day, 2001) and the moss *Physcomitrella patens* has 78 (Shen et al., 2012; Miki et al., 2014). In comparison, mammals have only 40-50 kinesins (Miki et al., 2001). Much of the expansion of the kinesin superfamily in plants is found in the kinesin-14 family, which in *Arabidopsis* contains 21 members separated into six subgroups, and includes kinesins with N-terminal, centrally located, and C-terminal motors. The current hypothesis is that the multitude of kinesin-14s in plants is required to compensate for the absence of cytoplasmic dynein-1 (Wickstead and Gull, 2007). Direct evidence supporting this

hypothesis is limited and retrograde motility has only been observed in *Physcomitrella* kinesin-14 VI under strict *in vitro* conditions (Jonsson et al., 2015).

Kinesins present in ciliated plants and the Marsilea male gametophyte

Comparative analyses have shown that the kinesin family in plants contains many of the same families as animals, including kinesins involved in ciliogenesis and motility. Additional plant specific and 'orphan' kinesins are also present. One of the major adaptions during the evolution of plants is the ability to live and reproduce on land. Conifers and angiosperms do not make any ciliated cells, while lower plants such as ferns, mosses, and related groups produce ciliated male gametes (Raven et al., 1999; Pryer et al., 2004; Brown et al., 2015) that swim to the female gametophyte for fertilization. So while ferns and mosses are adapted to life on land, they are still dependent upon water for fertilization. Kinesins involved in ciliogenesis are only found in plants that make ciliated cells such as the green alga *Chlamydomonas* (Richardson et al., 2006; Wickstead and Gull, 2006; Wickstead et al., 2010b), and embryophyte plants like the moss *Physcomitrella* (Shen et al., 2012), and the fern *Marsilea* (this chapter). Flowering plants like *Arabidopsis* do not have ciliated cells and kinesins involved in this process are absent (Reddy and Day, 2001). Although the literature on plant kinesins is growing, there currently exists a large gap in our understanding of the kinesin families in the embryophyte plants (which make ciliated gametes) and how the kinesin family has evolved with the adaption of plants to land. The full set of kinesins in ferns has never been analyzed and studies on the presence of the kinesins in ciliated plants have so far only been investigated in *Physcomitrella* (Shen et al., 2012).

Marsilea is a heterosporous semi-aquatic water fern with a sporophyte that resembles a four-leaf clover, typically found growing as an annual in vernal pools. As the sporophyte grows, it forms sporocarps (modified leaves) with sporangia that contain their meiotic products, megaspores and microspores, which are meiotic products. As the pools dry down during the summer, the spores mature and undergo a natural process of desiccation. The spores remain viable and quiescent for many years within the dry, hard sporocarps (Moran, 2004). After fracturing of the sporpcarp wall and upon rehydration, the megaspores develop into female gametophytes the microspores develop into male gametophytes, which produce egg cells and motile spermatozoids, respectively. I am interested in the processes that regulate male gametophyte development in *Marsilea*. In eleven hours, the male gametophyte transforms from a single undifferentiated cell to produce 32-corkscrew shaped motile spermatozoids, with each producing ~140 cilia (Sharp, 1914; Mizukami and Gall, 1966; Myles, 1975; Myles and Hepler, 1977). During the first four to five hours after rehydration, a series of asymmetric and symmetric cell division cycles occur to produce seven sterile cells and two primary spermatogenous cells. These divisions are responsible for establishing cell fate. The spermatogenous initials undergo four more division cycles to generate two clusters of 16 spermatids. After the divisions are complete, each spermatid differentiates into a spirally shaped, multiciliated spermatozoid. Ciliogenesis takes place on basal bodies that are placed at regular intervals along a coiled microtubule ribbon. Eventually each spermatozoid breaks free from the microspore wall and swims in a shallow helix to female gametophytes that have developed from megaspores to produce eggs. Development is post-

transcriptionally controlled. This means that transcription is not required for development and protein synthesis is instead regulated through transcript unmasking, splicing, and polyadenylation (Hart and Wolniak, 1998, 1999; Klink and Wolniak, 2001; Tsai and Wolniak, 2001; Tsai et al., 2004; Van der Weele et al., 2007; Deeb et al., 2010; Boothby and Wolniak, 2011; Boothby et al., 2013) (for review of gametophyte development see Chapter 1; Wolniak et al., 2011; 2015).

Next generation RNAseq and *de novo* transcriptome assembly were used to generate a reference transcriptome for the developing male gametophyte of *Marsilea vestita*. This transcriptome allows us to identify the presence and abundance of all transcripts throughout gametophyte development. For results presented here, I used the assembled reference transcriptome to analyze the diversity and abundance of kinesin transcripts that are present in the gametophyte during spermatogenesis. I found that the gametophyte produces 56 unique kinesin-like transcripts. This complement of kinesin mRNAs includes members of the kinesin-2, -4, -5, -7, -8, -9, -10, -12, and -14 families, as well as several 'orphan' and plant specific kinesins. Like *Physcomitrella* and *Chlamydomonas* (Wickstead and Gull, 2006, Shen et al., 2012), *Marsilea* male gametophytes have kinesins associated with ciliogenesis such as members of the kinesin-2, -9, and 'orphan'-III families present in the transcriptome. These kinesins are absent in *Arabidopsis* (Reddy and Day, 2001), which like all flowering plants do not make ciliated gametophytes. However, the kinesin family in *Marsilea* male gametophytes also resembles that of *Arabidopsis*, with an expanded group of kinesin-7s and a member of the kinesin-10 family, features which are absent in the kinesin family of *Physcomitrella* and *Chlamydomonas*. Overall, the kinesin

family identified from this transcriptome analysis of *Marsilea* male gametophytes appears to be an intermediate between that of *Physcomitrella* and *Arabidopsis*."

Results

Marsilea vestita has 56 kinesin-like transcripts

In order to identify kinesins sequences present in the *Marsilea* male gametophyte, I searched the reference transcriptome with the conserved kinesin motor domain (PF00225). This led to the identification of 127 sequences that contain a kinesin motor domain (Table 2-1). As expected, many of these sequences were identical except for small regions and may represent splicing intermediates or different isoforms of the same transcript. By combining these sequences and eliminating sequences that only contained partial motor domains, I found that the male gametophyte of *Marsilea* contains 56 unique kinesin transcripts (Table 2-2). It is possible that *Marsilea* has additional kinesins in the genome; but I am interested in the mechanisms that regulate rapid development in the male gametophyte so this was not analyzed. Of these 56 kinesin transcripts, 95% (53/56) contain a complete coding sequence (CDS). The high percentage of kinesins identified with a complete CDS gives us confidence in our overall transcriptome assembly.

Table 2-1. 127 kinesin sequences from the *Marsilea* transcriptome that show similarity to the conserved kinesin motor domain (PF00225), e < 0.00001.

Query	Target	Start	End	E-value	Notes
c38136_g1_i1	pfam00225	1537	2526	3.66E-143	Kinesin-12
c26263_g1_i2	pfam00225	1946	2920	3.35E-142	Kinesin-4
c28952_g1_i1	pfam00225	197	1198	2.56E-141	Kinesin-14
c26263_g1_i1	pfam00225	2138	3112	5.31E-141	Kinesin-4
c26263_g1_i3	pfam00225	2159	3133	7.11E-141	Kinesin-4
c27395_g1_i2	pfam00225	775	1737	1.07E-140	Kinesin-14
c18966_g1_i1	pfam00225	265	1266	1.16E-140	Kinesin-5
c18966_g1_i2	pfam00225	265	1266	1.27E-140	Kinesin-5
c18966_g1_i3	pfam00225	265	1266	1.81E-140	Kinesin-5
c78627_g1_i1	pfam00225	2379	3389	1.15E-138	Kinesin-5
c19072_g1_i1	pfam00225	1825	2826	8.35E-138	Kinesin-14
c20463_g1_i2	pfam00225	1901	2860	9.23E-136	Kinesin-14
c22572_g1_i1	pfam00225	1264	2238	5.27E-135	Kinesin-8
c11421_g1_i1	pfam00225	610	1650	9.67E-134	Kinesin-4
c68843_g1_i1	pfam00225	602	1624	1.22E-133	Kinesin-5
c20463_g1_i1	pfam00225	1901	2860	5.03E-132	Kinesin-14
c20463_g1_i3	pfam00225	1901	2860	1.82E-131	Kinesin-14
c78943_g1_i1	pfam00225	1545	2549	2.76E-131	Kinesin-14
c25940_g1_i1	pfam00225	2231	3235	2.80E-131	Kinesin-5
c39013_g1_i1	pfam00225	566	1561	2.89E-131	Kinesin-14
c21530_g1_i1	pfam00225	705	1709	2.41E-130	Kinesin-12
c31868_g1_i1	pfam00225	1804	2760	5.87E-130	Kinesin-14
c6848_g1_i1	pfam00225	608	1579	2.36E-129	Kinesin-7
c2216_g1_i1	pfam00225	1400	2359	3.05E-129	Kinesin-14
c23530_g1_i1	pfam00225	2833	3807	3.45E-129	Kinesin-7

c23530_g1_i3	pfam00225	2847	3821	3.83E-129	Kinesin-7
c23530_g1_i2	pfam00225	2859	3833	4.26E-129	Kinesin-7
c19024_g1_i2	pfam00225	3411	4400	6.57E-129	Kinesin-12
c2216_g1_i2	pfam00225	1400	2359	7.95E-129	Kinesin-14
c19024_g1_i1	pfam00225	3411	4400	1.77E-128	Kinesin-12
c19024_g1_i3	pfam00225	3411	4400	1.95E-128	Kinesin-12
c22678_g1_i1	pfam00225	3112	4059	2.23E-128	Kinesin-7
c31421_g1_i1	pfam00225	1508	2482	2.39E-128	Kinesin-8
c78969_g1_i1	pfam00225	816	1805	8.32E-128	Kinesin-12
c7540_g1_i2	pfam00225	1686	2645	9.36E-128	Kinesin-14
c7540_g1_i1	pfam00225	1808	2767	2.08E-127	Kinesin-14
c14624_g2_i1	pfam00225	2	844	8.96E-127	Partial CDS with motor domain
c27395_g1_i3	pfam00225	1754	2716	1.77E-126	Kinesin-14
c31183_g1_i4	pfam00225	622	1575	1.12E-125	ARK-LIKE
c22572_g1_i4	pfam00225	1389	2363	3.59E-125	Kinesin-8
c7137_g1_i2	pfam00225	1545	2534	3.97E-125	Kinesin-12, partial CDS
c7137_g1_i1	pfam00225	1545	2534	4.20E-125	Kinesin-12, partial CDS
c12285_g1_i2	pfam00225	1630	2586	4.58E-125	Kinesin-13
c12285_g1_i1	pfam00225	1861	2817	7.05E-125	Kinesin-13
c22572_g1_i3	pfam00225	1389	2363	1.02E-124	Kinesin-8
c22572_g1_i2	pfam00225	1389	2363	1.09E-124	Kinesin-8
c30896_g3_i2	pfam00225	663	1610	2.43E-124	Kinesin-14
c30896_g3_i3	pfam00225	663	1610	3.02E-124	Kinesin-14
c68831_g1_i1	pfam00225	908	1849	4.60E-124	Kinesin-7
c78621_g1_i1	pfam00225	2223	3179	1.02E-123	Kinesin-14
c16574_g1_i1	pfam00225	2836	3777	3.29E-123	Kinesin-7
c30896_g3_i1	pfam00225	663	1610	9.38E-123	Kinesin-14
c31064_g1_i1	pfam00225	1529	2494	2.56E-122	Kinesin-8
c18391_g1_i2	pfam00225	1097	2038	5.80E-122	Kinesin-7
c18391_g1_i3	pfam00225	1097	2038	1.03E-121	Kinesin-7

c18391_g1_i1	pfam00225	1097	2038	1.87E-121	Kinesin-7
c18951_g1_i2	pfam00225	2487	3551	2.75E-121	Kinesin-4
c18951_g1_i1	pfam00225	2500	3564	3.17E-121	Kinesin-4
c31183_g1_i9	pfam00225	1567	2604	1.14E-120	ARK-LIKE
c31183_g1_i5	pfam00225	1785	2822	2.14E-119	ARK-LIKE
c24958_g1_i2	pfam00225	2677	3738	5.99E-119	Kinesin-4, partial CDS
c24958_g1_i1	pfam00225	2746	3807	7.85E-119	Kinesin-4, partial CDS
c26633_g1_i1	pfam00225	1189	2211	8.57E-119	ARK
c26633_g1_i2	pfam00225	1189	2211	9.48E-119	ARK
c26419_g1_i1	pfam00225	2159	3148	1.51E-118	Kinesin-7
c11234_g1_i1	pfam00225	982	2001	4.96E-116	ARK
c31183_g1_i15	pfam00225	1567	2583	1.29E-115	ARK-LIKE
c18991_g1_i1	pfam00225	2217	3194	2.47E-115	Kinesin-7
c31183_g1_i10	pfam00225	1567	2505	1.67E-114	ARK-LIKE
c28098_g1_i1	pfam00225	1212	2153	3.49E-114	Kinesin-14
c28098_g1_i3	pfam00225	1212	2153	6.32E-114	Kinesin-14
c21618_g1_i1	pfam00225	2273	3235	6.46E-114	Kinesin-7
c28098_g1_i4	pfam00225	1212	2153	7.87E-114	Kinesin-14
c28098_g1_i2	pfam00225	1212	2153	1.66E-113	Kinesin-14
c28098_g1_i5	pfam00225	1212	2153	3.11E-113	Kinesin-14
c28048_g1_i1	pfam00225	2323	3282	4.26E-113	Kinesin-7
c28048_g1_i2	pfam00225	2335	3294	4.69E-113	Kinesin-7
c21627_g1_i2	pfam00225	1295	2251	4.37E-108	Kinesin-13
c21627_g1_i1	pfam00225	1419	2375	5.74E-108	Kinesin-13
c31427_g1_i6	pfam00225	2131	3087	8.37E-108	Kinesin-7
c28256_g1_i1	pfam00225	146	1147	3.88E-107	Kinesin-9
c31427_g1_i4	pfam00225	2131	3087	5.23E-107	Kinesin-7
c49008_g1_i1	pfam00225	616	1623	6.04E-107	Orphan-III
c31427_g1_i2	pfam00225	2212	3183	8.69E-107	Kinesin-7 II
c7029_g1_i1	pfam00225	1783	2799	1.34E-105	ARK

c31183_g1_i12	pfam00225	1567	2406	8.04E-104	ARK-LIKE
c31183_g1_i1	pfam00225	1567	2406	9.27E-104	ARK-LIKE
c31183_g1_i11	pfam00225	1567	2406	5.87E-103	ARK-LIKE
c31183_g1_i2	pfam00225	1716	2657	7.87E-103	ARK-LIKE
c14986_g1_i1	pfam00225	958	1755	1.38E-102	Kinesin-14 V, partial CDS
c31373_g1_i1	pfam00225	1018	1782	1.61E-101	Partial Motor Domain
c31833_g1_i1	pfam00225	524	1513	2.39E-93	Kinesin-10
c42178_g1_i1	pfam00225	443	1147	1.16E-92	Partial Motor Domain
c26386_g1_i1	pfam00225	2	685	2.96E-90	Partial Motor Domain
c24006_g1_i1	pfam00225	1871	2851	4.43E-90	Kinesin-12 I
c19861_g1_i2	pfam00225	2508	3212	8.72E-88	Partial Motor Domain
c19861_g1_i1	pfam00225	2544	3248	1.13E-87	Partial Motor Domain
c743_g1_i2	pfam00225	239	1207	1.04E-85	Kinesin-9
c743_g1_i1	pfam00225	338	1306	1.48E-85	Kinesin-9
c25940_g1_i2	pfam00225	2479	3243	6.83E-85	Kinesin-5
c21605_g1_i1	pfam00225	286	1275	2.00E-79	Kinesin-2
c27395_g1_i1	pfam00225	1754	2725	8.94E-79	Kinesin-14
c31724_g2_i1	pfam00225	2968	3897	1.05E-78	Kinesin-14
c31724_g2_i2	pfam00225	3020	3949	1.11E-78	Kinesin-14
c28127_g1_i3	pfam00225	428	1654	1.24E-73	Gap in motor domain
c28127_g1_i2	pfam00225	428	1654	4.58E-73	Gap in motor domain
c28127_g1_i1	pfam00225	428	1654	5.15E-73	Gap in motor domain
c31895_g1_i1	pfam00225	2163	3056	6.53E-71	Orphan-II
c82882_g1_i1	pfam00225	579	1109	2.83E-68	Partial Motor Domain
c31183_g1_i6	pfam00225	2019	2696	3.11E-67	ARK-LIKE
c31183_g1_i3	pfam00225	2033	2710	3.30E-67	ARK-LIKE
c12304_g1_i1	pfam00225	1082	2038	6.08E-62	Kinesin-13
c26765_g2_i1	pfam00225	1390	2373	1.27E-60	Orphan-IV
c12584_g1_i1	pfam00225	3	278	1.19E-48	Partial Motor Domain
c40463_g1_i1	pfam00225	6	239	4.95E-39	Partial Motor Domain

c31373_g2_i1	pfam00225	3741	3989	1.11E-35	Partial Motor Domain
c572_g1_i1	pfam00225	5	226	4.09E-33	Partial Motor Domain
c31183_g1_i14	pfam00225	1567	1818	8.43E-32	ARK-LIKE
c26386_g2_i1	pfam00225	4	276	3.64E-29	Partial Motor Domain
c11638_g1_i1	pfam00225	745	1020	4.44E-29	Partial Motor Domain
c14986_g3_i1	pfam00225	4	159	6.69E-27	Partial Motor Domain
c28098_g1_i6	pfam00225	1993	2250	4.00E-26	Kinesin-14
c56625_g1_i1	pfam00225	3	221	6.68E-24	Partial Motor Domain
c48167_g1_i1	pfam00225	4	204	2.24E-21	Partial Motor Domain
c45811_g1_i1	pfam00225	2	226	2.23E-19	Partial Motor Domain
c39901_g1_i1	pfam00225	2	241	2.23E-09	Partial Motor Domain
c46485_g1_i1	pfam00225	3	86	4.33E-08	Partial Motor Domain

Table 2-2. Fifty-six kinesin transcripts in the *Marsilea* male gametophyte, annotated

Accession	Kinesin	Len	ORF	KISc	e-value
KT986231	ARKa	4793	958-3948	1189-2211	1.12E-119
KT986232	ARKb	4491	718-3519	982-2001	5.07E-117
KT986233	ARKc	4106	1164-3626	1308-2324	3.37E-106
KT986234	ARK-LIKE	3164	172-2490	343-1380	1.85E-129
KT986235	Kinesin2	5448	103-2520	286-1275	2.67E-158
KT986236	Kinesin4-Ia	4304	1962-4304, partial	2127-3188	6.69E-119
KT986237	Kinesin4-Ib	3990	163-3624	427-1491	1.95E-133
KT986238	Kinesin4-Ic	5451	496-4851	610-1650	1.22E-134
KT986239	Kinesin4-II	3285	312-2807	366-1340	1.30E-143
KT986240	Kinesin5a	3753	181-3210	265-1266	3.70E-142
KT986241	Kinesin5b	3945	615-3719	711-1715	1.97E-132
KT986242	Kinesin5c	4420	491-3180	602-1624	1.12E-134
KT986243	Kinesin5d	3601	165-3063	213-1223	5.58E-140
KT986244	Kinesin7-Ia	4528	665-3829	1097-2038	9.55E-123
KT986245	Kinesin7-Ib	4702	632-4240	908-1849	8.44E-125
KT986246	Kinesin7-Ic	4701	583-4056	925-1866	8.87E-137
KT986247	Kinesin7-Id	5094	754-4428	1036-1983	5.47E-143
KT986248	Kinesin7-IIa	4049	774-3551	963-1919	1.08E-107
KT986249	Kinesin7-IIb	4017	610-3456	706-1683	1.55E-151
KT986250	Kinesin7-IIc	3522	224-3193	329-1306	2.01E-116
KT986251	Kinesin7-IId	3368	161-3010	221-1210	1.01E-119
KT986252	Kinesin7-IIe	3596	275-3166	362-1324	5.84E-115
KT986253	Kinesin7-III	4400	570-3797	594-1568	3.62E-130
KT986254	Kinesin7-IV	3676	584-3295	608-1579	1.46E-130
KT986255	Kinesin8-I	4422	1472-2319	1529-2494	3.65E-123
KT986256	Kinesin8-IIa	3176	71-2697	695-1669	1.14E-129
KT986257	Kinesin8-IIb	3470	694-2802	1264-2238	1.60E-136
KT986258	Kinesin9A	3688	209-2158	239-1207	7.71E-86

KT986259	Kinesin9B	3305	107-3133	146-1147	3.45E-108
KT986260	Kinesin10	3106	359-2608	524-1513	3.03E-94
KT986261	Kinesin12-Ia	5572	537-5246	1173-2162	1.12E-128
KT986262	Kinesin12-Ib	3650	664-2982	823-1761	1.40E-117
KT986263	Kinesin12-Ic	4148	825-4148, partial	1545-2534	2.89E-133
KT986264	Kinesin12-Id	5810	255-5465	816-1805	8.73E-128
KT986265	Kinesin12-Ie	6169	952-5787	1537-2526	3.14E-143
KT986266	Kinesin12-II	5802	264-4613	705-1709	2.05E-130
KT986267	Kinesin13a	3611	434-2872	1082-2038	1.60E-115
KT986268	Kinesin13b	4902	1210-3651	1843-2817	8.25E-166
KT986269	Kinesin13c	5991	819-3578	1419-2375	4.87E-108
KT986270	Kinesin14-Ia	3366	423-2843	1806-2801	1.50E-132
KT986271	Kinesin14-Ib	3130	225-2624	1545-2549	1.21E-132
KT986272	Kinesin14-Ic	3225	463-2886	1825-2826	2.24E-139
KT986273	Kinesin14-IIa	4103	500-3343	1901-2860	3.85E-133
KT986274	Kinesin14-IIb	4912	553-4185	2146-3105	4.92E-142
KT986275	Kinesin14-IIc	4836	558-4397	2223-3179	2.30E-124
KT986276	Kinesin14-IId	3968	80-3607	1754-2716	1.44E-127
KT986277	Kinesin14-IIIa	4693	733-4374	2335-3294	8.15E-143
KT986278	Kinesin14-IIIb	3838	218-3409	1079-2035	3.98E-131
KT986279	Kinesin14-IV	3830	1315-3453	1678-2619	3.37E-125
KT986280	Kinesin14-V	5298	895-4956	1402-2331	1.21E-87
KT986281	Kinesin14-VIa	4970	586-4458	3361-4308	1.29E-136
KT986282	Kinesin14-VIb	1755	40-1755, partial	958-1755	4.20E-104
KT986283	Orphan-I	2384	293-2131	731-1957	7.46E-74
KT986284	Orphan II	3761	466-3468	706-1599	6.05E-78
KT986285	Orphan III	3786	508-2490	616-1623	4.34E-108
KT986286	Orphan IV	3134	132-3133	762-1745	1.31E-60

I next constructed a phylogenetic tree to determine the complement of kinesins present in the gametophyte (Figure 2-1). To construct the tree, I used the conserved motor domain from all 56 kinesins found in *Marsilea* plus the entire kinesin families in *Arabidopsis thaliana* and *Physcomitrella patens*. I chose to use kinesins in *Arabidopsis* and *Physcomitrella* for comparison because in both these organisms the kinesin family has been has been previously characterized (Reddy and Day, 2001; Shen et al., 2012, Miki et al., 2014). A genome for the lycophyte *Selaginella* was recently completed (Banks et al., 2011), however, the kinesin family not been completely characterized and therefore *Selaginella* was not included in this analysis. Only the conserved kinesin motor domain from each protein was used for multiple sequence alignment (MSA) and subsequent tree building (Appendix I-1). To help identify well-known kinesin family groups, a representative member from each kinesin family in humans was also included in the phylogenetic analysis (Table 2-3).

The most widely accepted kinesin nomenclature (Lawrence et al., 2004), adapted and used for studies on plant kinesins (Shen et al., 2012) is also used here to describe the kinesin family in *Marsilea*. I found that the male gametophyte of *Marsilea* has members of the kinesin-2, -4, -5, -7, -8, -9, -10, -12, and -14 families, as well as several 'orphan' and plant specific kinesins, such as members of the armadillo repeat containing kinesin (ARK) family (Figure 2-1, Table 2-3). This complement of kinesins is similar to those found in other plants that produce ciliated spermatozoids, like *Physcomitrella* (Shen et al., 2012). The absence of traditional kinesin-3, -6, and -11 plus a large, diverse group of kinesin-14s is common feature among many plant species (Reddy and Day, 2001; Shen et al., 2012; Richardson et al., 2006).

To emphasize the importance of the kinesin family during gametogenesis, I also searched for dynein transcripts in the transcriptome. I found inner arm dynein transcripts and several sequence fragments with low similarity to IFT dynein; however, no transcripts were found that encode cytoplasmic dynein or outer arm axonemal dynein (see Appendix I for additional details and figures). Therefore it is likely that dynein plays a role in the motility of axonemens, but other motor proteins must compensate for the action of cytoplasmic dynein-1 during mitosis and intracellular transport. An alternative view is that other dyneins are present in the gametophyte as stored proteins, and completely absent as mRNAs explaining their absence in the transcriptome.

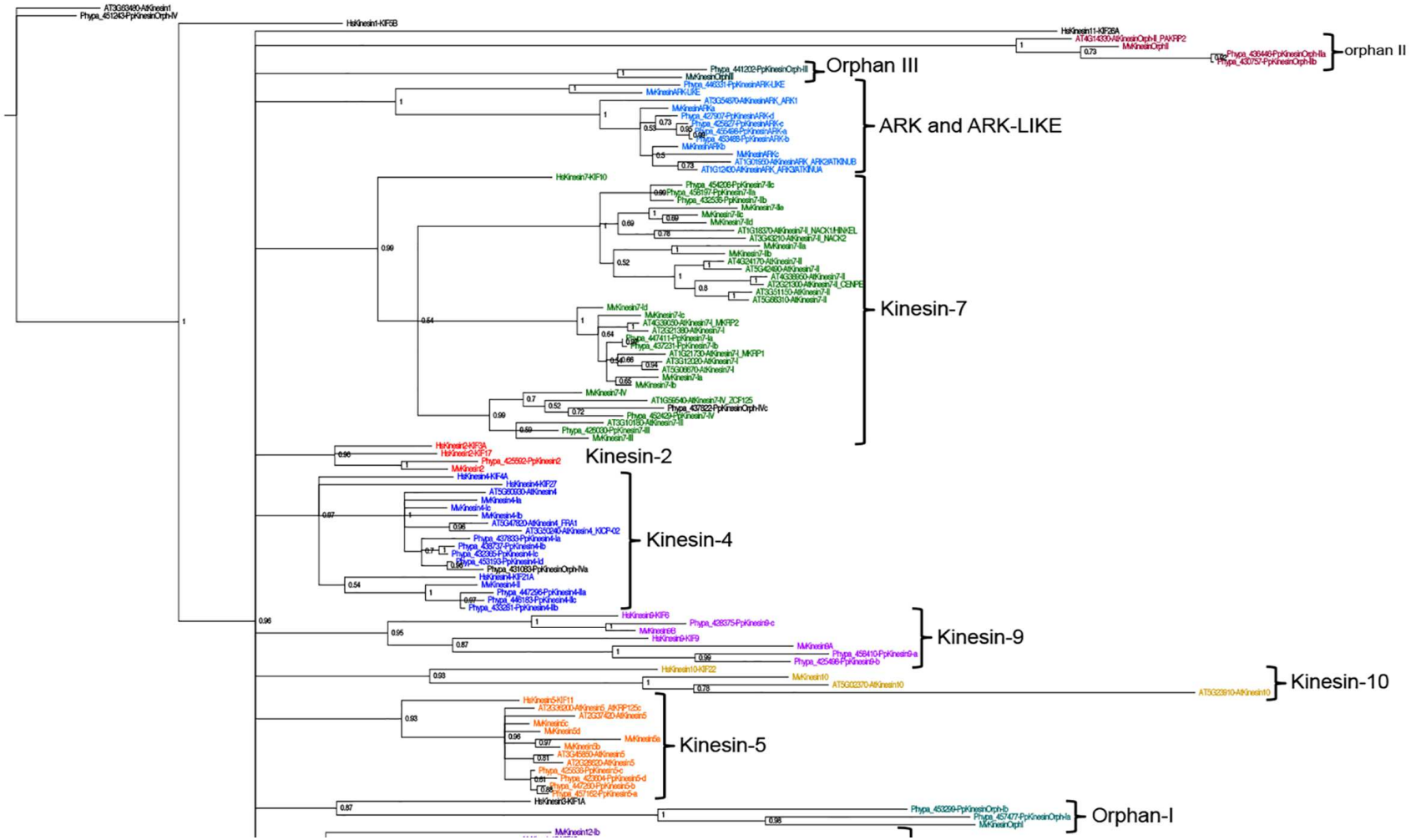


Figure 2-1. The kinesin family in *Marsilea.* Part 1 of 2. Maximum likelihood phylogenetic tree constructed with kinesin motor domains. Numbers represent percent bootstrap values for each node. Tomei and Wolniak, 2016.

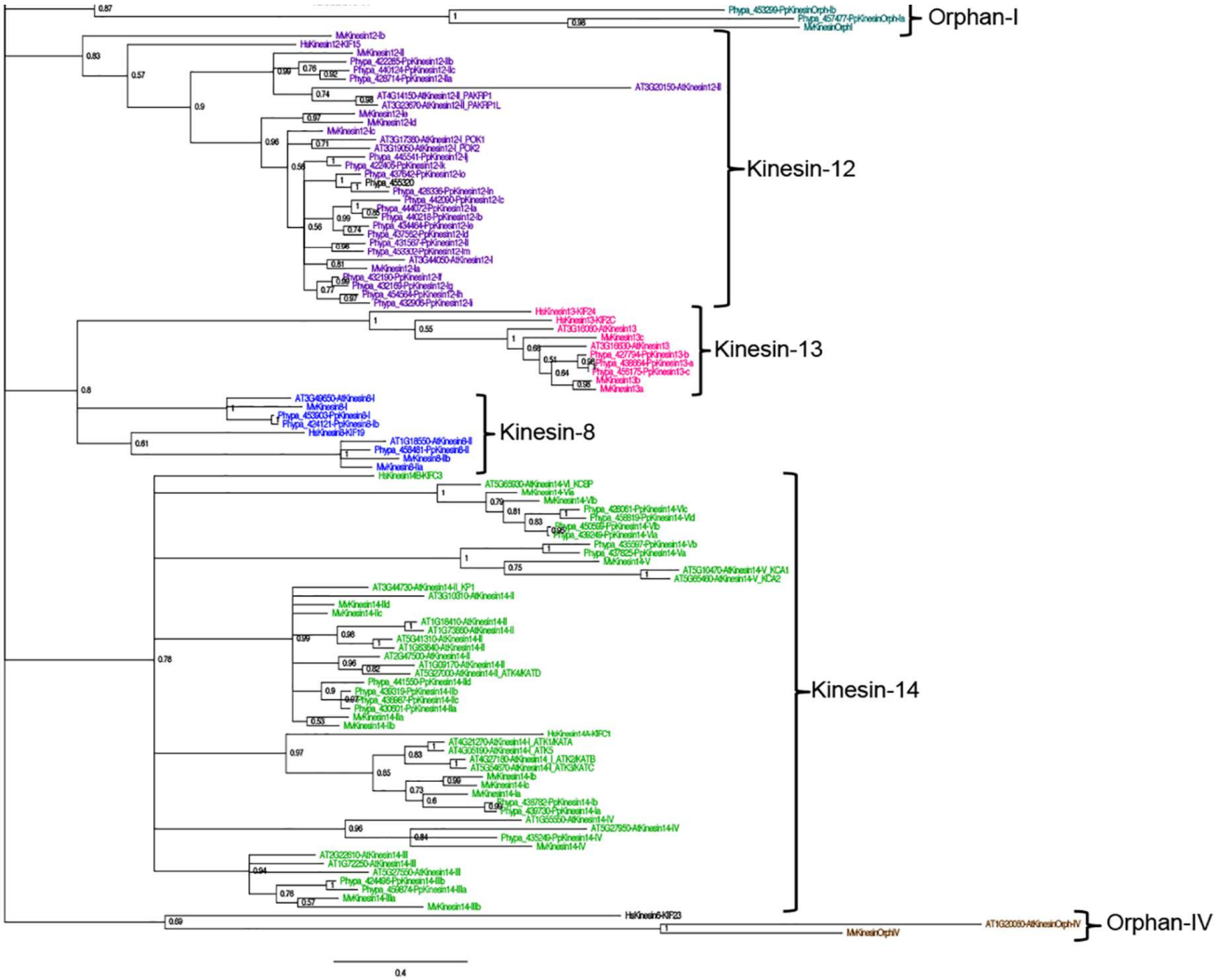


Figure 2-1. Part 2 of 2. Tomei and Wolniak, 2016.

Table 2-3. Kinesins from Humans, *Arabidopsis*, and *Physcomitrella* used to build a phylogenetic tree (Tomei and Wolniak, 2016).

Human Kinesins		**Arabidopsis Kinesins**		**Physcomitrella Kinesins**	
Accession	**Kinesin**	**Accession**	**Kinesin**	**Accession**	**Kinesin Name**
NP_004512	HsKinesin1—KIF5B	AT3G63480	AtKinesin1	Phypa_425592	PpKinesin2
NP_001287720	HsKinesin2—KIF3A	AT5G47820	AtKinesin4—FRA1	Phypa_437833	PpKinesin4-Ia
NP_001116291	HsKinesin2—KIF17	AT3G50240	AtKinesin4	Phypa_438737	PpKinesin4-Ib
NP_001230937	HsKinesin3—KIF1A	AT5G60930	AtKinesin4	Phypa_432365	PpKinesin4-Ic
NP_036442	HsKinesin4—KIF4A	AT2G36200	AtKinesin5—KRP125c	Phypa_453193	PpKinesin4-Id
NP_001166935	HsKinesin4—KIF21A	AT3G45850	AtKinesin5	Phypa_441211	PpKinesin4-Ie
NP_060046	HsKinesin4—KIF27	AT2G28620	AtKinesin5	Phypa_447296	PpKinesin4-IIa
NP_004514	HsKinesin5—KIF11	AT2G37420	AtKinesin5	Phypa_433281	PpKinesin4-IIb
NP_612565	HsKinesin6—KIF23	AT1G21730	AtKinesin7-I—MKRP1	Phypa_446183	PpKinesin4-IIc
NP_001804	HsKinesin7—KIF10	AT4G39050	AtKinesin7-I—MKRP2	Phypa_457162	PpKinesin5-a
NP_694941	HsKinesin8—KIF19	AT2G21380	AtKinesin7-I	Phypa_447260	PpKinesin5-b
NP_071737	HsKinesin9—KIF9	AT3G12020	AtKinesin7-I	Phypa_425536	PpKinesin5-c
NP_659464	HsKinesin9—KIF6	AT5G06670	AtKinesin7-I	Phypa_423604	PpKinesin5-d
NP_015556	HsKinesin10—KIF22	AT1G18370	AtKinesin7-II—NACK1	Phypa_447411	PpKinesin7-Ia
NP_056471	HsKinesin11—KIF26A	AT3G43210	AtKinesin7-II—NACK2	Phypa_437231	PpKinesin7-Ib
NP_064627	HsKinesin12—KIF15	AT4G38950	AtKinesin7-II	Phypa_458197	PpKinesin7-IIa

NP_006836	HsKinesin13—KIF2C	AT3G51150	AtKinesin7-II	Phypa_432536	PpKinesin7-IIb
NP_919289	HsKinesin13—KIF24	AT5G66310	AtKinesin7-II	Phypa_454208	PpKinesin7-IIc
NP_002254	HsKinesin14A—KIFC1	AT4G24170	AtKinesin7-II	Phypa_426030	PpKinesin7-III
NP_005541	HsKinesin14B—KIFC3	AT5G42490	AtKinesin7-II	Phypa_452429	PpKinesin7-IVa
		AT2G21300	AtKinesin7-II—CENPE	Phypa_453903	PpKinesin8-Ia
		AT3G10180	AtKinesin7-III	Phypa_424121	PpKinesin8-Ib
		AT1G59540	AtKinesin7-IV—ZCF125	Phypa_458481	PpKinesin8-II
		AT3G49650	AtKinesin8-I	Phypa_458410	PpKinesin9-a
		AT1G18550	AtKinesin8-II	Phypa_425498	PpKinesin9-b
		AT5G02370	AtKinesin10	Phypa_428375	PpKinesin9-c
		AT5G23910	AtKinesin10	Phypa_444072	PpKinesin12-Ia
		AT3G17360	AtKinesin12-I—POK1	Phypa_440218	PpKinesin12-Ib
		AT3G19050	AtKinesin12-I—POK2	Phypa_442090	PpKinesin12-Ic
		AT3G44050	AtKinesin12-I	Phypa_437562	PpKinesin12-Id
		AT4G14150	AtKinesin12-II—PAKRP1	Phypa_434464	PpKinesin12-Ie
		AT3G23670	AtKinesin12-II—PAKRP1L	Phypa_432190	PpKinesin12-If
		AT3G20150	AtKinesin12-II	Phypa_432169	PpKinesin12-Ig
		AT3G16060	AtKinesin13	Phypa_454564	PpKinesin12-Ih
		AT3G16630	AtKinesin13	Phypa_432906	PpKinesin12-Ii

	AT4G21270	AtKinesin14-I—ATK1	Phypa_445541	PpKinesin12-Ij
	AT4G27180	AtKinesin14_I—ATK	Phypa_422406	PpKinesin12-Ik
	AT5G54670	AtKinesin14-I—ATK3	Phypa_431567	PpKinesin12-Il
	AT4G05190	AtKinesin14-I—ATK5	Phypa_453302	PpKinesin12-Im
	AT5G41310	AtKinesin14-II	Phypa_426336	PpKinesin12-In
	AT1G63640	AtKinesin14-II	Phypa_437642	PpKinesin12-Io
	AT1G18410	AtKinesin14-II	Phypa_455320	PpKinesin12-Ip
	AT1G73860	AtKinesin14-II	Phypa_422514	PpKinesin12-IIa
	AT3G44730	AtKinesin14-II—KP1	Phypa_422285	PpKinesin12-IIb
	AT1G09170	AtKinesin14-II	Phypa_440124	PpKinesin12-IIc
	AT5G27000	AtKinesin14-II—ATK4	Phypa_428714	PpKinesin12-IId
	AT2G47500	AtKinesin14-II	Phypa_438664	PpKinesin13-a
	AT3G10310	AtKinesin14-II	Phypa_427794	PpKinesin13-b
	AT2G22610	AtKinesin14-III	Phypa_456175	PpKinesin13-c
	AT1G72250	AtKinesin14-III	Phypa_439730	PpKinesin14-Ia
	AT5G27550	AtKinesin14-III	Phypa_438782	PpKinesin14-Ib
	AT5G27950	AtKinesin14-IV	Phypa_43060	PpKinesin14-IIa
	AT1G55550	AtKinesin14-IV	Phypa_439319	PpKinesin14-IIb
	AT5G10470	AtKinesin14-V—KCA1	Phypa_43698	PpKinesin14-IIc

	AT5G65460	AtKinesin14-V—KCA2	Phypa_441550	PpKinesin14-IId
	AT5G65930	AtKinesin14-VI—KCBP	Phypa_459874	PpKinesin14-IIIa
	AT3G54870	AtARK—ARK1	Phypa_424496	PpKinesin14-IIIb
	AT1G01950	AtARK—ARK2	Phypa_435249	PpKinesin14-IV
	AT1G12430	AtARK—ARK3	Phypa_43782	PpKinesin14-Va
	AT4G14330	AtOrphan-II—PAKRP2	Phypa_435597	PpKinesin14-Vb
	AT1G20060	AtOrphan-IV	Phypa_439249	PpKinesin14-VIa
			Phypa_450599	PpKinesin14-VIb
			Phypa_428061	PpKinesin14-VIc
			Phypa_458819	PpKinesin14-VId
			Phypa_455498	PpARK-a
			Phypa_453488	PpARK-b
			Phypa_425827	PpARK-c
			Phypa_427907	PpARK-d
			Phypa_446331	PpARK-LIKE
			Phypa_431083	PpOrphan-IVa
			Phypa_451243	PpOrphan-IVb
			Phypa_437822	PpOrphan-IVc

Searching for kinesin-1 architecture in Marsilea

My initial analysis of the kinesin family in *Marsilea* did not reveal any transcripts that encode members of the kinesin-1 family. This is not entirely surprising since conclusive evidence for the presence of kinesin-1 in *Physcomitrella* is lacking (Wickstead and Gull, 2006; Shen et al., 2012) and the kinesin-1 identified in *Arabidopsis* and rice fails to function in a way that resembles its animal counterpart (Zhou et al., 2011; Wang et al., 2014). Gene models from *Physcomitrella* suggest that kinesin-1 and ARK-LIKE have similar structures (Shen et al., 2012). It is therefore possible that ARK-LIKE kinesin in *Physcomitrella* and *Marsilea* is a divergent version of the kinesin-1 present in *Arabidopsis*. To test this hypothesis, I constructed a new multiple sequence alignment (Appendix I-2) and a phylogenetic tree (Figure 2-2) that compares the motor domain of previously identified kinesin-1 proteins in *Arabidopsis* and *Chlamydomonas* plus members of the ARK-LIKE, 'orphan' I, and 'orphan' IV families in *Arabidopsis*, *Marsilea*, *Selaginella,* and *Physcomitrella*. I used these additional kinesins for comparison to determine if these orphaned kinesins are also share similarity to identified kinesin-1 sequences.

Using this analysis, I confirmed my previous observations that kinesin-1 and ARK-LIKE represent different kinesin families and that kinesin-1 does not exist in the transcriptome of the male gametophyte of *Marsilea* (Figure 2-2). However, in this analysis, I was able to align a kinesin in *Physcomitrella* (Phypa_451243) that was previously identified as a member of the kinesin-'orphan' IV family (Shen et al., 2012) with the kinesin-1 family, confirming earlier findings (Wickstead and Gull, 2006). In addition, a sequence in *Chlamydomonas* previously identified as a member

of the kinesin-1 family (Cre13.g588600.t1.1), more closely resembles members of the ARK-LIKE family, suggesting that kinesin-1 and ARK-LIKE do in fact share similar features. Two sequences previously identified as kinesin-‘orphan’ IV in *Physcomitrella* (Shen et al., 2012) cluster separately from kinesin-‘orphan’ IV sequences in *Marsilea* and *Arabidopsis* (Figure 2-2). This echoes findings in my original phylogenetic tree (Figure 2-1) where *Physcomitrella* kinesin-‘orphan’ IVa (Phypa_431083) groups with kinesin-4 I and kinesin-‘orphan’ IVc (Phypa_437822) groups with kinesin-7 IV, thereby restricting kinesin-‘orphan’ IV to *Marsilea* and *Arabidopsis*.

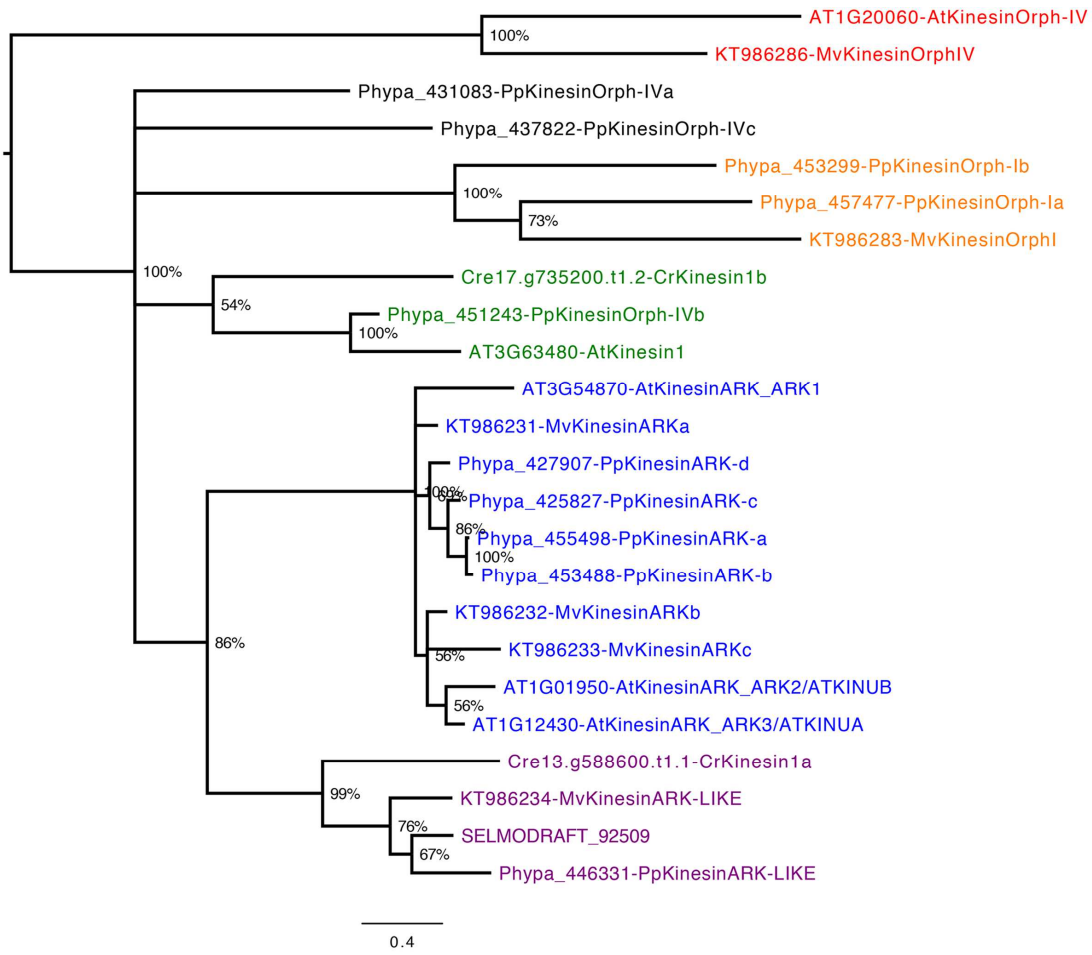


Figure 2-2. The male gametophyte of *Marsilea* does not make any transcripts that encode members of the kinesin-1 family. A phylogenetic tree of kinesin-1, ARK, ARK-LIKE, 'orphan'-I, and 'orphan'-IV sequences from *Arabidopsis*, *Marsilea, Selaginella, Physcomitrella*, and *Chlamydomonas* was constructed and used to determine if *Marsilea* contains kinesin-1. Kinesin-1 and ARK-LIKE represent different families and motors previously as members of the 'orphan'-IV family in *Physcomitrella* do not group with *Arabidopsis* and *Marsilea* 'orphan'-IV.

Comparative analysis of the kinesin family in Marsilea to other plants

The kinesin family in the male gametophyte of *Marsilea* resembles those in *Arabidopsis*, *Physcomitrella*, and *Chlamydomonas* but in different ways (Figure 2-3). *Arabidopsis* is a flowering plant that lacks centrioles and never makes ciliated cells; instead, its male gametophyte (pollen) extends a tube that allows amoeboid sperm cells to approach the egg. The moss *Physcomitrella* exists mainly as a gametophyte, and, makes large numbers of spermatozoids in each antheridium. However, unlike *Marsilea,* each spermatozoid in *Physcomitrella* has two ciliary axomemes (often incorrectly referred to as flagella), as opposed to the roughly 140 found on each *Marsilea* spermatozoid. It is important to note that centrioles are absent from vegetative cells, and ciliogenesis, which is restricted to the spermatids, requires the *de novo* the formation of basal bodies in a particle known as the blepharoplast (Mizukani and Gall, 1966; Hepler, 1976; Pryer et al., 2004; Wolniak et al., 2011, 2015). Unlike *Arabidopsis*, *Marsilea*, and *Physcomitrella*, *Chlamydomonas* is not a land plant. Instead, *Chlamydomonas* is a biflagellate green alga that is frequently used for studies on ciliogenesis and motility.

To compare the kinesin family in *Marsilea* to these organisms, I constructed a chart that separates these species by major events in evolution (Prigge and Bezanilla, 2010). These categories include land plants (*Arabidopsis*, *Marsilea*, and *Physcomitrella*), plants that have ciliated cells (*Marsilea, Physcomitrella*, and *Chlamydomonas*), plants that are only ciliated as male gametophytes (*Marsilea* and *Physcomitrella*), and vascular plants (*Arabidopsis* and *Marsilea*) (Figure 2-3A). Members of the kinesin-3, -6, and -11 families are missing in all of these organisms.

The absence of these kinesins is common among plants (Richardson, 2006). Kinesin families restricted to land plants include members of the kinesin-8 II, -14 IV, -14V, ARK, 'orphan' II, and 'orphan' IV families. Of these, ARK is the most well studied and is implicated regulating asymmetric division planes (Malcos and Cyr, 2011) and in nuclear positioning (Miki et al., 2015). Kinesin-2, kinesin-9, and 'orphan' III are only found in organisms that construct cilia (Wickstead and Gull, 2006; Wickstead et al., 2010b; Shen et al., 2012). My analysis confirms these results and adds kinesin-4 II and ARK-LIKE as additional kinesin that are restricted to ciliated organisms (Figure 2-3). Many of these kinesins and are not expressed in in *Physcomitrella* caulonemal cells (Miki et al., 2014) suggesting a role for them in the male gametophyte, possibly during ciliogenesis.

Kinesin-17 is also generally associated with ciliated organisms (Wickstead and Gull, 2006); however, transcripts that encode kinesin-17 are absent in *Marsilea* and *Physcomitrella.* These organisms are only ciliated as male gametophytes and contain members of the 'orphan' I family, which is absent in *Chlamydomonas* (Figure 2-3). From these results it is possible that the construction of ciliated male gametophytes requites a slightly different complement of kinesins than building cilia in algae.

Two kinesins, kinesin-10 and kinesin-'orphan' IV, are restricted to *Arabidopsis* and *Marsilea* (Figure 2-3). This suggests that kinesin-10 and kinesin-'orphan' IV have evolved in higher plants to perform functions that are perhaps specific to vascular plants. Members of the kinesin-4, -5, -7I, -7II, -7III, -7IV, -8I, -12I, -12II, -13, -14I, -14II, and -14IV are conserved in all the organisms studied and

likely represent the basic complement of kinesins found in green plants (Figure 2-3).

In addition to these general comparisons, the size of the kinesin family is also important. *Arabidopsis*, *Chlamydomonas*, and *Marsilea* all have a reduced group of kinesin-12s, compared to *Physcomitrella*. *Marsilea* and *Arabidopsis* each have six and *Chlamydomonas* has four members of the kinesin-12 family, whereas *Physcomitrella* has 20. Most notably, the kinesin-12 I subfamily appears to have the most expansion in *Physcomitrella* and consists of sixteen members. In *Arabidopsis*, *Chlamydomonas*, and *Marsilea* this subfamily contains only three, two, and five members, respectively (Figure 2-3B). Overall, the kinesin family in the male gametophyte of *Marsilea* appears to be intermediate between those of *Chlamydomonas, Physcomitrella* and *Arabidopsis*, which is consistent with the evolutionary relationship among these three plant species (Pryer et al., 2004).

Kinesins are not solely composed of a motor domain, but these proteins also have a range of additional domains that bind cargo or accessory proteins and regulate many of the interactions that are important for the function of specific family members. Therefore, in order to classify the kinesin family in *Marsilea* in more detail, I searched each kinesin transcript for additional conserved domains outside of the motor motif. I found kinesins with ARM repeats, SAM, CH, malectin, MyTH4, and FERM domains (Figure 2-4, Table 2-4). Many of these domains regulate protein-protein interactions. Kinesins with ARM repeats, SAM, CH, and malectin domains are present in land plants, where as kinesin-14 VI, which contains MyTH4 and FERM domains, is conserved in all plants as well as in green algae (Wickstead and Gull, 2006).

A

All Green Plants	Arabidopsis	Marsilea	Physcomitrella	Chlamydomonas
Plants with Cilia		Marsilea	Physcomitrella	Chlamydomonas
Land Plants with Ciliated Male Gametes		Marsilea	Physcomitrella	
All Land Plants	Arabidopsis	Marsilea	Physcomitrella	
Vascular Plants	Arabidopsis	Marsilea		

	All Green Plants	Plants with Cilia	Land Plants with Ciliated Male Gametes	All Land Plants	Vascular Plants
Kinesin-1					
Kinesin-2		■			
Kinesin-3					
Kinesin-4 I	■				
Kinesin-4 II		■			
Kinesin-5	■				
Kinesin-6					
Kinesin-7 I	■				
Kinesin-7 II	■				
Kinesin-7 III	■				
Kinesin-7 IV	■				
Kinesin-8 I	■				
Kinesin-8 II				■	
Kinesin-9		■			
Kinesin-10					■
Kinesin-11					
Kinesin-12 I	■				
Kinesin-12 II	■				
Kinesin-13	■				
Kinesin-14 I	■				
Kinesin-14 II	■				
Kinesin-14 III	■				
Kinesin-14 IV				■	
Kinesin-14 V				■	
Kinesin-14 VI	■				
ARK				■	
ARK-LIKE		■			
Orphan I			■		
Orphan II				■	
Orphan III		■			
Orphan IV					■
Kinesin-17					

B

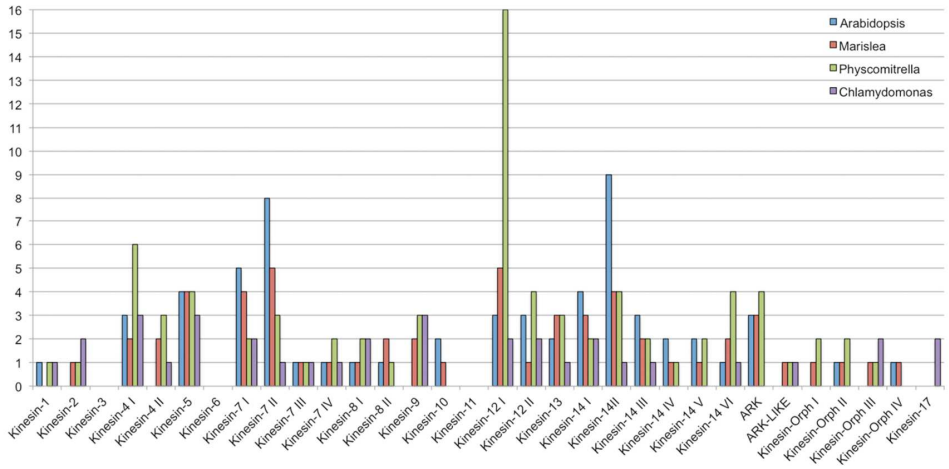


Figure 2-3. Comparing the kinesin superfamily in plants. (A) Chart that compares kinesins, separated by significant adaptations. Kinesin-3, -6, and -11 (red) are absent in all plants studied. Land plants share most kinesin families, however, plants that make ciliated cells contain a different complement of kinesins than non-ciliated plants. No conclusions can be made about kinesin-1 and kinesin-17 (blue) using this analysis. (B) Graph comparing the number of kinesins in each subfamily in *Marsilea* (red), *Arabidopsis* (blue), *Physcomitrella* (green), and *Chlamydomonas* (purple). Adapted from Tomei and Wolniak, 2016.

Figure 2-4. Illustrated transcript models for kinesins *Marsilea*. The motor domain and important accessory domains are shown (Fink and Hamilton, 2007) for each kinesin transcript identified in *Marsilea*. Members of the kinesin-2, -4, -5, -7, -8, -9, -12, -14IV, -14V, 'orphan' I, 'orphan' II, 'orphan' III, 'orphan' IV, ARK, and ARK-LIKE have N-terminal motors. Members of the kinesin-13, 14II, and -14III families have central motor domains and members of the kinesin-14I and -14VI families have C-terminal motors. Important accessory regions include ARM, a domain of unknown function (DUF3490), SAM, CH, malectin, MyTH4, and FERM domains. Kinesin transcripts with an incomplete CDS are noted with an asterisk (*). Tomei and Wolniak, 2016.

Table 2-4. Accessory domains present in *Marsilea* kinesins.

Kinesin	Domain	Location	e-value	Domain	Location	e-value
MvARKa	ARM	3145-3894	3.52E-15			
MvARKb	ARM	2755-3366	3.60E-15			
MvARKc	ARM	2844-3455	5.18E-12			
MvKinesin7-IIa	DUF3490	2997-3497	2.09E-59			
MvKinesin7-IIb	DUF3490	2926-3408	1.32E-58			
MvKinesin7-IIc	DUF3490	2624-3124	3.71E-64			
MvKinesin7-IId	DUF3490	2441-2941	6.62E-65			
MvKinesin7-IIe	DUF3490	2615-3106	1.83E-67			
MvKinesin13a	SAM	530-706	2.68E-15			
MvKinesin13b	SAM	1309-1485	2.36E-19			
MvKinesin14-IIa	CH	755-1015	6.23E-09			
MvKinesin14-IIb	CH	853-1140	7.84E-09			
MvKinesin14-IId	CH	185-496	1.54E-06			
MvKinesin14-IIIa	Malectin	1216-1647	6.01E-34			
MvKinesin14-VIa	FERM	2125-2451	9.62E-75	MyTH4	985-1461	1.28E-40

Discussion and Conclusions

Comparing the kinesin family in Marsilea to other plants

The kinesin family in the *Marsilea* male gametophyte is similar to those found in other plants. The absence of kinesin-3, -6, and -11, typically found in animal cells, and the addition of a diverse group kinesin-14s as well as several 'orphan' kinesins is a common feature across many plants (Reddy and Day, 2001; Richardson et al., 2006; Wickstead and Gull, 2006; Wickstead et al., 2010b; Shen et al., 2012). The lack of any transcripts encoding kinesin-1 in *Marsilea* is a bit surprising. Previous analyses

have not provided conclusive evidence for the presence of kinesin-1 in *Physcomitrella* (Wickstead and Gull, 2006; Shen et al., 2012); however, my analysis suggests that a kinesin previously identified as part of the kinesin-'orphan' IV family (Shen et al., 2012) is in fact similar to kinesin-1 in *Arabidopsis* (Figure 2-2) and that the kinesin-'orphan' IV family does not exist in *Physcomitrella* (Figure 2-1; 2-2). In *Arabidopsis* and rice kinesin-1 fails to function in a way that resembles its animal counterpart. Instead this kinesin is required for female gametogenesis (Zhou et al., 2011; Wang et al., 2014). It is therefore possible that the reason I was not able to identify any transcripts that encode members of the kinesin-1 family in *Marsilea* is due to the fact that this kinesin is not required for the formation of male gametophytes. Further analysis of the kinesin family in the *Marsilea* genome and sporophytes are required.

Comparative analyses consistently show that the kinesin family in a majority of land plants is much larger those families found in animals. For example, *Physcomitrella* has 78 kinesins (Shen et al., 2012; Miki et al., 2014) and *Arabidopsis* has 61 (Reddy and Day, 2001). This is compared to the 45-50 kinesins typically found in mammalian genomes (Miki et al., 2001). The transcriptome from the male gametophyte of *Marsilea* reveals the presence of at least 56 kinesins. It is important to note that the larger total number of kinesins in *Physcomitrella* is mostly attributed to the expansion of the kinesin-12 family (Figure 2-3B) (Shen et al., 2012; Zhu and Dixit, 2012). *Marsilea* may also have an expanded group of kinesin-12s in its genome and in its sporophyte, but this was not apparent in my transcriptome analyses, which was restricted to the male gametophyte. The absence of a large group of kinesin-12s

in the *Marsilea* gametophyte transcriptome suggests that an expanded kinesin-12 family is unnecessary for the formation of ciliated spermatozoids in embryophyte plants. However, because the dry spore contains large quantities of stored (pre) mRNAs and proteins (Hart and Wolniak, 1998, 1999), it is also possible that some kinesins are translated during spore desiccation and stored as proteins during quiescence. The transcripts that encode these stored proteins would not necessarily be represented in the transcriptome obtained from gametophytes after spore rehydration. Therefore additional kinesin proteins may be present in the gametophyte that went undetected in the transcriptome search.

It is unclear why the kinesin family is so large in plants. The current hypothesis suggests that that additional kinesins, specifically members of the kinesin-14 family, are needed in to compensate for the absence cytoplasmic dynein (Wickstead and Gull, 2007) and an organized centrosome in plant cells (Richardson et al., 2006; Shen et al., 2012). In support of this hypothesis, I was only able to identify transcripts that encode inner arm dynein in the *Marsilea* male gametophyte transcriptome (see Appendix I). Sequences encoding outer arm dynein, IFT dynein, and cytoplasmic dynein are absent. It has long been known that outer dynein arms in the axonemes from the spermatozoids of these organisms are extremely rare, or even nonexistent (Wolniak and Cande, 1980; Hyams and Campbell, 1985) even though appropriate binding sites exist on the axonemal microtubules for dynein attachments (Hyams, 1985).

Kinesin-14s are known for their c-terminal, minus end directed motors (Endow, 1999). *Marsilea* has 13 members of the kinesin-14 family (Figure 2-3B) that

contain not only this c-terminal motor domain (kinesin-14 I and -VI), but also n-terminal motors (kinesin-14 IV and -V), and motor domains that reside in the middle portion of the protein (kinesin-14 II and –III) (Figure 2-4). This pattern is reminiscent of the kinesin-14 family in both *Physcomitrella* (Shen et al., 2012) and *Arabidopsis* (Reddy and Day, 2001; Lee and Liu, 2004). In contrast, *Chlamydomonas* has a smaller overall kinesin-14 family and does not contain kinesin-14s with n-terminal motors (Wickstead and Gull, 2006). This suggests that the expansion of the kinesin-14 family to include n-terminal motors is a more recent evolutionary event and is possibly restricted to land plants. Recent evidence suggests that kinesin-14 II (Walter et al., 2015) and kinesin-14 VI (Jonsson et al., 2015) are able to transport cargo along microtubules in the minus direction and may in fact be functional replacements for cytoplasmic dynein in retrograde transport (Jonsson et al., 2015; Walter et al., 2015).

Comparisons between the kinesin family in *Marsilea*, *Arabidopsis*, *Physcomitrella*, and *Chlamydomonas* provide a substantial amount of information on the biology and cytoskeletal dynamics that regulate growth and development in each plant. Kinesin-2, -9, 'orphan' III, and kinesin-17 are restricted to organisms that produce ciliated cells at some point during their life cycles. Kinesin-2 is well established as the key motor protein necessary for eukaryotic anterograde IFT (Walther et al., 1994; Kozminski et al., 1995; Cole et al., 1998; Yokoyama et al., 2004) and although less fully understood, a role for kinesin-9 in motility has been documented (Demonchy et al., 2009). *Marsilea* and *Physcomitrella* each have one kinesin-2, two or more kinesin-9s, and one kinesin-'orphan' III. In contrast, *Chlamydomonas* has two members of the kinesin-2 and kinesin-'orphan' III families,

plus a single kinesin-17 (Figure 2-3B). Kinesin-17 in both *Marsilea* and *Physcomitrella* is absent. The presence of multiple kinesin-2 members is typical in cells that possess motile cilia since kinesin-2 heterotrimers are required for IFT. It is unclear why only a single kinesin-2 is present in *Marsilea* and *Physcomitrella;* however, a general reaction of cilia and IFT-associated proteins is observed in organisms with that are only ciliated at specific points during the life cycle (Marande and Kohl, 2011). My analysis also reveals that kinesin-4 II and ARK-LIKE are similarly restricted to ciliated plants and that the presence kinesin-'orphan' I is restricted even further to plants with ciliated male gametophytes (Figure 2-3). Although none of these kinesins are currently implicated in ciliogenesis, their restricted distribution and presence in the transcriptome from *Marsilea* male gametes suggests that they might be important for the processes that assemble motile ciliary axonemes in a variety of organisms. *Arabidopsis* never makes ciliated cells, using amoeboid sperm cells instead of freely swimming ciliated spermatozoids for reproduction, and it lacks members of all of these groups.

Kinesins that are present in all the plants analyzed here include members of the kinesin-4 I, -5, -8 I, -13, -14 I, -14 II, -14 III, -14 VI families, plus all subgroups of the kinesin-7 and -12 families (Figure 2-3). Many of these kinesins are implicated in mitosis in both plant and animal cells (see Chapter 1) suggesting the possibility of conserved functions for these kinesins in eukaryotes. In *Arabidopsis* and *Physcomitrella* kinesin-4 I, -7 II, and -12 II localize to and are important for organizing the phragmoplast microtubules during cell division (Takahashi et al., 2010; Oh et al., 2012; Sasabe et al., 2012; Miki et al., 2014; Zhu et al., 2015).

However, the evolution of the phragmoplast occurred after the separation of *Chlamydomonas* from the rest of the land plants (Prigge and Bezanilla, 2010). Instead, *Chlamydomonas* uses a different microtubule array, termed the phycoplast, for spatially regulating cytokinesis (Johnson and Porter, 1968; Pickett-Heaps, 1976). Some algae do use a phragmoplast-like structure for cytokinesis, including *Coleochaetales*, *Charales*, and *Zygnematales* (Sawitzky and Grolig, 1995; López-Bautista et al., 2003). If these conserved kinesins evolved roles in phragmoplast organization from the *Chlamydomonas* phycoplast or the phragmoplast of other algae remains to be studied. The one exception here is kinesin-7 I. Members of this subfamily are not implicated in mitosis (Vanstraelen et al., 2006; Miki et al., 2014). Instead the expression of kinesin-7 I is restricted to mitochondria (Itoh et al., 2001).

Kinesins only present here in land plants and that are absent in *Chlamydomonas* include members of the kinesin-8 II, -14 IV, -14 V, ARK, -'orphan' II, and -'orphan' IV families. Of these the best studied are kinesin-14 V, ARK, and kinesin-'orphan' II. These kinesins have diverse range of functions, including crosslinking actin and microtubule arrays to mediate chloroplast localization (Klotz and Nick, 2012; Shen et al., 2015), during nuclear localization (Miki et al., 2015) and the selection of asymmetric division planes (Malcos and Cyr, 2011), plus in the transport of vesicles along phragmoplast microtubules (Lee et al., 2007), respectively.

Marsilea and *Arabidopsis* both have members of the kinesin-10 and kinesin-'orphan' IV families. Kinesins in these families are absent in *Chlamydomonas* and *Physcomitrella* restricting the distribution of these kinesins to vascular plants (Wickstead et al., 2010b) (Figure 2-3). In animal cells kinesin-10 is binds to

chromosome arms and is necessary for their alignment during metaphase (Tokai et al., 1996; Tokai-Nishizumi et al., 2005). In *Arabidopsis* kinesin-10 is upregulated during mitosis (Vanstraelen et al., 2006); however, functional analyses of kinesin-10 or 'orphan' IV have not yet been performed.

Accessory domains that are present in *Marsilea* kinesin transcript maps can also be found in kinesins present in *Arabidopsis* and *Physcomitrella. Chlamydomonas* kinesins do not contain many of these accessory domains except the MyTH4 and FERM domains of kinesin-14 VI. This suggests that kinesins containing ARM, RING, SAM, CH, and malectin domains are more recent evolutionary adaptions to the kinesin family and are necessary for specific functions of kinesins in multicellular organisms and/or land plants.

Chapter 3: Patterns of Abundance Correlate with the Essential Functions of Kinesin Motors during Spermatogenesis

Note: The following has been partially adapted from Tomei and Wolniak, Cytoskeleton, 2016. DOI 10.1002/cm.21285.

Introduction

Plant kinesins participate in many of the same functions as animal kinesins including intracellular transport, microtubule organization, spindle assembly, chromosome motility, and ciliogenesis (see Chapter 1; Lee and Liu, 2004; Zhu and Dixit, 2011; Lee et al., 2015); however, the kinesin story in plants is more complex than in animals. Outside of the conserved motor domain and family specific neck region, the remainder of the kinesin protein is highly divergent, and outside of the motor and neck domains, plant and animal kinesins have few similarities. These regions contain important domains that direct interactions with cargo or accessory proteins and therefore are vitally important for directing the function of individual kinesin proteins. Many of these additional domains are not shared between plant and animal kinesins and are often species specific (Wickstead et al., 2010b). ARM repeats, actin binding CH domains, MyTH4, and FERM domains, all necessary for directing specific types of protein-protein interactions, are only found in plant kinesins and some are even further restricted to land plants (see Chapter 2). It is therefore difficult, if not impossible, to infer the specific function of plant kinesins based solely on animal counterparts. Only kinesin-5 is thought to have analogous

roles during the formation of bipolar spindles in plant and animal cells (Bannigan et al., 2007), although the degree of conservation both in the proteins and in the roles they play has been the subject of debate (Miki et al., 2014; Lee et al., 2015).

The large numbers of kinesin proteins present in plants make it difficult to study the kinesin family comprehensively and there is limited information on the function of individual kinesin motors. *Arabidopsis* has 61 kinesin motor proteins (Reddy and Day, 2001) and 23 of these kinesins are upregulated during mitosis (Vanstraelen et al., 2006). In a study the endogenous localization of every kinesin protein expressed in *Physcomitrella* caulonemal cells was tracked, 43 of the 78 kinesins present in the genome (Shen et al., 2012) were localized in or with mitotic structures and all but kinesin-5 showed distinct localization patterns from animal homologs (Miki et al., 2014). Complicating efforts to attribute functions to all of these mitotic kinesins is the fact that a significant amount of functional redundancy was observed and kinesins of the same family do not always appear to share the same function. For example, *Physcomitrella* has five members of the kinesin-4 I family and only two, kinesin-4 Ia and kinesin-4 Ic, localize to mitotic structures (Miki et al., 2014). Therefore, it is not possible to assign a function to kinesin-4 I or even to classify kinesin-4 I as a purely mitotic kinesin. Similarly, members of the kinesin-4 I, -7 II, -7 III, -8 I, -8 II, -13, -12 I, -12 II, and 'orphan' II all localize to the phragmoplast equator (Miki et al., 2014) making it difficult to untangle specific roles for each kinesin during phragmoplast organization and cytokinesis.

These comprehensive analyses of the kinesin family have paved the way for additional studies and discrete functions have been assigned to a few isolated kinesin

motors. Kinesin-12 I and kinesin-14 VI (KCBP) are recruited to the PPB and are important for establishing the positional information of the PPB after its disappearance (Lipka et al., 2014; Buschmann et al., 2015). Kinesin-5 and kinesin-14 I localize to spindle microtubules and are required for the assembly of organized bipolar spindles (Marcus et al., 2003; Ambrose and Cyr, 2007; Bannigan et al., 2007; Miki et al., 2014). Kinesin-7 III can be found at the kinetochore throughout mitosis (Miki et al., 2014). Kinesin-7 II and kinesin-'orphan' II contribute to cargo transport at the phragmoplast and with kinesin-12 II are essential for the generation of interdigitated antiparallel phragmoplast microtubules (Lee and Liu, 2000; Nishihama et al., 2002; Strompen et al., 2002; Soyano et al., 2003; Pan et al., 2004; Lee et al., 2007; Hiwatashi et al., 2008; Takahashi et al., 2010; Oh et al., 2012; Sasabe et al., 2012; Miki et al., 2014). Kinesin-14 II and ARK are implicated in nuclear positioning and may be important for positioning asymmetric division planes (Frey et al., 2010; Malcos and Cyr, 2011; Miki et al., 2015).

Although significant advances have been made, many of these kinesins have only been studied in one system and many kinesins do not have conserved roles in *Arabidopsis* and *Physcomitrella*. A major reason for this is because the majority of studies on kinesins have been done in *Physcomitrella* caulonemal cells. While these cells are excellent for imagining mitotic structures and rapidly divide every five to six hours, they lack a key organizational feature of plant cell division, the PPB. Instead, the cell division in *Physcomitrella* caulonemal cells is a highly self-organized process that involves a multitude of proteins to generate the force required for spindle and phragmoplast assembly; many of which may be absent in plant cells that use the PPB

for division plane selection (Lloyd and Chan, 2006; Bannigan et al., 2008; Müller et al., 2009; Goshima and Kimura, 2010). It is therefore difficult to extrapolate the function of mitotic kinesins from *Physcomitrella* to other plants.

In addition to mitotic kinesins, plant kinesin motor proteins are also involved in general microtubule organization and cell morphogenesis. Unfortunately, there are few examples that directly illustrate the role of non-mitotic kinesins outside of ciliogenesis in *Chlamydomonas* where kinesin-2, -9, and -13 are necessary for intraflagellar transport, motility, and axoneme length control, respectively (Yokoyama et al., 2004; Piao et al., 2009; Scholey, 2013). Kinesin-14 VI also localizes to basal bodies in *Chlamydomonas,* although its function in ciliary growth or regulation is unknown (Dymek et al., 2006). Most of our current knowledge on these kinesins in plants is from genomic analyses, which restrict kinesin-2, -9, and – 'orphan' III to ciliated organisms (Wickstead and Gull, 2006; Wickstead et al., 2010b; Shen et al., 2012; Tomei and Wolniak, 2016), and functional studies on cilia-associated kinesins have not been performed on land plants outside of *Marsilea* (see Chapter 3 and Chapter 4). In extending beyond ciliogenesis, members of the kinesin-7, kinesin-14, and ARK families also have defied roles in non-mitotic processes. In *Arabidopsis* kinesin-7 I and kinesin-14 II localize to mitochondria (Itoh et al., 2001; Ni et al., 2005) and ARK localizes to microtubule plus ends where it assists in sustaining the rapid microtubule polymerization required for unidirectional growth in root hairs (Jones et al., 2006; Yang et al., 2007; Sakai et al., 2008; Eng and Wasteneys, 2014). In *Physcomitrella* kinesin-14 V mediates the chloroplast light-avoidance response through stabilizing cortical actin filaments (Shen et al., 2015).

Mitosis and ciliogenesis represent key developmental processes during male gametophyte development in *Marsilea,* and underlie the establishment of cell fate through division plane selection and the morphological changes associated with spermatid differentiation. In this gametophyte, mitosis and ciliogenesis are temporally separated and the mechanisms that regulate the transition from mitosis to differentiation are tightly controlled (Boothby et al., 2013). All cell divisions occur and are completed in the first five hours after spore hydration. The first two hours of this time are marked by asymmetric divisions cycles that establish cell fate in the gametophyte and distinguish spermatogenous initials from sterile cells. The spermatogenous initials then undergo rounds of symmetric divisions to produce 32 spermatids. During the last 5.5-6 hours of development, each spermatid differentiates forming a coiled nucleus and ribbon of microtubules that is studded with basal bodies. These basal bodies become the sites of ciliogenesis enabling each spermatid to become a motile spermatozoid (Myles et al., 1977; 1982). By investigating kinesin motor proteins during male gametophyte development in *Marsilea* I am able to uncover information about the functions of specific kinesins in plants and to study the mechanisms that underlie important regulatory stages of development (namely mitosis and ciliogenesis and the transition between the two) using one class of proteins.

In this chapter, I used transcriptome datasets to analyze the abundance of kinesin transcripts throughout spermatogenesis and was able to correlate patterns of transcript abundance with kinesin function. EdgeR (Robinson et al., 2010) analyses of contig abundance in replicate samples from different stages of development for these

kinesin transcripts show changes in kinesin mRNA abundance during gametophyte development. Functional silencing analyses show kinesin mRNAs that are abundant early in development encode proteins with essential roles during the mitotic division cycles that give rise to seven sterile cells and 32 spermatids. In contrast, kinesin mRNAs that become abundant later in development primarily encode proteins with roles in spermatid differentiation, including ciliogenesis. Based on these results, it is possible that kinesin motor proteins maintain and control important rate-limiting steps that regulate male gametophyte development in *Marsilea*.

Results

Kinesin transcripts change in abundance during development

In order to make predictions about the function of individual kinesins in *Marsilea*, I tracked the abundance of each transcript during development. Here a transcript is defined as a particular mRNA sequence that is found in the transcriptome that encodes a kinesin-like protein. Sequences that were mostly identical, save for areas that appeared to be different splicing intermediates or isoforms, were combined to create each transcript (see Chapter 2). Transcript abundance was tracked by analyzing the transcriptome assembled (Grabherr et al., 2011) from polyA(+)RNA isolates obtained at 1-2 hours, 3-5 hours, and 6-8 hours of gametophyte development. During each of these stages, progress through different developmental landmarks occurs. At 1-2 hours of development, asymmetric mitotic divisions that separate spermatogenous initials from sterile cells occur to establish cell fate. During the next

two hours of development (3-5h post hydration), each spermatogenous initial undergoes four symmetric rounds of cell division to produce sixteen spermatogenous cells. The last stage of development consists of the differentiation of each spermatid into a corkscrew shaped, spermatozoid with about 140 motile cilia (for review, see Chapter 1; Wolniak et al., 2011; 2015). RNA was isolated during the interval of 6-8 hours after the spores were hydrated; earlier work from our lab demonstrated that many of the transcripts undergo degradation more than 8 hours into gametophyte development (Tsai et al., 2004).

FPKM values for were calculated for each transcript mapped to our combined reference transcriptome using Cufflinks (Trapnell et al., 2010). EdgeR (Robinson et al., 2010) analysis was then used to calculate changes in transcript abundance between each time interval of development (1-2h vs. 3-5h, 3-5h vs. 6-8h, and 1-2h vs. 6-8h) from RNA-seq counts in replicate samples. In this comparison, I initially generalized changes in abundance into three main categories, as a) transcripts that increased in abundance during development, b) those that decreased in abundance during development, or c) those that did not change significantly during development (FDR<0.05). I found that almost all, ~91% (51/56), of kinesin transcripts exhibit at least a two-fold change in abundance during development (-1.0 < logFC > 1.0). 39% (22/56) of kinesins increase while 52% (29/56) decrease in abundance as gametophyte development proceeds (Figure 3-1A).

Kinesin mRNAs that are enriched early in development and then decrease in abundance as spermatogenesis proceeds include members of the ARK, kinesin-4I, -5, -7I, -7II, -7III, -7IV, -8II, -10, -12I, -12II, -13, - 14I, -14II, -14V, ‘orphan’ II, and

'orphan' IV (Figure 3-1B). Many of these mRNAs encode proteins with roles during mitosis in plants (see Chapter 1). Kinesin mRNAs that are enriched at later time points do so by increasing in abundance during development. These include members of the ARK, ARK-LIKE, kinesin-2, -4I, -4II, -5, -7I, -7II, -8I, -9, -12I, -13, -14II, 14IV, and 'orphan' III (Figure 3-1B). Of these, kinesin-2, kinesin-9, and 'orphan' III are only found in organisms that are ciliated (Wickstead and Gull, 2006; Wickstead et al., 2010b) and kinesin-2 and -9 have clear roles in ciliogenesis (see Chapter 4, Walther et al., 1994, Kozminski et al., 1995, Cole et al., 1998, Yokoyama et al., 2004, Demonchy et al, 2009). mRNAs that decrease in abundance are not detected later in development because they are translated or destroyed. While mRNAs that increase in abundance become available for detection at later time points through unmasking and polyadenylation, presumably for in order for them to be translated.

Next, I generated a heatmap that compares changes in transcript abundance for each distinct stage of development (Figure 3-2). Specifically, changes in kinesin transcript abundance that occur during the mitotic stage of development (1-2h vs. 3-5h), during differentiation (the 3-5h vs. 6-8h), and over the entire course of development (1-2h vs. 6-8h) were analyzed. Changes in abundance are represented as $\log_2$ of the fold change (logFC) between two time points. Negative values for logFC represent decreases (shown in blue) in abundance, while positive values represent increases (shown in red). No change and non-significant changes in abundance are depicted in yellow. No kinesin transcripts show statistically significant decreases in abundance from the 1-2h to the 3-5h time intervals and only one, kinesin-13a, decreases significantly from the 3-5h to the 6-8h time intervals. In general, significant

decreases in abundance occur over the first 8 h of development (1-2h v 6-8h time interval) but are not specific to any one stage of spermatogenesis (Figure 3-2). Many kinesin transcripts show significant increases in abundance during development, either between the 1-2h and 3-5h intervals or between the 3-5h and 6-8h time intervals (Figure 3-2, Table 3-1). The increases suggest these kinesins may be important for events that occur between the 1-2h and 3-5h time intervals, division cycles and/or blepharoplast formation, or between 3-5h and 6-8h time intervals, when spermatid maturation and ciliogenesis is occurring. The time-directed abundance changes suggest that there is precise regulation of unmasking, processing and translation of these transcripts (Van der Weele et al., 2007; Deeb et al., 2010; Boothby et al., 2013; Wolniak et al., 2015).

I also found a small subset of kinesin transcripts that do not change significantly in abundance during the 8 h of development when transcripts are detectable in the gametophytes (Tsai et al., 2004). These include members of the kinesin-8II, -13, -14III, -14VI, and 'orphan' I families. Many of these kinesins have high RNAseq counts and FPKM values throughout development (Table 3-1).

Many kinesin families in *Marsilea* consist of more than one member and each of these members tends to have a different pattern of abundance during development (Figure 3-1, 3-2). For example there are three transcripts that encode members of the kinesin-13 family in *Marsilea*. Of these three transcripts, one decreases in abundance, one increases, and one does not change. It is therefore nearly impossible to make a generalized prediction about the function of kinesin-13 during development based on the observed patterns of abundance. Instead, it is likely that each transcript encodes a

different kinesin-13 protein with separate roles in mitosis and in differentiation. In support of this hypothesis, kinesin-13, a microtubule depolymerizer (Desai et al., 1999), participates in regulating microtubule length during both mitosis and ciliogenesis (Walczak et al., 1996; Maney et al., 1998; Desai et al., 1999; Kline-Smith et al., 2004; Rogers et al., 2004; Mayr et al., 2007; Varga et al., 2009; Wickstead et al., 2010a; Weaver et al., 2011; Vasudevan et al., 2014). Similar situations are observed with kinesin-ARK, -4I, -5, -7I, -7II, -8II, -12I, -14II, -14III, and -14VI. Exceptions are the kinesin-9 and kinesin-14 I families. All transcripts that encode members of the kinesin-9 family increase in abundance, while all members of the kinesin-14 family decrease in abundance.

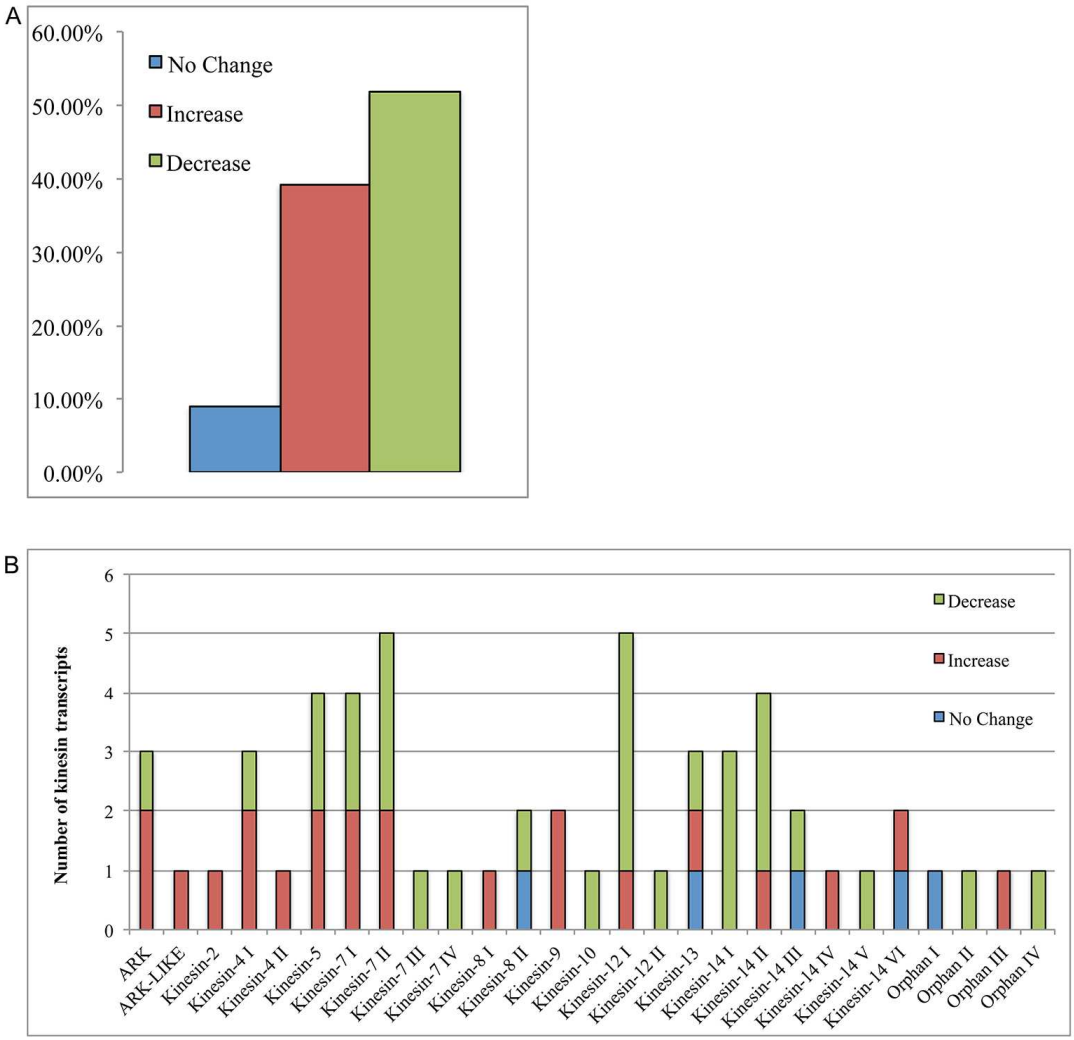


Figure 3-1. Kinesin transcripts change in abundance during spermatogenesis. (A-B) For these graphs a transcript is defined as the combined mRNA sequences that encode a particular kinesin-like protein. Sequences that were mostly identical, save for areas that appeared to be different splicing intermediates or isoforms, were combined to create each transcript (A) Percentage of kinesin transcripts that increase (red), decrease (green), and do not change (blue) in abundance. 39% (22/56) of kinesins increase and 52% (29/56) decrease in abundance. (B) Kinesin subfamilies grouped by changes in transcript abundance. Many families have members with different patterns of transcript abundance. Tomei and Wolniak, 2016.

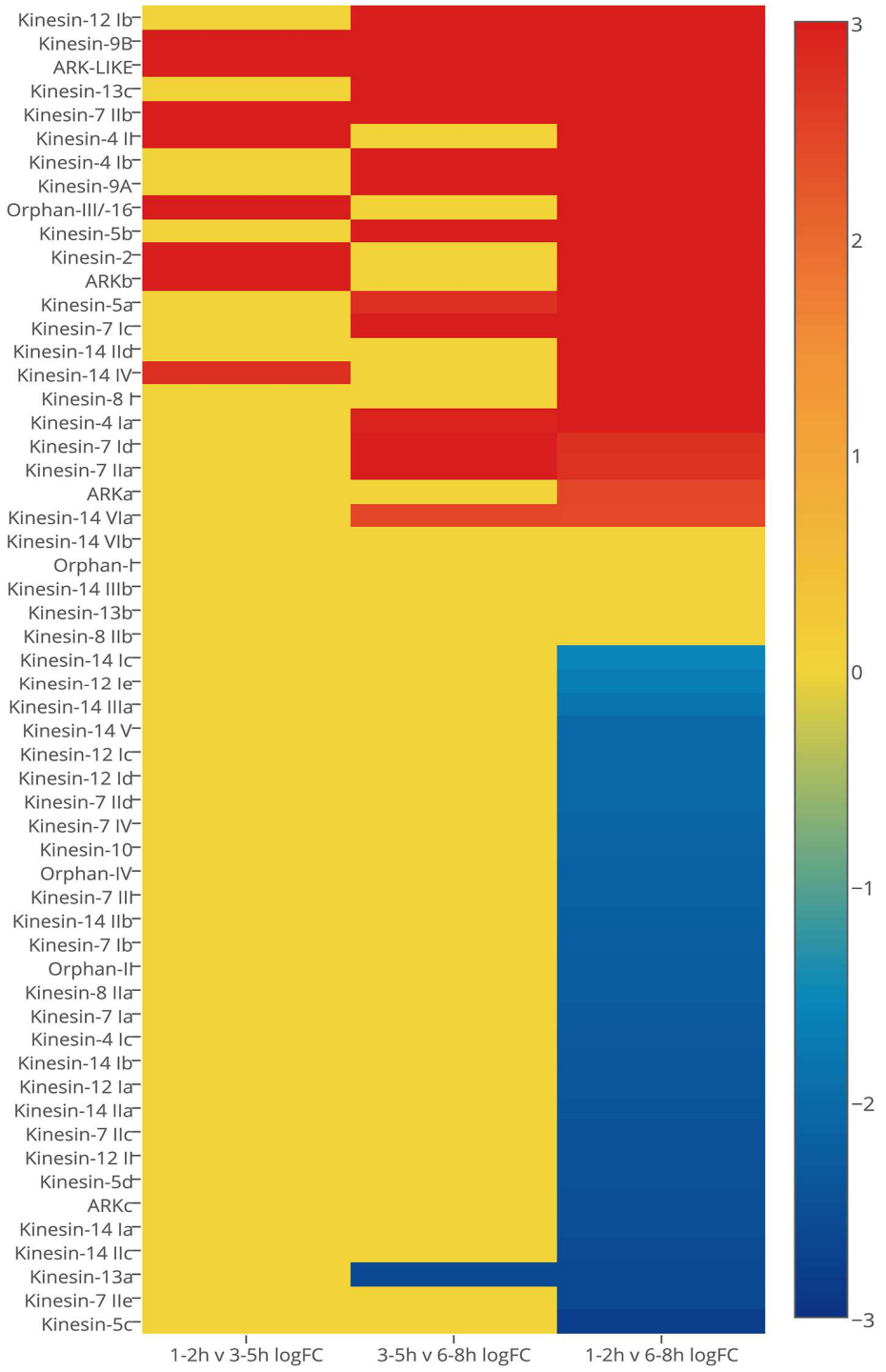


Figure 3-2. Heatmap of changes in transcript abundance. Increases in abundance are shown in increasing shades of red and decreases are shown in increasing shades of blue. Non-significant changes in abundance are yellow. Changes were calculated between the 1-2h and 3-5h time intervals, 3-5h and 6-8h time intervals, and over the entire course of development (between the 1-2h and 6-8h time intervals). Transcripts are grouped with the largest increases in abundance between the 1-2h and 6-8h time interval at the top. Tomei and Wolniak, 2016.

Table 3-1. LogFC and FPKM values for all 56 kinesin sequences present in *Marsilea*, separated by developmental trend.

Kinesin	**1-2 v 3-5h logFC**	**1-2 v 3-5h FDR**	**3-5 v 6-8h logFC**	**3-5 v 6-8h FDR**	**1-2 v 6-8h logFC**	**1-2 v 6-8h FDR**	**1-2h FPKM**	**3-5h FPKM**	**6-8h FPKM**	
Kinesin-9B	8.29	2.17E-10	4.22	2.46E-03	12.64	1.65E-73	0.01	0.70	45.12	Increases throughout development
ARK-LIKE	8.61	1.37E-07	3.43	1.38E-02	12.18	9.49E-61	0.00	0.74	21.02	
Kinesin-7 IIb	3.07	1.51E-02	5.02	7.43E-05	8.20	1.66E-32	0.27	1.25	110.01	
Kinesin-14 IId	0.14	1.00E+00	3.57	2.31E-01	3.88	3.49E-06	0.01	0.70	45.12	Increases from the 1-2 to the 6-8h time interval
Kinesin-8 I	1.50	8.96E-01	1.79	1.26E-01	3.41	4.73E-11	0.00	0.74	21.02	
ARKa	0.69	1.00E+00	1.66	1.42E-01	2.45	3.08E-12	0.27	1.25	110.01	
Kinesin-4 II	6.34	3.72E-12	0.61	7.34E-01	7.06	1.45E-36	0.05	2.23	9.07	Increases from the 1-2 to the 3-5h time interval
Orphan-III	4.80	3.94E-07	1.10	3.87E-01	5.99	3.91E-28	0.40	6.71	34.43	
Kinesin-2	3.73	3.75E-07	1.66	3.14E-01	5.48	2.01E-29	0.66	10.68	40.30	
ARKb	3.74	1.21E-04	1.19	3.50E-01	5.00	4.25E-24	0.73	5.84	31.66	
Kinesin-14 IV	2.77	4.40E-02	0.82	5.38E-01	3.70	1.12E-10	0.26	0.92	4.97	
Kinesin-12 Ib	0.10	1.00E+00	12.75	1.44E-05	13.03	1.59E-83	0.01	0.01	99.05	Increases from the 3-5 to the 6-8h time interval
Kinesin-13c	2.25	2.70E-01	6.80	2.28E-06	9.15	1.01E-36	0.16	0.41	126.84	
Kinesin-4 Ib	1.98	2.80E-01	4.83	8.37E-05	6.94	3.67E-54	0.72	1.53	116.21	
Kinesin-9A	0.36	1.00E+00	5.95	2.26E-05	6.43	9.71E-37	1.26	0.86	145.58	
Kinesin-5b	0.23	1.00E+00	5.56	8.67E-03	5.92	2.85E-23	0.11	0.07	8.76	

Kinesin-5a	1.96	7.57E-01	4.83	9.09E-10	4.83	9.09E-10	0.15	0.31	6.05	
Kinesin-7 Ic	1.68	5.40E-01	3.00	1.40E-02	4.81	8.43E-33	0.49	0.84	18.43	
Kinesin-4 Ia	-0.09	1.00E+00	2.95	4.71E-02	3.02	4.15E-11	0.27	0.14	2.97	
Kinesin-7 Id	-2.66	1.00E+00	5.28	1.81E-02	2.75	2.18E-02	0.13	0.01	1.17	
Kinesin-7 IIa	-0.87	6.64E-01	3.45	3.09E-02	2.73	1.19E-08	0.41	0.11	5.12	
Kinesin-14 VIa	-0.12	1.00E+00	2.46	1.88E-02	2.43	9.91E-11	22.04	18.96	148.76	
Kinesin-13b	-0.23	1.00E+00	-0.25	6.98E-01	-0.42	3.97E-01	61.51	58.23	62.12	
Orphan-I	0.39	1.00E+00	-1.07	1.00E+00	-0.57	3.57E-01	2.37	1.68	2.17	No change in abundance
Kinesin-14 VIb	-2.05	1.00E+00	1.31	1.00E+00	-0.59	6.71E-01	0.09	0.01	0.08	
Kinesin-8 IIb	-0.95	5.25E-01	-0.02	1.00E+00	-0.89	2.43E-02	34.54	11.29	24.97	
Kinesin-14 IIIb	-0.52	1.00E+00	-0.44	1.00E+00	-0.89	4.87E-02	38.05	18.29	27.31	
Kinesin-14 Ic	-1.05	4.07E-01	-0.62	1.00E+00	-1.55	1.86E-05	17.81	4.89	8.06	
Kinesin-12 Ie	-0.96	4.89E-01	-0.82	1.00E+00	-1.67	2.20E-05	8.83	2.58	3.65	
Kinesin-14 IIIa	-0.16	1.00E+00	-1.74	5.02E-01	-1.82	2.25E-05	11.75	6.39	4.44	
Kinesin-14 V	-1.09	3.72E-01	-1.06	1.00E+00	-2.03	1.59E-07	4.43	1.12	1.44	
Kinesin-12 Ic	-0.96	5.34E-01	-1.14	1.00E+00	-2.05	3.68E-06	14.98	4.98	4.80	Decreases from the 1-2 to the 6-8h time interval
Kinesin-12 Id	-0.85	5.74E-01	-1.28	1.00E+00	-2.05	5.24E-04	10.20	3.29	3.20	
Kinesin-7 IId	-0.40	9.75E-01	-1.81	5.32E-01	-2.05	9.89E-05	1.18	0.48	0.37	
Kinesin-7 IV	-1.22	3.00E-01	-1.01	1.00E+00	-2.14	2.12E-06	20.66	5.13	6.20	
Kinesin-10	-0.79	6.07E-01	-1.48	7.72E-01	-2.15	8.57E-08	13.61	4.42	4.04	
Orphan-IV	-0.93	5.01E-01	-1.37	9.62E-01	-2.17	9.79E-07	5.91	1.67	1.74	

Kinesin-7 III	-0.91	5.21E-01	-1.36	9.34E-01	-2.18	1.60E-06	17.25	5.43	5.01	
Kinesin-14 IIb	-1.43	3.29E-01	-0.84	1.00E+00	-2.21	3.30E-05	69.96	18.73	19.62	
Kinesin-7 Ib	-0.88	5.18E-01	-1.44	8.36E-01	-2.23	7.23E-07	13.55	4.26	3.80	
Orphan-II	-1.01	5.14E-01	-1.29	9.61E-01	-2.25	1.29E-05	43.21	14.11	11.83	
Kinesin-8 IIa	-0.88	5.81E-01	-1.45	7.56E-01	-2.26	1.15E-08	36.01	12.25	10.03	
Kinesin-7 Ia	-1.46	1.61E-01	-0.94	1.00E+00	-2.31	7.47E-07	14.47	2.95	3.84	
Kinesin-4 Ic	-1.76	9.06E-02	-0.65	1.00E+00	-2.35	3.08E-08	47.29	9.06	12.23	
Kinesin-14 Ib	-1.27	2.70E-01	-1.21	1.00E+00	-2.36	4.64E-09	14.86	3.44	3.82	
Kinesin-12 Ia	-1.24	3.45E-01	-1.20	1.00E+00	-2.36	1.04E-07	31.64	8.69	8.05	
Kinesin-14 IIa	-1.06	4.00E-01	-1.43	8.19E-01	-2.38	1.19E-09	13.50	3.68	3.44	
Kinesin-7 IIc	-1.39	2.19E-01	-1.15	1.00E+00	-2.46	6.73E-09	42.68	10.09	10.22	
Kinesin-12 II	-1.19	4.40E-01	-1.33	8.61E-01	-2.47	2.15E-07	38.95	11.75	9.31	
Kinesin-5d	-0.82	7.57E-01	-1.71	5.12E-01	-2.48	1.47E-07	52.42	20.71	12.46	
ARKc	-0.98	5.21E-01	-1.56	6.58E-01	-2.49	1.07E-07	33.08	10.73	7.85	
Kinesin-14 Ia	-0.97	5.23E-01	-1.60	6.02E-01	-2.50	3.21E-09	39.64	12.80	9.37	
Kinesin-14 IIc	-1.03	5.01E-01	-1.60	6.00E-01	-2.56	6.46E-10	30.55	9.69	6.91	
Kinesin-7 IIe	-1.20	3.07E-01	-1.48	7.92E-01	-2.60	1.50E-08	23.10	5.85	5.00	
Kinesin-5c	-1.63	2.14E-01	-1.21	9.44E-01	-2.79	3.64E-10	101.01	24.11	19.34	
Kinesin-13a	-1.31	3.55E-01	-2.33	8.60E-03	-2.58	6.58E-08	71.27	19.97	15.73	Decreases from 3-5 to 6-8h.

Functional analysis of kinesins with different patterns of transcript abundance

In order to determine the function of individual kinesins more clearly during male gametophyte development in *Marsilea*, I performed RNAi knockdowns of kinesins that displayed different patterns of transcript abundance during development as a means to correlate patterns of transcript abundance with kinesin functions during spermatogenesis. Here I analyze the function of transcripts that encode members of the kinesin-4, -12, -13, -14, ARK, ARK-LIKE, and orphan III families during male gametophyte development in *Marsilea*. I performed knockdowns by adding dsRNA (Figure 3-3A), constructed from unique regions in each kinesin to gametophyte populations at the time of spore hydration. Microspores were grown for 2, 5, and 8 hours, fixed, embedded in methacrylate, and sectioned. Sections were stained with toluidine blue (TBO), DAPI, anti-centrin, and anti-tubulin antibodies and examined with brightfield and fluorescence microscopy to observe any morphological changes or defects in development after knockdown. The effectiveness of RNAi was then measured using RT-PCR for each transcript after knockdown. After each knockdown, the presence of the transcript was undetectable while the abundance of centrin mRNA from these gametophytes did not change (Figure 3-3B). Each of these knockdowns produced a distinct phenocopy, indicating that different kinesins perform specific and unique functions during development. Kinesin-2 and kinesin-9 were also functionally analyzed; the results of these experiments are shown in Chapter 4. RT-PCR shows that centrin transcripts do not change in abundance from 1-2h, 3-5h, and 6-8h of development (Figure 3-3C).

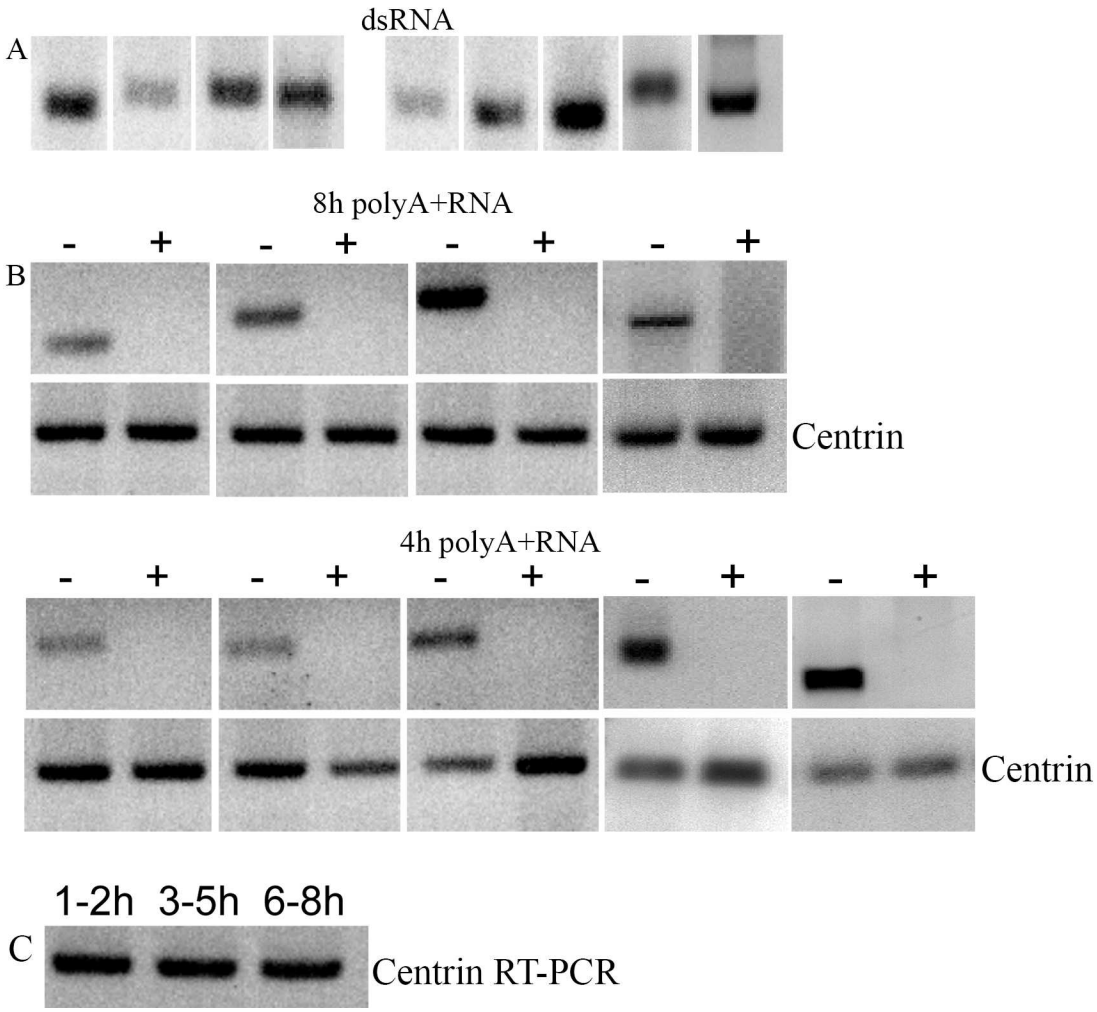


Figure 3-3. Kinesin dsRNA and RT-PCR after knockdown
(A) dsRNA constructed from ARK-LIKE, -'orphan' III, -14VIa, and -13c respectively, using polyA+RNA isolated at 8h as a template and dsRNA constructed from kinesin-4Ic, -13a, -13b, -12 II, and –ARKc respectively, using polyA+RNA isolated at 4h as a template. (B) RT-PCR for ARK-LIKE, -'orphan' III, -14VIa, -13c, -4Ic, -13a, -13b, -12 II, and ARKc respectively, before (-) and after (+) the addition of dsRNA to knockdown each kinesin. The presence of each transcript cannot be detected after knockdown. Centrin mRNA (bottom panel) does not change after the addition of dsRNA. (C) Centrin transcripts do not change in abundance during development.

Controls are shown at 2, 5, and 8 h of gametophyte development (Figure 3-4). At 2 hours, plastid (p) containing jacket cells (j) are formed through a series of asymmetric divisions near the edges of the microspore (Figure 3-4A). At the end of these division cycles, the sterile cells eventually surround two primary spermatogenous cells (sp). By 5 h, a series of symmetrical mitotic divisions produce 32 spermatogenous cells. The nucleus is clearly visualized in sections stained with TBO as a pink oval near the center of each spermatogenous cell (Figure 3-4B). Differentiation occurs after the divisions are complete, and by 8 hours of development the nucleus has elongated and formed a coil that is no longer visible with TBO staining. At this stage, each spermatid (sp) reshapes into a helix, due to the coiling of the nucleus and microtubule ribbon (Figure 3-4C).

To observe the process of spermatid differentiation, gametophytes were allowed to develop for 8 hours and were stained with DAPI (Figure 3-4 D, E), to observe nuclear elongation, with anti-tubulin antibodies (Figure 3-4 D), to observe the microtubule ribbon, and with anti-centrin antibodies (Figure 3-4 E), to observe the presence and distribution of basal bodies in the spermatids (Deeb et al., 2010). During normal spermatid differentiation, the basal bodies become situated at regular intervals along the microtubule ribbon and the nuclear coil. These prepositioned basal bodies later serve as templates for the growth of ciliary axonemes.

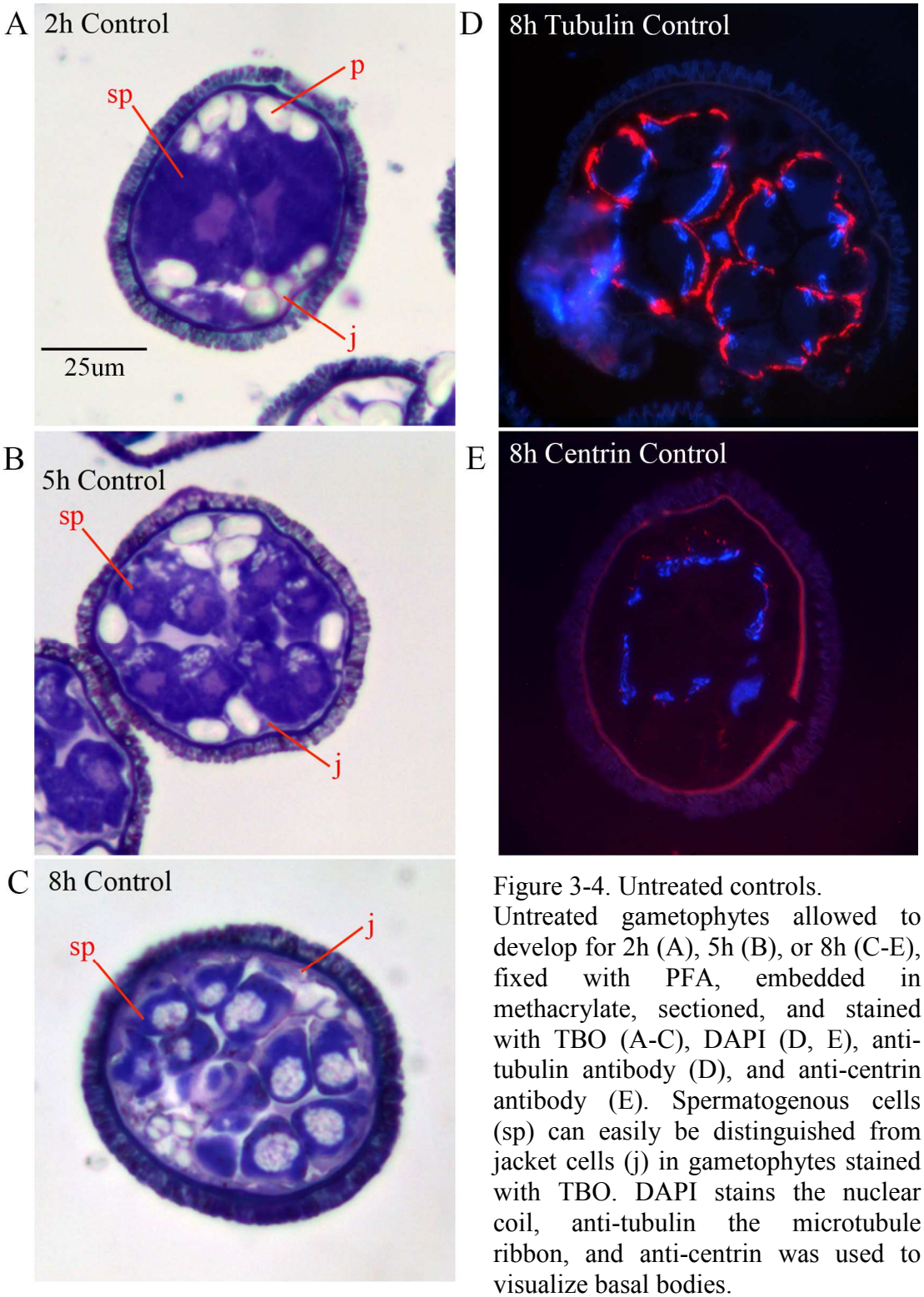


Figure 3-4. Untreated controls. Untreated gametophytes allowed to develop for 2h (A), 5h (B), or 8h (C-E), fixed with PFA, embedded in methacrylate, sectioned, and stained with TBO (A-C), DAPI (D, E), anti-tubulin antibody (D), and anti-centrin antibody (E). Spermatogenous cells (sp) can easily be distinguished from jacket cells (j) in gametophytes stained with TBO. DAPI stains the nuclear coil, anti-tubulin the microtubule ribbon, and anti-centrin was used to visualize basal bodies.

Kinesin-4 I

The *Marsilea* male gametophyte has three transcripts that encode members of the kinesin-4 I family (Figure 3-1). Of these transcripts, two increase in abundance during spermatogenesis while one decreases. In animals, kinesin-4 (Kif4) binds to chromosome arms (Wu and Chen, 2008) and is important for regulating microtubule dynamics at the spindle midzone (Bieling et al., 2010). I therefore decided to analyze kinesin-4 Ic, which is most abundant during the stage of development associated with mitosis and decreases in abundance from the 1-2h to the 6-8h time intervals (Figure 3-2). This pattern of transcript abundance was validated with RT-PCR (Figure 3-5A). At eight hours of development, kinesin-4 Ic knockdowns have abnormally large spermatogenous cells compared to controls, although spermatogenous cells can clearly be distinguished from jacket cells. The spermatogenous cells are unevenly distributed throughout the microspore indicating problems with the symmetric divisions that produce spermatogenous cells (Figure 3-5B). Knockdowns visualized at two hours post hydration appear morphologically normal compared to controls (Figure 3-5C); however, five hour gametophytes exhibit similar defects to eight hour gametophytes with large and unevenly distributed spermatogenous cells (Figure 3-5D). Visualized at 8h, anti-centrin staining is more diffuse than controls with only a few anti-centrin aggregates that resemble basal bodies in each cell (Figure 3-5E). It is also clear from this image that spermatids have failed to complete the last few rounds of mitosis, as cells are larger than controls. At 8h post hydration anti-α-tubulin staining appears similar to controls, although somewhat more disorganized than normal (Figure 3-5F).

Thus, kinesin-4 Ic appears to be necessary for proper spermatogenous cell divisions, but not for the earlier asymmetric divisions or during differentiation in the formation of the microtubule ribbon and blepharoplast. This implies that kinesin-4 Ic is required for events that occur after the establishment of polarity (1-2h time interval) and before spermatid differentiation (6-8h time interval). Based on these results it is likely that kinesin-4 Ic is important for regulating symmetric, spermatogenous cell divisions that occur three to five hours post hydration.

Kinesin-12 II

Like kinesin-4 Ic, kinesin-12 II is most abundant during the mitotic stage of development and decreases in abundance from 1-2 to the 6-8h time interval (Figure 3-2, 3-6A). The male gametophyte of *Marsilea* makes only one transcript that encodes a member of the kinesin-12 II family (Figure 3-1); however, there are five transcripts that encode kinesin-12 I. In *Arabidopsis* and *Physcomitrella*, kinesin-12 II, also known as PAKRP, localizes to the plus ends of phragmoplast microtubules during cytokinesis and are required for phragmoplast organization (Lee and Liu, 2000; Pan et al., 2004; Lee et al., 2007; Oh et al., 2012; Miki et al., 2014). After RNAi knockdowns of kinesin-12 II in *Marsilea*, gametophytes did not show any obvious defects in development and sections visualized eight hours post hydration look similar to controls (Figure 3-6B).

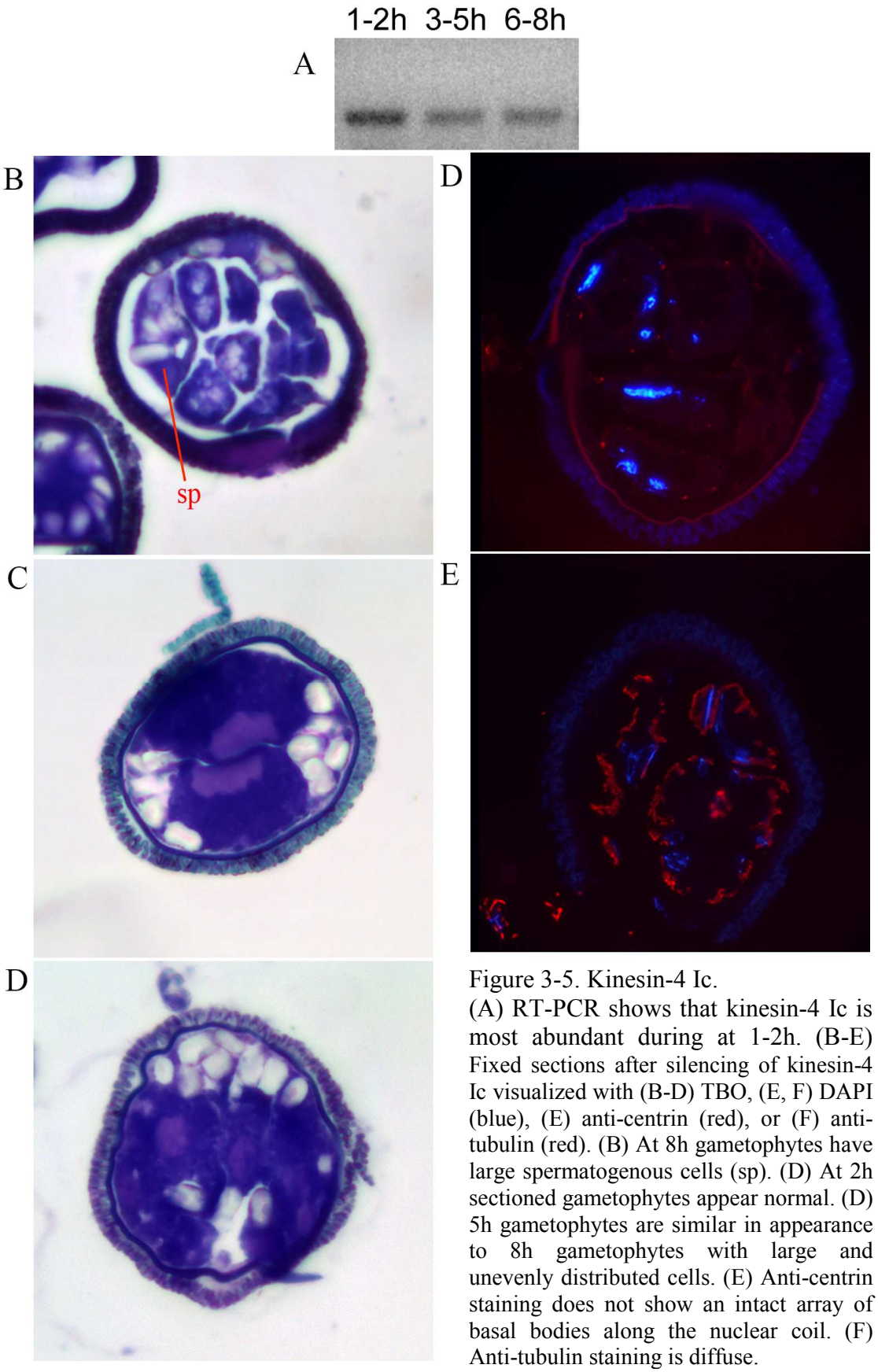


Figure 3-5. Kinesin-4 Ic.
(A) RT-PCR shows that kinesin-4 Ic is most abundant during at 1-2h. (B-E) Fixed sections after silencing of kinesin-4 Ic visualized with (B-D) TBO, (E, F) DAPI (blue), (E) anti-centrin (red), or (F) anti-tubulin (red). (B) At 8h gametophytes have large spermatogenous cells (sp). (D) At 2h sectioned gametophytes appear normal. (D) 5h gametophytes are similar in appearance to 8h gametophytes with large and unevenly distributed cells. (E) Anti-centrin staining does not show an intact array of basal bodies along the nuclear coil. (F) Anti-tubulin staining is diffuse.

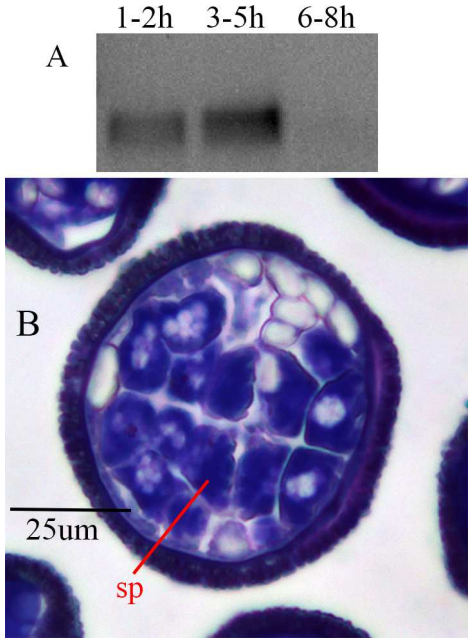


Figure 3-6. Kinesin-12 II.
(A) RT-PCR shows that kinesin-12 II is most abundant during the first five hours of development and then decreases during the 6-8h time interval. (B) Fixed sections after silencing of kinesin-12-II visualized with TBO 8 hours post hydration. Kinesin-12 II silencing does not have any produce any notable morphological defects in development.

Kinesin-13

In animals, kinesin-13 is a non-processive kinesin that contains a centrally located motor domain and a short, lysine rich, neck region responsible for depolymerizing microtubules (Ovechkina et. al., 2002; Ogawa et. al., 2004; Moores et. al., 2006). Kinesin-13 (Kif2) is required during mitosis to organize spindle microtubules (Manning et. al., 2007; Verhey and Hammond, 2009). A separate member of the kinesin-13 family, Kif24, lacks this neck domain and contains an N-terminal SAM domain. Kif24 does not appear to depolymerize mitotic microtubules and instead is involved in regulating the size of the centrosome and plays a role in ciliogenesis (Kobayashi et. al., 2011). In plants, kinesin-13 also exhibits microtubule-depolymerizing activity (Oda and Fukuda, 2013a; Deng et al., 2015) and is important during both mitosis and ciliogenesis. *Physcomitrella* kinesin-13 localizes to the spindle midzone during metaphase, anaphase, and cytokinesis (Miki et al., 2014) and in *Chlamydomonas* kinesin-13 regulates flagellar assembly and disassembly (Piao et al., 2009). However, in *Arabidopsis* kinesin-13 has not been shown to be important for mitosis and instead influences Golgi morphology and distribution (Lu et al., 2005; Wei et al., 2009).

Marsilea encodes three members of the kinesin-13 family, each with a different pattern of transcript abundance (Figure 3-1). Kinesin-13a is most abundant during the 1-2h time interval and is the only kinesin transcript to decrease significantly from 3-5 to 6-8 hours post hydration. FPKM values for kinesin-13b remain high throughout development (Table 3-1) and the levels of this transcript do not significantly change during gametogenesis. Kinesin-13c increases in abundance

and opposite pattern to kinesin-13a. Kinesin-13c increases specifically from the 1-2h to the 3-5h time interval of development (Figure 3-2). These opposing patterns of transcript abundance were investigated and validated with RT-PCR (Figure 3-7A). Since kinesin-13 is involved in mitosis and ciliogenesis, the fact that *Marsilea* has three kinesin-13 transcripts each with unique and opposing patterns of abundance makes this kinesin family an interesting target for functional analysis during gametogenesis.

Translated protein alignments reveal that kinesin-13 in *Marsilea* is more similar to Kif24 than Kif2. All *Marsilea* kinesin-13s lack the short neck region typically associated with microtubule depolymerization (Figure 3-7B). In addition like kif24, both kinesin-13a and kinesin-13b contain an N-terminal SAM domain that is important for mediating protein-protein interactions (see Chapter 2). These are features that are shared by other plant kinesin-13s (Oda and Fukuda, 2013; Deng et al., 2015). *Marsilea* kinesin-13c does not possess a SAM domain (see Chapter 2).

Kinesin-13a and kinesin-13b show major defects in mitosis and cell division after knockdown. At eight hours of development, knockdowns of kinesin-13a have disorganized division planes, large and misshaped nuclei, and plastids are localized to spermatogenous cells. This indicates a problem with the asymmetric divisions early in development that produce jacket cells (Figure 3-7C). Kinesin-13b has a similar phenocopy; however, the defects in development are more severe. There is only one large nucleus in the gametophyte, no division planes are visible, and plastids can be found throughout the cytoplasm. Development in these knockdowns has stopped prior

to any divisions (Figure 3-7D). However, the silencing of kinesin-13c did not produce any discernable defects in development when visualized at 8 hours (Figure 3-7E).

Visualization with anti-α-tubulin antibody, anti-centrin antibody, and DAPI at eight hours of development showed obvious defects in spermatid differentiation after silencing of kinesin-13a or kinesin-13b. After knockdown of kinesin-13a (Figure 3-7F) or kinesin-13b (Figure 3-7G) the microtubule ribbon and the nuclear coil are severely disorganized compared to controls. Anti-centrin antibody staining showed larger than normal centrin spots and the expression of centrin throughout the gametophyte (Figure 3-7I, J). These large centrin spots could be enlarged blepharoplasts that failed to dissociate completely during the process of basal body dispersion. Spermatid differentiation is normal after silencing of kinesin-13c (Figure 3-7H, K), confirming results obtained with TBO.

To determine the exact timing of these severe defects in development, kinesin-13 knockdowns were also fixed and sectioned at two (Figure 3-7L-N) and five (Figure 3-7O-Q) hours after spore hydration and visualized with TBO. At both two and five hours of development, kinesin-13a (Figure 3-7L, O) and kinesin-13b (Figure 3-7M, P) exhibit major morphological defects and look similar to knockdowns visualized at eight hours. This indicates that these kinesins are important during the asymmetric divisions that establish cell fate during the first two hours of development. Similar problems in development were observed in both kinesin-13a and kinesin-13b knockdowns (Figure 3-7D, M, P). In contrast, kinesin-13c knockdowns proceeded through development normally and do not show any morphological changes after silencing (Figure 3-7E, N, Q).

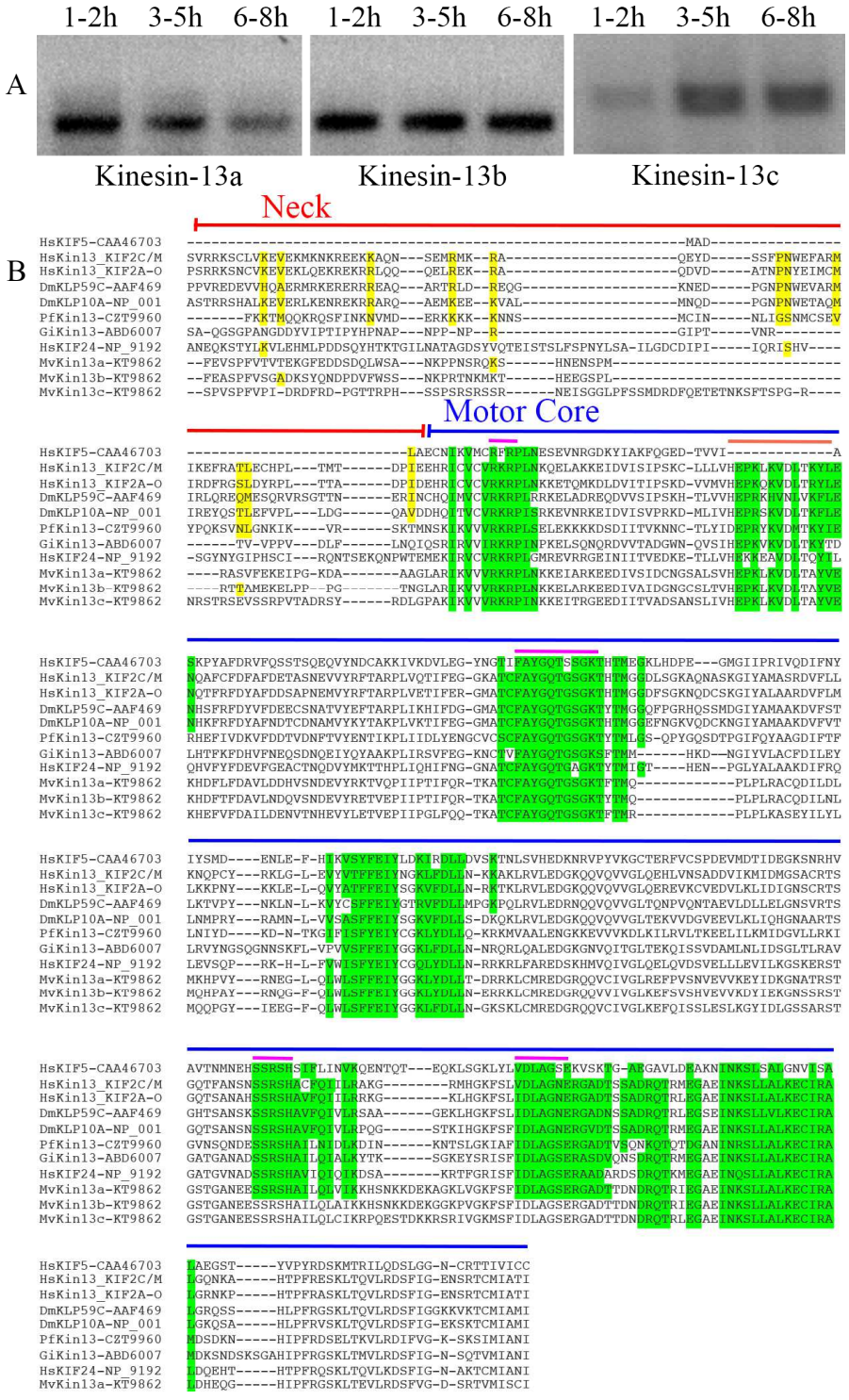


Figure 3-7. Kinesin-13. Part 1 of 3.
(A) RT-PCR of kinesin-13a, -13b, and -13c. Kinesin-13a is most abundant during the first 5h, -13b does not change, and -13c is most abundance during the 3-5h and 6-8h time intervals. (B) Alignments of the kinesin-13 neck and motor domain with conserved regions in yellow and green, respectively. Pink lines represent nucleotide binding regions and the orange line highlights a kinesin-13 specific motor insert. *Marsilea* kinesin-13s do not possess a conserved neck showing that they are more similar to kif24 than kif2.

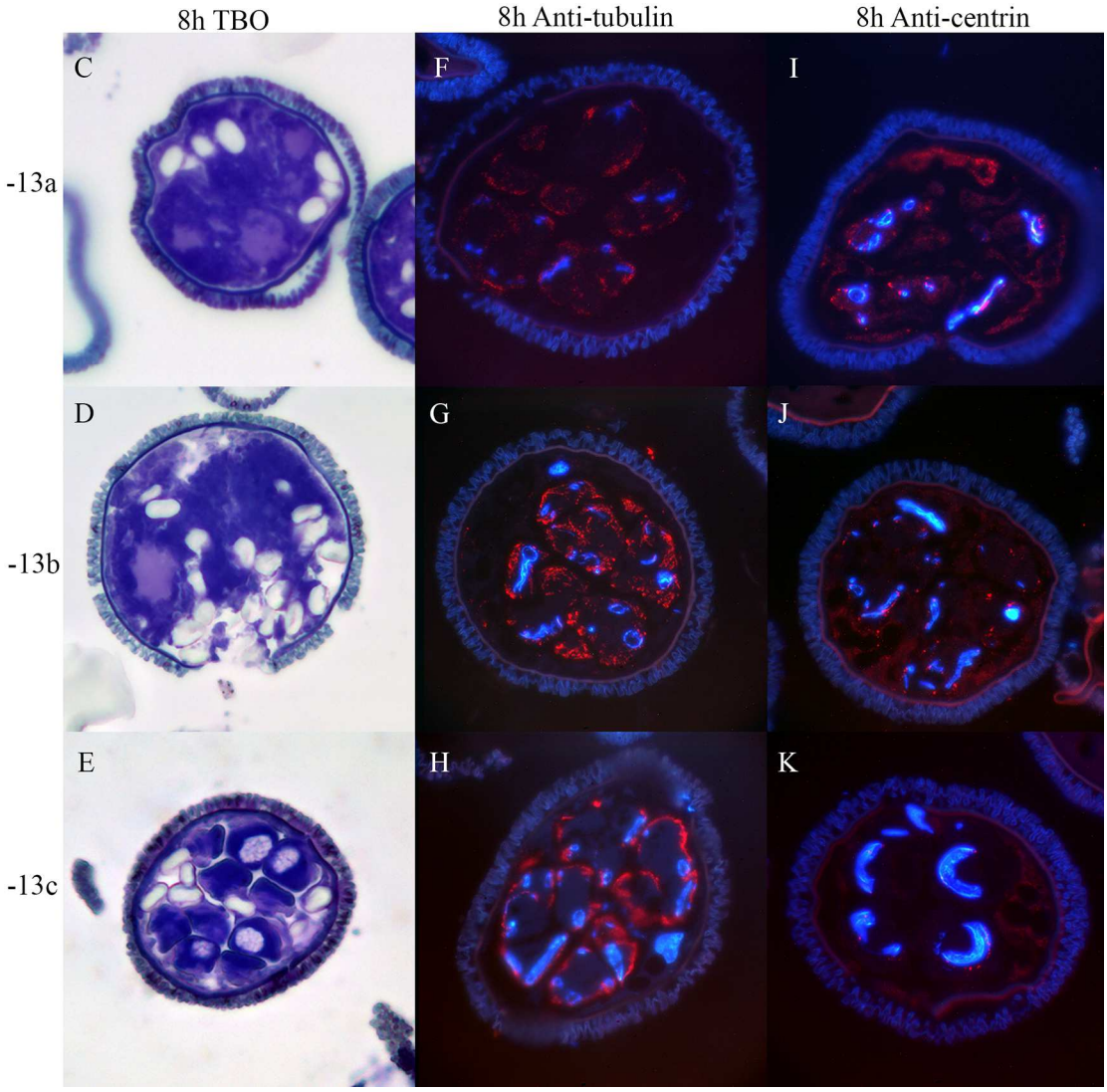


Figure 3-7. Kinesin-13. Part 2 of 3.
Knockdowns of (C, F, I) kinesin-13a, (D, G, J) -13b and (E, H, K) -13c visualized at 8h post hydration with (C-E) TBO, (F-K) DAPI (blue), (F-H) anti-tubulin antibody (red), and (I-K) anti-centrin antibody (red). Silencing of kinesin-13a and -13b result in severe defects in development with a failure to distinguish spermatogenous cells from jacket cells and an absence of differentiation with the incomplete formation of the nuclear coil, microtubule ribbon, and basal bodies. Silencing of kineins-13 does not appear to have an effect in gametophytes visualized at 8 hours of development.

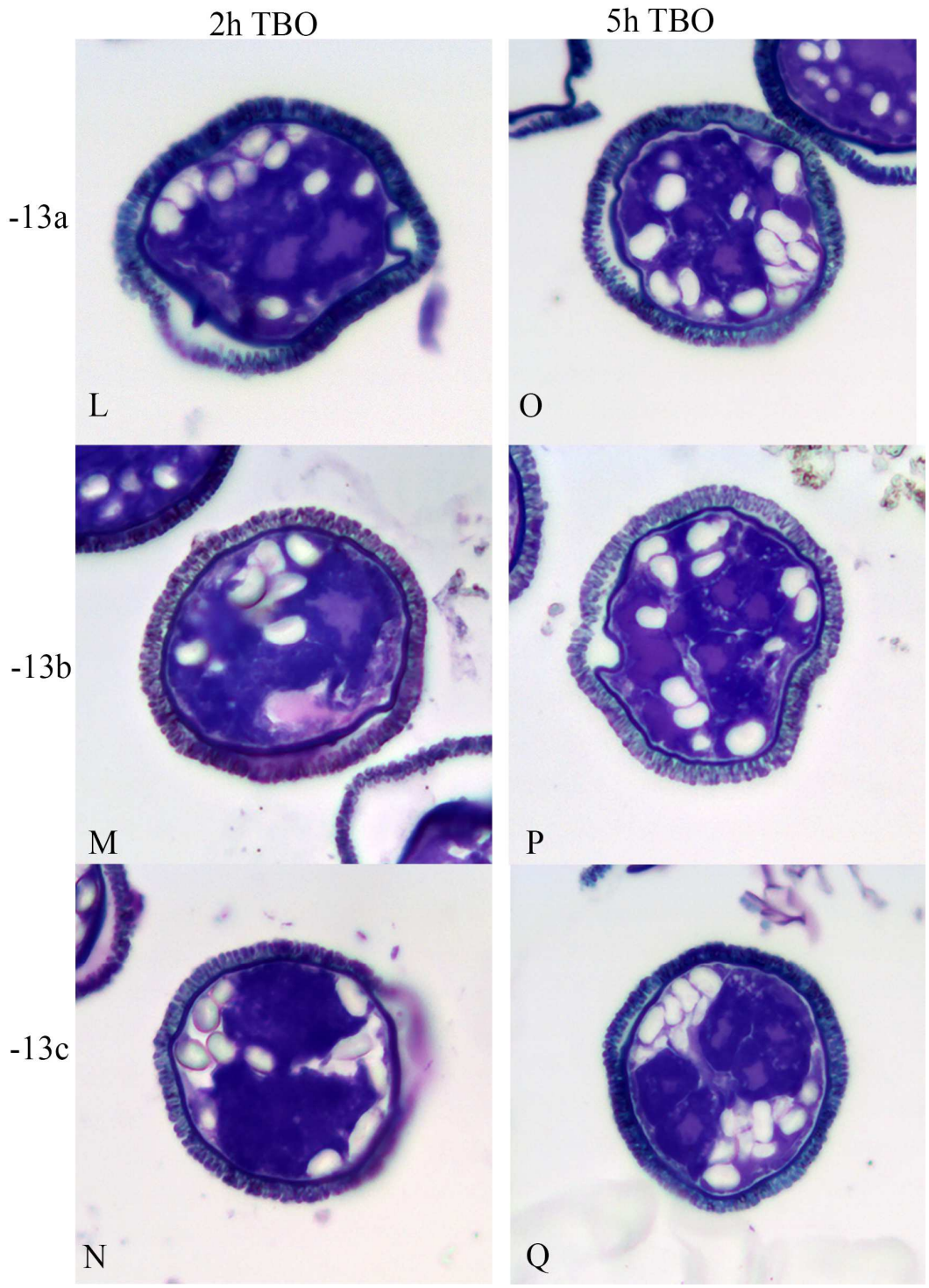


Figure 3-7. Kinesin-13. Part 3 of 3.
Knockdowns of (L, O) kinesin-13a, (M, P) -13b, and (N, Q) -13c visualized at (L-N) 2h or (O-Q) 5h post-hydration and visualized with TBO. At both 2 and 5h, kinesin-13a and -13b exhibit major morphological defects and jacket cells are absent. Kinesin-13c knockdowns proceeded through development normally.

Kinesin-14 VI

Land plants have a highly diversified group of kinesin-14s (see Chapter 1 and 2). Of the numbers kinesin-14 family members that exist in plants, kinesin-14 VI (KCBP) is likely the best studied. Kinesin-14 VI is involved in establishing memory at the PPB and for general microtubule organization in *Arabidopsis* (Bowser and Reddy, 1997; Oppenheimer et al., 1997; Vos et al., 2000; Lazzaro et al., 2013; Buschmann et al., 2015). In *Chlamydomonas* kinesin-14 VI is localized to spindle poles, phycoplast microtubules, basal bodies, and at the ciliary membrane (Dymek et al., 2006). In *Physcomitrella*, kinesin-14 VI is only weekly expressed during cytokinesis and is not considered to play an important role in mitosis (Miki et al., 2014). *Marsilea* has two transcripts that encode members of the kinesin-14 VI family (Figure 3-1B). In *Marsilea*, kinesin-14 VIa increases in abundance specifically during the time interval associated with spermatid differentiation (from the 3-5h to the 6-8h time interval) while abundance levels of kinesin-14 VIb do not change (Figure 3-2). mRNA levels of kinesin-14VIa were validated with RT-PCR (Figure 3-8A). This pattern of abundance makes it unlikely that kinesin-14 VIa is involved in mitosis.

To test this hypothesis, developing gametophytes were treated with kinesin-14 VIa dsRNA and visualized with TBO at eight hours of development. Knockdowns of kinesin-14 VIa have rounded spermatids and large, oval nuclei (Figure 3-8B). This is reminiscent of an earlier stage of development prior to differentiation and suggests that kinesin-14 VIa is required for spermatid differentiation. In accordance with this observation, 8h gametophytes visualized with DAPI (Figure 3-8 C, D) also have large round nuclei and do not have a staining pattern that is consistent with the formation of

the nuclear coil. The microtubule ribbon (Figure 3-8 C) is absent in these cells. Knockdowns of kinesin-14 VIa have a large aggregate of centrin staining (Figure 3-8 D) that resemble blepharoplast particles. The blepharoplast is a cytoplasmic particle that forms during the last mitotic division and functions like a centrosome (Sharp, 1914; Hepler, 1976). As spermatids differentiate, the blepharoplast serves as the site for *de novo* basal body assembly (Hepler, 1976). In this knockdown, no further maturation of the blepharoplast (for basal body formation) was observed.

ARK and ARK-LIKE

Armadillo repeat kinesins (ARK) are plant specific and contain ARM domains that are important for mediating protein-protein interactions. In plants, ARKs are important in nuclear localization, asymmetric division, and in general microtubule organization (Malcos and Cyr, 2011; Miki et al., 2014; 2015). ARK-LIKE kinesins are similar to ARKs; however, they are restricted to ciliated organisms (see Chapter 2) and lack the important ARM domains associated with ARK kinesins (Shen et al., 2012). *Marsilea* has three transcripts that encode ARK kinesins and one ARK-LIKE kinesin. ARKa, ARKb, and ARK-LIKE all increase in abundance, while ARKc decreases in abundance during gametogenesis (Figure 3-1B). Specifically, ARKc decreases in abundance over the entire course of development (from the 1-2h to the 6-8h time interval) and ARK-LIKE does the opposite and increases in abundance during the same time frame (Figure 3-2). These patterns of transcript abundance were verified with RT-PCR (Figure 3-9A). I chose to examine the role of ARKc and ARK-LIKE during gametogenesis in *Marsilea* because of their opposing patterns of

abundance and suggested roles in positioning asymmetric division planes and ciliogenesis, respectively.

Knockdown of ARKc in *Marsilea* did not show any obvious defects at eight hours of development (Figure 3-9B). It is therefore difficult to determine if ARKc in *Marsilea* functions like other ARKs proteins in nuclear migration and in positioning asymmetric divisions planes. Further analysis of ARKa and ARKb are required to definitely answer this question. At 8 hours after knockdown of ARK-LIKE, spermatids have a round appearance and do not possess the characteristic helical shape that is present after differentiation. However, large round nuclei in these cells are not visible indicating that some nuclear elongation has occurred (Figure 3-9C). Spermatid differentiation after knockdown of ARK-LIKE is likely to be incomplete.

To investigate the phenocopy associated with the knockdown of ARK-LIKE the localization of the microtubule ribbon, nuclear coil, and centrin was investigated after silencing. The localization of the microtubule ribbon is correct in these cells, although it appears more disorganized and much more diffuse than in controls (Figure 3-9D). Centrin staining is localized to spermatogenous cells indicating that no loss of cell fate has occurred during development. In the spermatogenous cells, centrin forms aggregates that are reminiscent of basal bodies, however, they are larger than in controls and do not become dispersed as small punctate dots, like normal basal bodies (Figure 3-9E). This pattern of centrin expression points to defects in differentiation, but not in cell specification (Tsai and Wolniak, 2001; Tsai et. al., 2004).

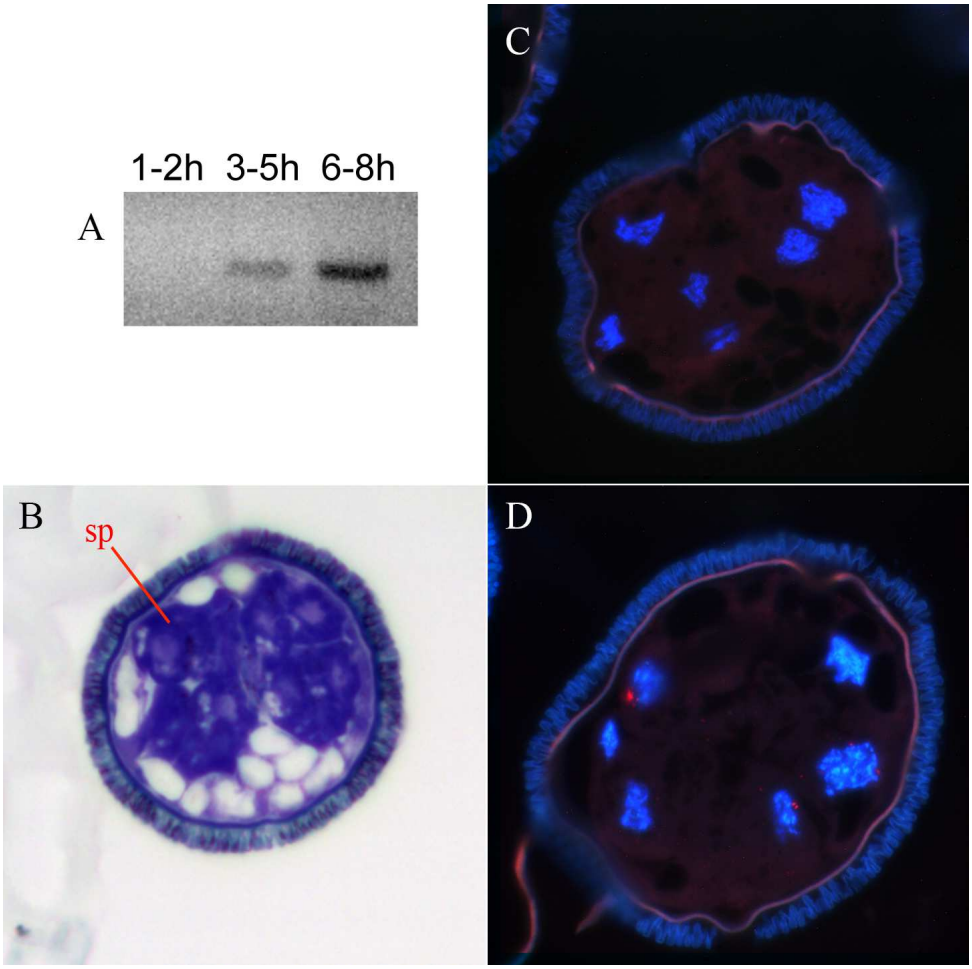


Figure 3-8. Kinesin-14 VI.
(A) RT-PCR showing that kinesin-14 VIa transcripts increase in abundance. Knockdowns at 8h post hydration visualized with (B) TBO, (C-D) DAPI, (C) anti-tubulin, or (D) anti-centrin. Silencing of kinesin-14 VI does not have any effect on cell division planes or cell fate, but spermatids fail to differentiate and do not produce coiled nucleus, a microtubule ribbon, or basal bodies.

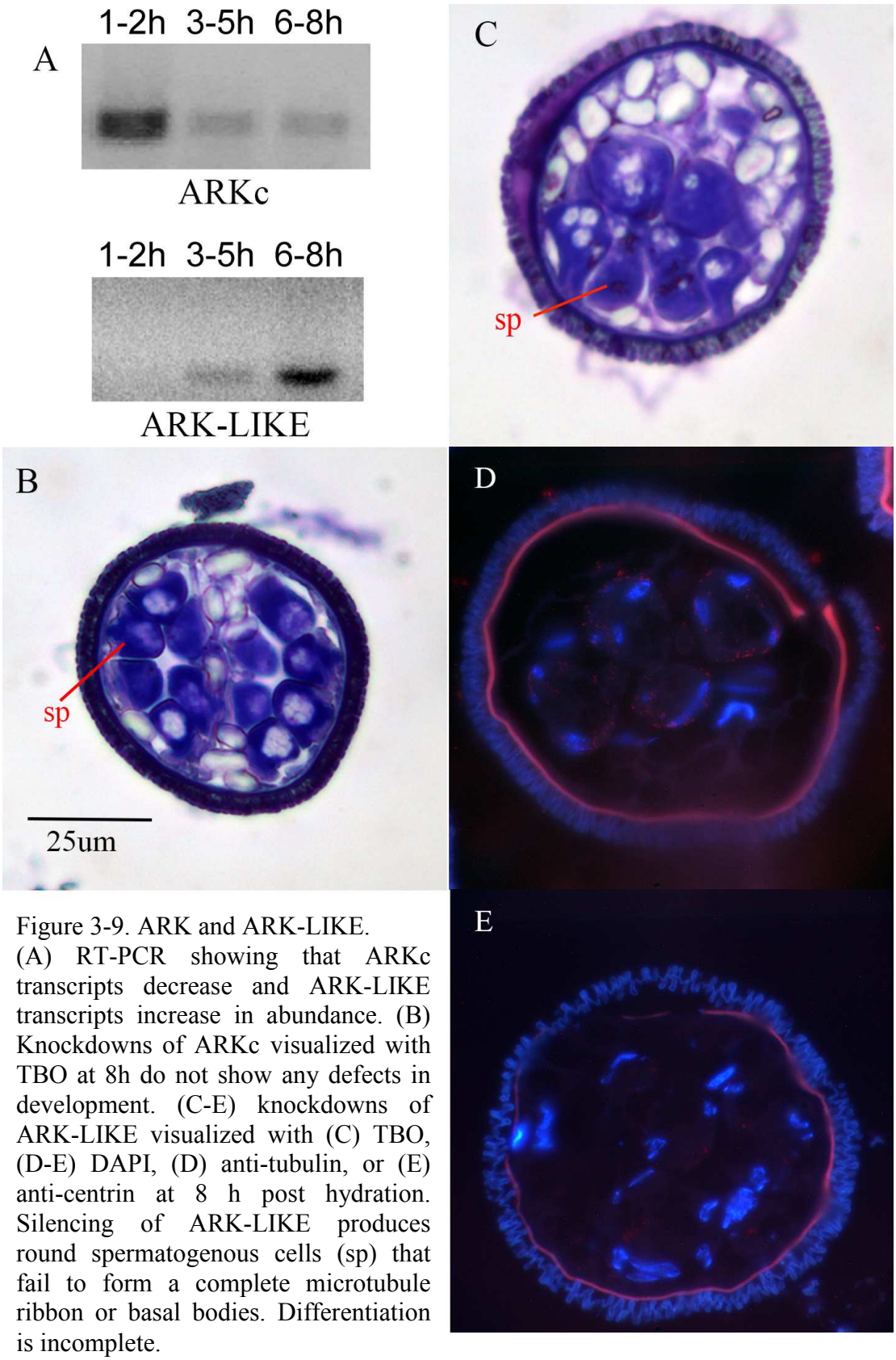


Figure 3-9. ARK and ARK-LIKE.
(A) RT-PCR showing that ARKc transcripts decrease and ARK-LIKE transcripts increase in abundance. (B) Knockdowns of ARKc visualized with TBO at 8h do not show any defects in development. (C-E) knockdowns of ARK-LIKE visualized with (C) TBO, (D-E) DAPI, (D) anti-tubulin, or (E) anti-centrin at 8 h post hydration. Silencing of ARK-LIKE produces round spermatogenous cells (sp) that fail to form a complete microtubule ribbon or basal bodies. Differentiation is incomplete.

Kinesin-'orphan' III

Similar to ARK-LIKE, kinesin-'orphan' III is also restricted to ciliated organisms; however, the presence of kinesin-'orphan' III is more highly conserved outside of plants (Wickstead and Gull, 2006; Wickstead et al., 2010b). *Marsilea* has one transcript that encodes kinesin-'orphan' III and this transcript significantly increases in abundance between the 1-2h and 3-5h time intervals (Figure 3-2, 3-10A). This increase suggests a role for this kinesin during the events that occur after the establishment of polarity in the gametophyte and possibly during the differentiation of spermatids into motile spermatozoids. After the knockdown of 'orphan'-III there are no major changes in development in gametophytes visualized at eight hours post hydration, although one plastid localized inside a spermatid (Figure 3-10B).

For the majority of kinesin knockdown, the defects in development observed after RNAi were manifested as arrested development at a stage that matched the time when the transcript became abundant in untreated gametophytes (Table 3-2). This set of results suggests that each kinesin becomes rate limiting for further development prior to or at the time of arrest.

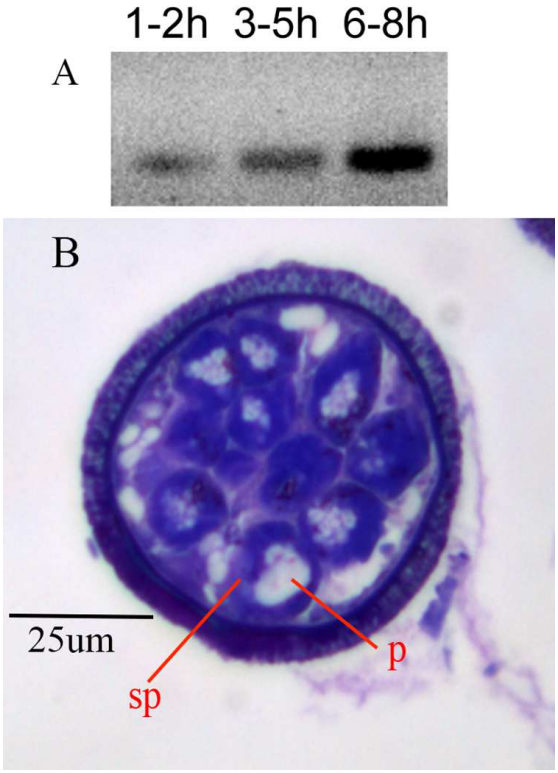


Figure 3-10. Kinesin-'orphan' III. (A) RT-PCR of 'orphan' III showing that transcripts increase in abundance during development. (B) Silencing of 'orphan' III produces gametophytes with no major morphological defects in development, although one plastid (p) can be found mis-localized to a spermatogenous cell (sp).

Table 3-2. Summary of the affect of kinesin knockdowns on spermatid development in *Marsilea*

	Phenocopy after RNAi	**Developmental Arrest**	**Developmental Process**
Kinesin-13b	No organized division planes, absence of mitosis, no jacket cells present	In the first 90 minutes of development	No mitotic divisions
Kinesin-13a	No organized division planes, plastids present in spermatogenous cells	Between 1 and 3 hours	No organized divisions to distinguish jacket from spermatogenous cells
Kinesin-4 Ic	Large and unevenly distributed spermatogenous cells	Between 3 and 5 hours	Skipped symmetric cell divisions that give rise to 32 spermatids
Kinesin-14 VIa	Spermatogenous cells have round appearance and round nuclei	Before 6 hours of development	No differentiation of spermatids
ARK-LIKE	Spermatids do not have characteristic shape, round nuclei not visible	Between 6 and 8 hours	Incomplete differentiation of spermatids
Kinesin-12 II	Normal progression through the first eight hours of development	No apparent arrest in development	Undetermined
Kinesin-13c	Normal progression through the first eight hours of development	No apparent arrest in development	Undetermined
ARKc	Normal progression through the first eight hours of development	No apparent arrest in development	Undetermined
'Orphan' III	Normal development, although one plastid located inside a spermatogenous cell	No apparent arrest in development	Undetermined

Discussion and Conclusions

Kinesin transcripts change in abundance during gametophyte development

Similar to many rapidly developing systems, spermatogenesis in *Marsilea* does not require significant amounts of transcription (Hart and Wolniak, 1998, 1999; Klink and Wolniak, 2001; Tsai and Wolniak, 2001; Tasi et al., 2004; Deeb et al., 2010; Boothby and Wolniak, 2011; Wolniak et al., 2015) though exit of mature gametes from the spore wall may require some mRNA synthesis (Hart and Wolniak, 1998; Klink and Wolniak, 2003). Previous work from our laboratory has shown that patterns of transcript abundance and availability are important for regulating rapid development. pre-mRNAs are stored as intron-containing, masked transcripts until spore rehydration, when they are unmasked, processed, and subsequently translated in the developing gametophyte (Tsai et al., 2004; Boothby et al., 2013; Wolniak et al., 2015). My analysis of the kinesin family in *Marsilea* shows that almost all (91%) kinesin transcripts change in abundance during development. I suspect that kinesin transcripts that decrease (52%) in abundance as gametophyte development progresses become undetectable, presumably by destruction after translation, while kinesin transcripts that increase (39%) in abundance during development become detectable by unmasking, splicing, and polyadenylation at a specific time intervals after spore hydration when their translation is required for development (Deeb et al., 2010; Boothby and Wolniak, 2011; Boothby et al., 2013; Wolniak et al., 2015).

I found that almost all kinesin mRNAs that decrease in abundance during development in *Marsilea* encode proteins with known roles in plant mitosis and cytokinesis (see Chapter 1). The only exception to this is kinesin-7 I. Transcripts that

encode 50% (2/4) of the kinesins in this subfamily decrease in abundance during spermatogenesis in *Marsilea* (Figure 3-1A). However, members of the kinesin-7 I subfamily are not known to be involved in plant cell mitosis and instead are expressed in mitochondria (Itoh et al., 2001). In contrast, animal kinesin-7 (CENP-E) is a kinetochore-associated motor important for localizing and positioning chromosomes at the metaphase plate during mitosis (Yardimci et al., 2008). The significance of this in *Marsilea* has not been functionally studied, though kinesin-7 II in *Physcomitrella* shows a similar pattern of localization to CENP-E (Miki et al., 2014) and the single kinesin-7 III motor present in the *Marsilea* male gametophyte decreases in abundance as the gametophyte exits its phase of successive cell division cycles. It is therefore possible that plant kinesin-7 III is most similar to animal CENP-E and that kinesin-7 I is a more divergent subgroup of this large kinesin family in plants.

Decreases in kinesin transcript abundance are generally not specific for any particular time period of development. Almost all of the kinesins that decrease in abundance do so over the entire course of spermatogenesis, between the 1-2h and the 6-8h time intervals (Figure 3-2). The only exception to this is kinesin-13a. This transcript decreases specifically between the 3-5h and 6-8h time interval of development and levels of kinesin-13a are highest 1-2h post hydration (Figure 3-2, Table 3-1). This suggests a specific role for kinesin-13a during the asymmetric divisions that establish cell fate in the microspore, when transcript levels are highest. In support of this, functional studies point to a role for kinesin-13a during the early division cycles that establish cell fate in the gametophyte (Figure 3-7).

Many of the kinesin transcripts that increase in abundance in *Marsilea* are not

up-regulated during mitosis in *Arabidopsis* (Vanstraelen et al., 2006) or are non-mitotic or not expressed in *Physcomitrella* caulonemal cells (Miki et al., 2014). It appears then, that these transcripts do not encode mitotic kinesins. Of these, kinesin-2 and -9 have established roles in ciliogenesis and motility and are only found in ciliated organisms (Wickstead and Gull, 2006). The fact that both of these kinesins increase in abundance during gametophyte development suggests that they are also involved in ciliogenesis in *Marsilea* (see Chapter 4 for functional analysis of kinesin-2 and kinesin-9 during ciliogenesis). Kinesin-4 II, ARK-LIKE, and kinesin-'orphan' III are also restricted to ciliated organisms (see Chapter 2) and increase in abundance in during gametophyte development. These kinesins are not currently known to function during ciliogenesis or inside cilia; however, their restricted distribution and patterns of abundance make them interesting targets for further investigation. Functional analysis of ARK-LIKE confirms the involvement of this kinesin in spermatid differentiation (Figure 3-9).

Transcripts that increase in abundance significantly show these rises during precise time intervals of development (Figure 3-2). This suggests that the mechanisms that regulate the increase in the abundance of specific transcripts (unmasking, splicing, polyadenylation) are tightly controlled and temporally regulated. Many kinesin transcripts increase specifically from 1-2 to 3-5 hours post hydration, suggesting they are not required for asymmetric divisions, but may be needed for symmetric spermatogenous cell divisions, for blepharoplast formation, or for later events during differentiation and ciliogenesis. Some transcripts also specifically increase between 3-5 and 6-8 hours post hydration, indicating a restricted role in

differentiation, cell morphogenesis, and ciliogenesis.

In addition to kinesins that increase and decrease in abundance, a few kinesin transcripts do not change exhibit significant changes in abundance during gametophyte development. FPKM values for these transcripts are generally high throughout development (Table 3-1). Examples of this include transcripts that encode kinesin-8 IIb, -13b, and -14 IIIb. Kinesin-13b is needed early in development to establish cell fate and polarity (Figure 3-7). In *Marsilea*, there are three transcripts that encode kinesin-8 and another three that encode kinesin-13. In each case, one transcript increases in abundance, one decreases, and one does not change significantly. The significance of this in *Marsilea* is unclear, but both kinesin-8 and kinesin-13 are known for their ability to depolymerizing microtubules (Desai et al., 1999; Moores and Milligan, 2007). This makes them important players in mitosis and in regulating the length of ciliary axonemes (Varga et al., 2006; Blaineau et al., 2007; Mayr et al., 2007; Piao et al., 2009; Varga et al., 2009; Delgehyr et al., 2012; Niwa et al., 2012; Wang et al., 2013). It is intriguing to speculate that the microtubule depolymerizing activity of kinesin-8 and kinesin-13 is conserved in *Marsilea* and that specific transcripts change in abundance in accordance with their functions during either mitosis or ciliogenesis.

Patterns of kinesin transcript abundance correlate with protein function

My analysis shows that transcripts that encoding kinesin-4 Ic, kinesin-12 II, kinesin-13a, and ARKc in *Marsilea* decrease in abundance during gametophyte development. Of these, kinesin-4 Ic and kinesin-13a are shown to be required for the mitotic cell divisions that give rise to seven sterile cells and 32 spermatids (Table 3-

2). Kinesin-4 Ic decreases significantly over the course of development and is required during the symmetric cell divisions that give rise to 32 spermatids (Figure 3-5). Kinesin-13a decreases specifically from the 3–5 h to the 6–8 h time interval and is the only kinesin showing this pattern of transcript abundance. Knockdowns show that this kinesin is required much earlier in development during the asymmetric divisions that distinguish spermatogenous cells from jacket cells (Figure 3-7). These results are generally consistent with the established roles of these kinesins during mitosis, although the precise involvement of kinesin-4 and kinesin-13 in plant mitosis has yet to be clearly demonstrated (see Chapter 1). In animals, kinesin-4 (Kif4) binds to chromosome arms (Wu and Chen, 2008) and is important for regulating microtubule dynamics at the spindle midzone (Bieling et al., 2010). One member of the kinesin-4 family in *Physcomitrella* has a similar localization pattern (nucleus, chromosomes, and midzone) to animal Kif4 (Miki et al., 2014). Kinesin-13 (Kif2) has microtubule depolymerizing activity (Desai et al., 1999) and members of this family are required for spindle assembly during mitosis (Ems-McClung and Walczak, 2010). This evidence suggests conserved function for both kinesin-4 and kinesin-13 during mitosis.

In contrast, kinesin-12 II and ARKc decrease in abundance and did not show any morphological changes in development after knockdown (Table 3-2). In *Arabidopsis* and *Physcomitrella* Kinesin-12 II localizes to the phragmoplast and is important for microtubule organization during cytokinesis (Lee and Liu, 2000; Pan et al., 2004; Lee et al., 2007; Oh et al., 2012; Miki et al., 2014). Since there is only one kinesin12-II transcript present in *Marsilea* (Figure 3-1), I suspected this kinesin

would be needed during cytokinesis and that knockdowns of kinesin-12 II would show defects during cell division. The lack of a detectable phenocopy in kinesin-12 II knockdowns (Figure 3-6) suggests that kinesin-12 II functions redundantly with other phragmoplast kinesins or that defects in cell division were minor and went unnoticed in our detection system. A similar fate can be prescribed to ARKc, which is the only ARK kinesin to decrease in abundance during gametogenesis in *Marsilea* (Figure 3-2). ARK is the only kinesin that is known to be important for positioning asymmetric divisions in plants (Malcos and Cyr, 2011) and I suspected that knockdowns of ARKc would display significant abnormalities during the asymmetric divisions that establish cell fate. However, knockdowns visualized at eight hours post hydration did not show any defects in development (Figure 3-9B). Further analysis of ARKa and ARKb are needed to determine if a conserved role for ARKs during asymmetric divisions and in nuclear migration exists.

Knockdowns of kinesins that increase in abundance, kinesin-13c, ARK-LIKE, kinesin-'orphan' III, and kinesin-14 VIa, showed unique phenocopies in the *Marsilea* gametophyte (Table 3-2). Kinesin-13c increases significantly between the 3-5 and 6-8 hour time intervals of development (Figure 3-2). Since kinesin-13 is known to be important for ciliogenesis in *Chlamydomonas* (Piao et al., 2009; Wang et al., 2013), I suspected kinesin-13c knockdowns would exhibit problems with basal body formation and spermatid differentiation. Instead, kinesin-13c knockdowns appeared morphologically normal (Figure 3-7). ARK-LIKE kinesin increases throughout development (Figure 3-2) and is apparently required for the normal differentiation of spermatids. After knockdown, most spermatids do not develop their characteristic

helically shaped cell bodies. Moreover, round nuclei, present in spermatids prior to differentiation, are not visible, which suggests that differentiation is abnormal or incomplete (Figure 3-9). Kinesin-'orphan' III increases specifically from the 1–2 to 3–5 hour time interval (Figure 3-2). These knockdowns proceed through the normal numbers of cell divisions and the spermatids differentiate normally (Figure 3-10). These are the first functional studies on both ARK-LIKE kinesin and kinesin-'orphan' III.

Most kinesin mRNAs that do not change in abundance are present throughout gametophyte development at high levels (Table 3-1). Knockdowns of kinesin-13b become arrested early in development and no cell divisions are apparent (Figure 3-7, Table 3-2). This phenocopy is distinct from knockdowns of both kinesin-13a and kinesin-13c, leading to the conclusion each kinesin-13 present in *Marsilea* performs unique, yet complementary, functions. Kinesin-13a and kinesin-13b are important in organizing division planes early in development, which kinesin-13c is not involved in this process and may be required later in development. From these results, it appears that high levels of kinesin-13b are required for development to proceed in an organized fashion.

Kinesin-14 VIa (KCBP) presents an interesting illustration of how these studies in *Marsilea* can be used to make conclusions about the functions of plant kinesins. This kinesin is highly conserved in plants (see Chapter 2) and contains MyTH4 and FERM accessory domains in addition to a c-terminal kinesin motor (Richardson et al., 2006). *Marsilea* contains only one transcript that encodes kinesin-14 VI and this transcript increases in abundance during the later stages of

development when the division cycles have been completed (Figure 3-2). Previous studies show conflicting roles for kinesin-14 VI during both cell division and ciliogenesis, depending on the system. In *Arabidopsis*, kinesin-14 VI is involved in mitosis (Buschmann et al., 2015), but in *Physcomitrella* it is only weakly observed at the nuclear envelope during cytokinesis (Miki et al., 2014). In *Chlamydomonas*, kinesin-14 VI has dual roles in mitosis and in the flagellum (Dymek et al., 2006). Recently *Physcomitrella* kinesin-14 VI has been implicated in retrograde cytoplasmic transport, although the cargo moved by this kinesin remains unknown (Jonsson et al., 2015). My analysis of kinesin-14 VIa points to the importance of this kinesin during spermatid differentiation, but does not address the possible role of this kinesin in retrograde transport. Knockdowns suggest that kinesin-14 VIa is required for spermatid differentiation since development is arrested before gamete maturation reaches completion (Figure 3-8, Table 3-2). Therefore kinesin-14 VIa in *Marsilea* appears to have a more similar function to kinesin-14 VI in *Physcomitrella* and in *Chlamydomonas* that it's *Arabidopsis* counterpart.

In the 1980s, it was suggested that tubulin was limiting for development in the male gametophyte of Marsilea and that the translation of tubulin was required for ciliogenesis (Pennell et al., 1986, 1988). Although some translation of tubulin occurs during late phases of gametophyte development (Hart and Wolniak, 1999; Klink and Wolniak, 2003), silencing experiments suggested that tubulin was not truly rate limiting for gamete formation to reach normal completion (Klink and Wolniak, 2001). Here, through RNAseq and functional silencing assays, I now posit that several kinesins may serve in key rate-limiting roles during development, especially

during the formation of the ciliary axonemes in motile spermatozoids. Next I will investigate this possibility further by looking into the role of other important regulators of ciliogenesis (kinesin-2, kinesin-9, flagellar dynein, IFT proteins, etc.) during development of the male gametophyte of Marsilea.

Chapter 4: Kinesin-2 and Kinesin-9 have Atypical Functions during Ciliogenesis in the Male Gametophyte of *Marsilea vestita*

Note: The following has been adapted from an article in revision at BMC Cell Biology, 2016.

Introduction

Cilia, intraflagellar transport, and kinesin motor proteins

Ciliogenesis is the process of by which cilia axonemes, extensions of singlet and doublet microtubules, are assembled at the distal end of a basal body and are surrounded by a specialized membrane to protrude from the cell surface. Cilia function to either power cell motility or serve a sensory function and receive outside signal. Motile cilia have a highly conserved '9+2' microtubule organization, with nine doublet microtubules surrounding two central pair singlet microtubules. Doublet microtubules are attached to inner and outer arm dynein arms. This generates sliding between the microtubule doublets (Sale and Satir, 1977; Sale, 1986; Wargo et al., 2004; Wirschell et al., 2009). This sliding is converted to bending by nexin, which connects neighboring doublet microtubules, and allows cilia to power motility (Summers and Gibbons, 1971; Nicastro et al., 2006; Heuser et al., 2009). Radial spoke proteins also attach to the doublet microtubules and transiently interact with central pair microtubules (Warner and Satir, 1974; Goodenough and Heuser, 1985; Smith and Lefebvre, 1997; Mitchell and Sale, 1999; Omoto et al., 1999). Cilia exhibit

a three-dimensional beat shape, where a power stroke is manifested along the entire length of the axoneme, and a recovery stroke is initiated at the base and is propagated distally (Wolniak and Cande, 1980). In contrast, flagellar beat is a planar event, where the power stroke is a sinusoidal wave shorter than the length of the axoneme. The wave initiates at the proximal portion of the axoneme, and moves distally. Radial spokes maintain the beating of cilia (Witman et al., 1978; Afzelius and Eliasson, 1979) by transmitting signals that regulate the activity of dynein arms in different portions of the axoneme for the propagation of the power and recovery strokes (Patel-King et al., 2004; Smith and Yang, 2004; Yang et al., 2006). Sensory cilia lack the central pair microtubules and the dynein arms, which are required for motility.

Like the structure of ciliary axonemes, the processes involved in ciliogenesis are also highly conserved in eukaryotes. Proteins important for axoneme assembly and function are localized in the axoneme by intraflagellar transport (IFT). IFT was first observed in *Chlamydomonas* (Bloodgood, 1977) and since as been confirmed in a broad range of eukaryotic organisms, from protists to mammals. During IFT, kinesin-2 is responsible for transporting cargo in the anterograde direction towards microtubule plus-ends, located at the distal ends of forming axonemes (Scholey, 2013). IFT dynein, also referred to as cytoplasmic dynein-2 or dynein-1b, is responsible for IFT in the opposite direction, towards microtubule minus-ends located at the basal bodies (Pazour et al., 1999; Porter et al., 1999). Kinesin-2 is only found in organisms that are ciliated at some point throughout the life cycle (Wickstead and Gull, 2006; Wickstead et al., 2010b). Although IFT is the primary means by which axonemes are assembled, other mechanisms are known to exist. In *Plasmodium*

falciparum and the sperm flagella of *Drosophila* axonemes are assembled using a mechanism independent of IFT. In this case, complete axonemes form in the cytoplasm, rather than within membrane extensions, and without the involvement of kinesin-2 (Han et al., 2003; Sarpal et al., 2003; Briggs et al., 2004).

Several kinesin-2 isoforms are important for IFT. Kinesin-2 isoforms assemble into a heterotrimeric complex that is responsible for IFT in motile cilia. The complex consists of a kinesin-2α, a kinesin-2β, and a kinesin associated protein (KAP) important for cargo binding (Scholey, 2013). In *Chlamydomonas*, mutations in the heterotrimeric kinesin-2 complex block IFT and ciliary assembly does not occur normally (Huang et al., 1977; Walther et al., 1994; Kozminski et al., 1995; Cole et al., 1998). Separate kinesin-2 homodimers also exist and consist of two kinesin-2γ subunits. KAPs are not currently known to associate with homodimeric kinesin-2 (Scholey, 2013). Kinesin-2γ, also known as OSM-3 in *C. elegans* or KIF17 in mammals, works in conjunction with heterotrimeric kinesin-2 to assemble sensory cilia (Signor et al., 1999; Setou et al., 2000; Snow et al., 2004; Evans et al., 2006; Imanishi et al., 2006; Pan et al., 2006).

Kinesin-9 proteins have a conserved role axoneme assembly and motility. Like kinesin-2, kinesin-9 is only found in ciliated organisms (Wickstead and Gull, 2006; Wickstead et al., 2010b). Phylogenetic analysis shows the existence of two kinesin-9 subfamilies, kinesin-9A and kinesin-9B (Wickstead and Gull, 2006; Demonchy et al, 2009). Kinesin-9A localizes to central pair microtubules (Bernstein et al., 1994; Yokoyama et al., 2004) and is required for motility. Mutations in kinesin-9A result in cilia with reduced beat frequency (Yokoyama et al., 2004; Demonchy et

al, 2009). This is due to the interaction between kinesin-9A and hydin, another central pair protein that is required for motility (Lechtreck and Witman, 2007; Lechtreck et al., 2008). Currently, little is known about kinesin-9B; however, in *T. brucei*, kinesin-9B localizes to basal bodies and is necessary for the construction of the paraflagellar rod (Demonchy et al, 2009). Two other kinesins are also restricted to ciliated organisms, kinesin-'orphan' III (kinesin-16) and kinesin-17 (Wickstead and Gull, 2006; Shen et al., 2012). The function of these kinesins either in ciliogenesis or motility is unknown (Figure 3-10; Tomei and Wolniak, 2016).

Ciliogenesis in the Marsilea male gametophyte

Although the processes that regulate the generation, structure, and function of cilia are highly conserved, not all organisms contain cilia. The loss of cilia is most pronounced during the evolution and adaption of land plants from green algae. Conifers and angiosperms do not make any ciliated cells, while lower plants such as ferns, mosses, and related groups produce ciliated male gametes (Raven et al., 1999; Pryer et al., 2004; Brown et al., 2015). These gametophytes are the only cells in the organisms that possess basal bodies and axonemes. Basal body formation in these organisms occurs in the spermatids as a *de novo* process; these plants lack centrioles in their cells (Wolniak et al., 2011; 2015; Prayer et al., 2014). Water is required for the male gametophyte of these organisms to swim to the female gametophyte, so while these organisms are adapted to life on land, they remain dependent upon water for fertilization. In contrast, the male gametophyte in conifers and angiosperms (pollen) extends a tube that allows amoeboid sperm cells to approach the egg. In conjunction with the loss of cilia in these organisms, the absence of proteins

important for IFT and motility is also observed. For example, *Arabidopsis*, a flowering plant commonly used as an experimental genetic model system, does not contain members of the kinesin-2, -9, -orphan III, or -17 families. *Chlamydomonas*, the moss *Physcomitrella*, and the water fern *Marsilea* contain members of all these kinesin families, except kinesin-17, which is only present in *Chlamydomonas* (Reddy and Day, 2001; Richardson et al., 2006; Wickstead and Gull, 2006; Wickstead et al., 2010b; Shen et al., 2012; Tomei and Wolniak, 2016). Due to the absence of cilia and IFT proteins in many land plants, the majority of research on ciliogenesis in plants has been conducted in *Chlamydomonas*. This is despite the fact that the conserved '9+2' microtubule organization of motile axonemes was first observed in the spermatozoid from a fern (Manton and Clarke, 1951).

Here, I am using spermatogenesis in *Marsilea* as a model for *de novo* ciliogenesis in order to fill this important gap and expand our knowledge on plant ciliogenesis. This provides us the unique ability to study the construction, organization and motility of ciliary apparatus produced in gametes of a land plant. Moreover, this gametophyte provides insights on the mechanisms that evolved to regulate *de novo* ciliogenesis in specialized cells of otherwise nonmotile organisms. Ciliogenesis in *Marsilea* occurs on basal bodies that form *de novo* during the differentiation of spermatids into motile spermatozoids (Sharp, 1914; Hepler, 1976; Myles and Hepler, 1977). Each spermatozoid contains about 140 cilia (Figure 1-1E) and is able to swim in shallow ponds to reach the female gametophyte for fertilization. Basal bodies are arranged regular intervals along a microtubule and nuclear coil that forms during the differentiation of spermatids into motile

spermatozoids, producing corkscrew shaped cells (Figure 1-3) (Sharp, 1914; Myles and Hepler, 1977). At first, basal bodies are oriented so cilia diverge from each other (Figure 1-4A). Basal bodies then rotate 90° so that the cilia protrude vertically in two regular rows (Figure 1-4B) (Myles et al., 1978; Myles and Hepler, 1982). An extension of cytoplasm grows around the anterior end of each spermatid and eventually fuses together to surround each cell. This creates an internal, but extracellular space that contains the microtubule ribbon and organelle coil plus all of the cilia (Figure 1-4C). Since ciliogenesis occurs in an extracellular space, IFT is suspected to be important for cilia assembly in *Marsilea*. After development is complete, spermatozoids break free from the surrounding cytoplasm leaving behind a thin, vesicle-like structure (Myles and Hepler, 1977).

During gametophyte development, ciliogenesis is restricted to the last two to four hours of an eleven-hour developmental program (Sharp, 1914; Hepler, 1976; Myles and Hepler, 1977; Myles et al., 1978; Wolniak et al., 2011; 2015). The first five hours are dedicated to producing 32 spermatid cells and seven sterile cells within each microspore (Figure 1-2). Using the transcriptome and the temporal separation of ciliogenesis from earlier phases of development, it is easy to determine which transcripts are enriched during the stage of development associated with ciliogenesis. The working hypothesis is that transcripts present during this stage of development become available for detection, and potentially translation, through RNA unmasking, the splicing of retained introns, and then, polyadenylation (Boothby and Wolniak, 2011; Boothby et al., 2013). Since kinesin-2 and kinesin-9 are vitally important for the structure and function of cilia and are abundant in the male gametophyte

transcriptome during the time interval associated with spermatid morphogenesis and differentiation (Chapter 2; Tomei and Wolniak, 2016), I was interested in investigation the potential roles of these proteins during ciliogenesis in *Marsilea*. Here I show that unlike many other systems, the male gametophyte of *Marsilea* has only one kinesin-2 that is apparent in its transcriptome. It is most similar to the kinesin-2 found in *Physcomitrella* and is divergent from the typical heterotrimeric kinesin-2 associated with IFT. MvKinesin-2 is required for two separate events in this gametophyte. It is necessary for cytokinesis in spermatogenous cells and is also important for regulating the length of cilia during later phases of spermatid maturation. The *Marsilea* gametophyte has two transcripts that encode members of the kinesin-9 family; one is similar to the kinesin-9A and the other is most like kinesin-9B. In the gametophyte, we show that MvKinesin-9A is involved in the proper positioning of basal bodies that are required for ciliogenesis and it is necessary for motility. MvKinesin-9B is not required for ciliogenesis and is instead needed for the timely differentiation of motile spermatozoids in the rapidly developing gametophyte.

Results

Characterization of kinesin-2 and kinesin-9 family in Marsilea

In order to determine whether kinesins are necessary for ciliogenesis during male gametophyte development in *Marsilea*, I searched our transcriptome for members of the kinesin-2 and the kinesin-9 families using *Physcomitrella* and

Chlamydomonas kinesins-2 and kinesin-9 sequences (Figure 4-1). I used these sequences as the basis of my search because both *Chlamydomonas* and *Physcomitrella* have the capacity to make ciliary axonemes and are two of the more closely related species to *Marsilea* with annotated kinesin families (Shen et al., 2012; Miki et al., 2014). Moreover, kinesin-2 and kinesin-9 are known to be involved in ciliogenesis in *Chlamydomonas* (Huang et al., 1977; Bernstein et al., 1994; Walther et al., 1994; Kozminski et al., 1995; Cole et al., 1998; Lechtreck et al., 2007). This search allowed me to identify one candidate kinesin-2 transcript and two candidate kinesin-9 transcripts (Figure 4-1). The results were verified and confirmed by comparing them to all the kinesin-like sequences found in the *Marsilea* gametophyte reference transcriptome (see Chapter 2, Tomei and Wolniak, 2016).

A

>XP_001697037 (Cr_FLA8)
MASGGECVKVAVRCRPLNGKEKGDNRATIVEVDNKTGQVTLNNPKGDEPPKTFTFDNAFDWNVTQRDVYDVVARPIVNSVMDGYNGTIFAYGQTGTGKTHTMEGFP
TPELQGIIPNCFDHVFETVNSSTGKQWMVRASYLEIYNEEVRDLLSKDPKNKLELKEHKDSGVYVKGLNAFVVKGVPELKNVLEVGKKNRSVGATLMNQDSSRSHSIFT
ITIETIEQTQAQPEGHIRVGKLNLVDLAGSERQSKTGATGDRLKEATKINLSLSALGNVISALVDGKSGHVPYRDSKLTRLLQDSLGGNTKTIMCANMGPADWNYDETLS
TLRYANRAKNIKNKPKINEDPKDAMLREFQDEIARLKAALEAEGGALPEGFATGPGGEIIVEKVVQVPKALDASFLEQMRKDMEEQMKKELASQQAAALNDEQLQKVK
EEAAAKAKAEAARLEEEKKKAEEEAARMQRKQQKIKAEMDKKSLDAEQIRAEKEALAKKLKAMESKILKGDQAGGLAEVTKKKEEELKRKEQELERRRKEEEEQRKK
IQVMEEQQLAMEDKYKDKADEADQKTKKLKKLWKKFQEVNAEVEDMYKEFQREKEDLLESIRMLQDQMQLKDMVIEAFIPPEEVQKVMKRAHWDDEREVWVLERL
SDIGKRETAQGASRRPVSASGQRRPTSDFAKLANAMGDMNPRFKSENILNLELDLPERTTYDYEGPGVDPRVQAAINAAFAEDGELIFVGSEQNVHLGDASAAAARPDS
AKKRPASARKGTKK
>XP_001701510 (CrFLA10)
MPPAGGGSESVKVVVRCRPLNGKEKADGRSRIVDMDVDAGQVKVRNPKADASEPPKAFTFDQVYDWNCQQRDVFDITARPLIDSCIEGYNGTIFAYGQTGTGKSHTME
GKDEPPELRGLIPNTFRYVFEIIARDSGTKEFLVRSSYLEIYNEEVRDLLGKDHSKKMELKESPDRGVYVKDLSQFVCKNYEEMNKVLLAGKDNRQVGATLMNQDSSRS
HSIFTITIECIEKLESAAAQKPGAKKDDSNHVRVGKLNLVDLAGSERQDKTGATGDRLKEGIKINLSLTALGNVISALVDGKSGHIPYRDSKLTRLLQDSLGGNTKTVMVA
NIGPADWNYDETMSTLRYANRAKNIQNKPKINEDPKDAMLRQFQEEIKKLKEQLAARAAGGGGPITMPSGGGSPTQKIVERTEEVDPDIDAIKAQMRAELEAKMKSDIS
TEALDKAREEAEAAAKKQLQAIIDDQGKTEAQKKAARDALKKQAEEARAIAGAIEKEKQEKAVLESRIKEMEGKIVVGGVNMLEKVDELKQKSEDIKREAAIRKRQEE
EAKRRLEELQAAQVDADAKFASLDEEINVKSRQLKKLFEKYQGKKGELADLQEQFQREREGMLEDYRILTQQIKLKNLIIACFIPPDYQDKIMQHCHWQDYDSSWNIDCI
AYAGNAVRTNQELQAQEDKEHDAAAENERLKNCFFSYEQFEAAGAGSKQGGGGGGGGGGAARPGSSAGRAVGSAARRTGGKAGGKDITDIGSLRDSVNWGDDDDK
KKGAIPKAKGLVKDTVDPRLRASKLK
>Phypa_425592 (PpKinesin-2)
MKERGRGHGSGLTRTSSTGSSLSSGGGSARRAERVQVVVRCRPMLVKENAEGRNNCVLVDTVGSTIQVKNLKQPEQEPKLFTFDKTYDATSTQKQLYDDVAHPIVHSV
MCGYNGTVLAYGQTASGKTFTMDGLDDPPEMRGIIPQAFEGIFTHIQDSQSSDNFLVRASYLEIHNEEIRDLLATGSQSSSRLELKENVEGNVVYVKNLTSITVQSVADISHL
LTVGKKSRSVGATLMNQDSSRSHSIFTITVEASARSSSAETDGSMHIRVGKLNLVDLAGSERLNKTGATGDRFRELTNINWSLSALGNVISALVDDKSSHVPYRDSKLTR
LLQDSLGGNTRTVMIANIGPADYNYDESVSTLRYANRAKSIKNKPRINEDPKDAILREFQEEIARLRAQLQASSPVLDERTPNKIDPSSPSAVDLELHMQQLRAKMQEDM
EHQVFLHKSMSDQAMACIKADFEQKTQSEMQALKAEKERSDEEKQRIAQQLQQQHVELQSHYQALAREKEDRDVLAAKLRALEEKLVHGTNNDNEKLLEKAKQKEV
ELALREQQLQEKENMDEERQRKIAELEEAQLMAEEKCNTMEEEVEMKTRKLRKLMARYQQSKNDINALRTELQDTIHEFQRERADMFLSLRSLDQQLQLKNFLIDKFIS
PEDLTKVMRRVHWDEENEIWNLVQNAPVRSLPYNGPTRVSIDGFTKQPLLRPNSAIGRSRPPLRQPEQTLSQSERDRYLSNTSSVHVHSPRKGAGRSKAIDEGKIGCRVD
NILKKDQDVSEGRNHFEFGDNSQLSSSKVDIEHALREFRVDNKPSFTWDREPQQVTPAAAGARPKSARTKQHNPRPRTAR
>Phypa_425498 (Pp_Kinesin9A)
MPYVDDALLSRIRVYLRLRPSVKPSPAINIESETHRVLIDVEKSIGGGPPKAYVNQIVFNVDNIVQVYVHRGIIPRAIQQIFEEKEAKPEAGIVVHMSYMEIYQEGLYDLLQ
KRRDDLMIIEDNQLLNVRGLAKVRVETETEALKWFQEGEKSRSFGNHFLNSLSSRSHTILTFYMERRVARVSTQLALQVAKLNLVDLAGVERLKKTKGDTGSLMRKEA
CINNKTLSFLEQTIFALRLKKAHIPFRHSKVTTLLKESLGNNHKTVFMVCAWPEEYFLDETIGALRFAQRVKYLKIFQVTHKKPDCADTTRKQQLEIATLKQELALKDAL
NGRPGLGYDNLT
>Phypa_458410 (Pp_Kinesin9A)
MPSGEPLSSIGIKVHVRIRPTAKPSPMFHIEDQTNTVLIDLLKQNGGGAPESFVDQIAFSVNSITQSTDQSEVYESCGRALVKDFLEGYNATFIAYGQVGSGKTYTMAGDM
KVNAHRGFIARAIHQIFEEKEADPGSGIVLYISYLEIYQERSKDLMIIEESGYVYIRGLAKIPVETEAQALMCFSEGEKQRSYACHQINQVSSRSHTIFTLCMEKRVGRFKTE
YDTVVAKLNLVDLAGFERLKKTNTSTGGRMRVEACSINKSLCLLEQAVYAIKQGQEYVPFRQSKISIILKEALSGNCRTELILCLWPEEYFLDETVRRQNLKTHSRKYFVR
HAQEIANLQQELALKDALQGRPGLSFDDLTLENYSLYSRSLKYLCELK
>Phypa_428375 (Pp_Kinesin9B)
MQLFVSSTKFSAMGAGFDSTIDIYLRVRPISSGAKAVLELNQEEGRVTWTIPRHVSLGLANHQREHFTFKFTGLFDMESKQDEVFQKVAHKVVIGSLDGYNGTIFAYGQT
GSGKTYTITGGSERYVDRGIIPRTISLIFSEIAERSEYAYTLHFSYMEVYNETGYDLLNPDHETKALEDLPKDFILANEPIIANYQFANAFRVATNPDVHFLQNPAGLGRNR
WNSNEEEALNLVFVGDTNRIISSTPMNMASSRSHCIFTAHILACKVGEETVRKSKLHLVDLAGSERVWKTGVDGQVIVALQEKFQGKMRTHIPYRNSMMTSVLRDSIGG
NCLTVMIATVTIAQDQLPETISTCRFAQRVAMISNQVTLNEEVDPNLLIKRLKQQIGDLKGEIALLRGENENRPPLSASEIENVRSRVIGFLSDKGVDTDLHCGGSMMHIHA
AFQILKEMLKHGETQTIKSVQDDNGSQVSSESTVVKTLQVKVSDLQYQLQQRDNEIQILVAFIRKREAAARNTVRSASVQSLTDRPPSTSCRGSRTEEKGGEMKRSQLLA
EKNVTPRPSKSADFHQPKSTELDVDLGVAKLPVTSGREMDKDKAFELFSKGNDKMEAIEENKSLLIAMCANAKALGEKVNRARDRTYKEGFSELRDIKKEIEHLHKLLE
HNGAQLQADFEVYWSAMTAAPASTETSRKAPIIPRLQISSAIGPQHQANQSSSAEYKPLFPFSSNSLSARGSHCSPLSVSDPRSGSRGGNDLRALALASSKEHSKYVKSPSS
SGAGQVLNNLDNLLQPIRRQQLKESVNTAGLQKVPLTGNAAADADIIAFYKARSGVLKKVSS
>XP_001701617 (Cr_KLP1)
MVKQAVKVFVRTRPTATSGSGLKLGPDGQSVSVNVPKDLSAGPVNNQQEQFSFKFDGVLENVSQEAAYTTLAHEVVDSLMAGYNGTIFAYGQTGAGKTFTMSGGGTA
YAHRGLIPRAIHHVFREVDMRADKMYRVHVSYLEIYNEQLYDLLGDTPGTSDALAVLEDSNSNTYVRGLTLVPVRSEEEALAQFFLGEQGRTTAGHVLNAESSRSHTVF
TIHVEMRTSDAASERAVLSKLNLVDLAGSERTKKTGVTGQTLKEAQFINRSLSFLEQTVNALSRKDTYVPFRQTKLTAVLRDALGGNCKTVMVANIWAEPSHNEETLST
LRFASRVRTLLTTDLALNESNDPALLLRRYERQIKELKAELAMRDTLSGKGRVSYDDLTDDELRELHATCRRFLHGEAEPEDLPADSMKRVRETFKALRAVHVAIKADM
ATQMATLRRATEEGSGAAARGGDSAGPSGVGDVDLRATGGFTVGHAPLDARPPVRSELGSPGAGASGAEALGEPRSPGGGLHAQASSHTDAGSNWGDAGPLSSPGGT
RLAGIFGVSGDRNAVFRRYKVDVGEGRELAASLKAASIALADTKASIRSLGASVNDAKQRIDELSSALALRRGATPAGGDGEVLDSEAYALMQELKSAKSRYRTDFDSL
KSAREELEPQIQAVAVARAGLLEAFDRWAAAQSDTTLKRMATAGRAMSGIAPGEEDEMDAGEQFERMQIARISERDPDSLAFHTALKRTGAAVSRPATVATGGNAKA
AAMATRKMEHTQAVNRGLAR
>Cre01.g036800.t1.1 (Cr_Kinesin9B)
MAPGPEVAGIDIFVRVKPVPKPSPRLGIDNSENKVEFNIPRNEAAGLVNNQREHFEFRFNGILQADAKQDEVFERVARPVVMGAMEGYNGTIFAYGQTGSGKTFTITGGP
ERYVDRGIIPRSISAIFSEISKRHDYQYSVHISYLEIYNNEGYDLLDAEREIKALEDLQQVHVGEGEDGTVSYRNLSMYRANNEEEALNLLFLGDTNRTISETPMNQASSRS
HCIFTIHVEARKTGEDVVRRSKLNLVDLAGSERVSKTGVDGTTLREAKYINLSLHYLEQVIIALQEKSMGMNRPHIPYRNSMMTMALKDSLGGNCRTVMVATINSAQDQ
LDESISTCRFAQRVAMVRNTVLLNEELDPSLIIRRLKQELRDLKEEVKMLRGEEEERGPLTPDELVRLQGQVETYVADNSPEASLNLGGSMMMIRAVFEVFKRLLRTGGF
KMAGSGGAGGSGSGAGGGGGEPGTPGRAGGAGEGGGGGGDSAGLQDQVRKLKLQVAQRDNEIGILVSMLKRREGAGAKAGPVLSSPSTSINGPPVPGMGAAAAAGG
AGGGLGAGPSGVDGSGAGPGPGSAAAAGGGGGPADELAVLMNTNLLADRNKAFELFRKSYRQNEVIEENKQLLKTKYDSAKSLGAAVNDSKGRINELRSAIEQRRMQ
RGAAAVAAGMSPEQLTDDDPEESRCKELMEQEKARYRDAFNQLRELKKEIEHLHLLLEQSRTRLQRDFEQWMGLMLRQQQQQQQQAAAGLPPSGPSPAMSPMRPSAA
AQQPGMPRQAWGDAGAAPSPSASPAPGPGPSSSGGSLTRGPSPGGSAAAPPPLPGAGGQWATPVGLRASGGNSPALGSHGYGNGHPSGMHGLGPGPGPGPGSGAASPA
VTRTGSGVAVQQHAAAHSSGGPSMQGVDAAVLEMARPFLTGNPDADADIVKFYEAKAKLMQKLSAG

B

Make Database
/ncbi-blast-2.2.29+/bin/makeblastdb -in /Cr_Pp_Kinesin2_Kinesin9.fasta -dbtype prot –out /Cr_Pp_Kinesin2_9_data

Blastx
/ncbi-blast-2.2.29+/bin/blastx -query /REF3Trinity.fasta -db /Cr_Pp_Kinesin2_9_data -num_threads 2 -evalue 1e-100 -outfmt 6 >/ALL_KINESIN2_9.outfmt6

Query Accession	Subject	Percent ID	Alignment length	Mismatches	Gap Openings	Q. Start	Q. End	S. Start	S. End	e-value	Bit score	ID
KT986258	XP_001701617	34.49	603	347	8	335	2011	10	608	5.00E-101	332	CrKLP1
KT986258	Phypa_458410	41.21	381	193	6	323	1453	12	365	8.00E-109	285	PpKinesin-9A
KT986235	Phypa_425592	60.71	700	244	10	289	2313	41	734	0.00E+00	749	PpKinesin-2
KT986235	XP_001701510	45.07	670	317	11	289	2211	17	664	1.00E-156	494	FLA10
KT986235	XP_001697037	60.61	363	131	5	289	1368	14	367	6.00E-143	455	FLA8
KT986259	Phypa_428375	45.65	758	282	11	107	2182	13	706	0.00E+00	597	PpKinesin-9B

Figure 4-1. Identification of kinesin-2 and kinesin-9 sequences in *Marsilea*.
(A) Kinesin-2 and kinesin-9 protein sequences from *Physcomitrella patens* and *Chlamydomonas reinhardtii* that were used to search the *Marsilea* transcriptome. (B) Stand alone blast was used to search the transcriptome for kinesin-2 and kinesin-9 sequences. Results show that there is one kinesin-2 sequence (KT986235) and two kinesin-9 sequences (KT986258, KT986259) in the male gametophyte transcriptome of *Marsilea.*

Unlike other species, only one kinesin-2 has been identified in *Physcomitrella* (Shen et al, 2012). Similarly, only one was found in *Marsilea* (Figure 4-1). However, this single kinesin-2 in *Marsilea* shares many similarities with other eukaryotic kinesin-2s. The translated kinesin-2 sequence from *Marsilea* (MvKinesin-2) recognizes members of the kinesin-2 family from other ciliated eukaryotes. Reciprocal Blastp analysis shows that MvKinesin-2 recognizes CrFLA8 (XP_001697037) and CrFLA10 (XP_001701510) with an expectancy (e)-value of 0.0. Phylogenetic analyses of the motor domain of established kinesin-2s in plants and animals (Table 4-1) and multiple sequence alignment (Appendix I-3) demonstrate that the single kinesin-2 motor in *Marsilea* cannot be placed in the standard kinesin-2α (-2A), -2β (-2B), or -2γ (-2C) subgroups (Figure 4-2). This is similar to the findings for both FLA10 and FLA8 in *Chlamydomonas* and for the kinesin-2 from *Physcomitrella*. MvKinesin-2 is most similar to the kinesin-2 in *Physcomitrella.* This is not surprising since the only ciliated cells in *Marsilea* and *Physcomitrella* are the male gametes. It is unclear whether the single kinesin-2 in *Marsilea* functions as a homodimer, a heterotrimer with yet unidentified partners, or as a single protein.

The male gametophyte of *Marsilea* has two transcripts that encode kinesin-9-like proteins. Phylogenetic analysis of the motor domain of established kinesin-9s in plants and animals (Table 4-1) and multiple sequence alignment (Appendix I-4) demonstrate that the kinesin-9s in *Marsilea* can be separated into kinesin-9A and kinesin-9B subfamilies (Figure 4-3). This provides further evidence for the existence of two distinct kinesin-9 subfamilies. Reciprocal Blastp analysis shows that translated kinesin-9A (MvKinesin-9A) and kinesin-9B (MvKinesin-9B) sequences in *Marsilea*

recognize other members of the kinesin-9 family. MvKinesin-9A is similar to CrKLP1 (XP_001701617) and TbKIF9A (XP_846252) with an e-value of 4e-112 and 8e-50, respectively. MvKinesin9B recognizes CrKIF9B (Cre01.g036800.t1.1) and TbKIF9B (XP_846346) with an e-value of 0.0 and 6e-89, respectively.

MvKinesin-2, MvKinesin-9A, and MvKinesin-9B have a typical structure found in the conserved kinesin motor and ATP-binding domain architectures including the P-loop, switch I, and switch II motifs. Non-motor regions of MvKinesin-2, MvKinesin-9A, and MvKinesin-9B are not as highly conserved and possibly point to differences kinesin function and cargo binding (Figure 4-4).

RT-PCR using poly(A+)-RNA isolated at 1-2h, 3-5h, and 6-8h of development shows that MvKinesin-2, MvKinesin-9A, and MvKinesin-9B transcripts increase in abundance from the 1-2h to the 6-8h time interval (Figure 4-5). This replicates our findings using RNAseq counts and edgeR analysis (see Chapter 2; Tomei and Wolniak, 2016). This pattern of transcript availability and abundance suggests that kinesin-2 and kinesin-9 transcripts are unmasked, spliced, and polyadenylated prior to the time interval associated with ciliogenesis during gametophyte development (Wolniak et al., 2015). It is unclear why kinesin-9B transcripts decrease during 3-5h time interval. We have seen in our transcriptome analysis that the middle time point of development (3-5 hours post hydration) appears to be a transition time between the early stage of development, marked by cell division, and the later portion of development, which is dedicated to spermatid differentiation and ciliogenesis. GO terms that are enriched during this transition time include the proteasome and ubiquitin ligase components. It is possible that kinesin-9B

transcripts are destroyed during this time period of development and then remade later during ciliogenesis.

Table 4-1. Kinesin-2 and kinesin-9 sequences used for phylogenetic analysis.

ID	Subfamily	Accession	Species
Mv_Kinesin-2	2	KT986235	*Marsilea vestita*
Am_Kinesin-2A	2A	XP_396164	*Apis mellifera*
Am_Kinesin-2B	2B	XP_393174	*Apis mellifera*
Am_Kinesin-2C	2C	XP_395281	*Apis mellifera*
Ce_KLP-11	2B	NP_001023139	*Caenorhabdatis elegans*
Ce_KLP-20	2A	NP_497178	*Caenorhabdatis elegans*
Ce_OSM-3	2C	NP_001023308	*Caenorhabdatis elegans*
Cr_FLA10	2	XP_001701510	*Chlamydomonas reinhardtii*
Cr_FLA8	2	XP_001697037	*Chlamydomonas reinhardtii*
Dm_KIF3C	2C	NP_651939.4	*Drosophila melanogaster*
Dr_KLP64D	2A	NP_523934	*Drosophila melanogaster*
Dr_KLP68D	2B	NP_524029	*Drosophila melanogaster*
Gg_KIF3B	2B	NP_001012852	*Gallus gallus*
Gl_Kinesin-2D	2	XP_001708236	*Giardia lamblia*
Gl_Kinesin-2D	2	XP_001706504	*Giardia lamblia*
Hs_KIF17	2C	NP_001116291	*Homo sapiens*
Hs_KIF3A	2A	NP_008985	*Homo sapiens*
Hs_KIF3B	2B	NP_004789	*Homo sapiens*
Hs_KIF3C	2B	NP_002245	*Homo sapiens*
Lm_Kinesin-2D	2D	XP_001682337	*Leishmania major*
Lm_Kinesin-2D	2D	XP_001685383	*Leishmania major*
Pt_Kinesin-2	2	XP_001455773	*Phaeodactylum tricornutum*
Pt_Kinesin-2	2	XP_001426973	*Phaeodactylum tricornutum*
Pt_Kinesin-2	2	XP_001427404	*Phaeodactylum tricornutum*
Pt_Kinesin-2	2	XP_00142818	*Phaeodactylum tricornutum*
Pt_Kinesin-2	2	XP_001428184	*Phaeodactylum tricornutum*
Pt_Kinesin-2	2	XP_001429325	*Phaeodactylum tricornutum*
Pt_Kinesin-2	2	XP_001429366	*Phaeodactylum tricornutum*
Pp_Kinesin2	2	Phypa_425592	*Physcomitrella patens*
Sp_KRP85	2A	NP_999777	*Strongylocentrotus purpuratus*

Sp_KRP95	2B	NP_999817	*Strongylocentrotus purpuratus*
Tb_Kinesin-2D	2	Tb11.01.5490	*Trypanosoma brucei*
Tb_Kinesin-2D	2	Tb927.5.2090	*Trypanosoma brucei*
Tt_Kinesin-2	2	XP_001014287	*Tetrahymena thermophila*
Tv_Kinesin-2	2	XP_001276971	*Trichomonas vaginalis*
Tv_Kinesin-2	2	XP_001300992	*Trichomonas vaginalis*
Tv_Kinesin-2	2	XP_001315568	*Trichomonas vaginalis*
Tv_Kinesin-2	2	XP_001319907	*Trichomonas vaginalis*
Tv_Kinesin-2	2	XP_001579747	*Trichomonas vaginalis*
Mv_Kinesin-9A	9A	KT986258	*Marsilea vestita*
Mv_Kinesin-9B	9B	KT986259	*Marsilea vestita*
Am_Kinesin-9B	9B	XP_006561916	*Apis mellifera*
Cr_KLP1	9A	XP_001701617	*Chlamydomonas reinhardtii*
Cr_Kinesin-9B	9B	Cre01.g036800.t1.1	*Chlamydomonas reinhardtii*
Gl_Kinesin-9A	9A	XP_001705615	*Giardia lamblia*
Gl_Kinesin-9B	9B	XP_001707755	*Giardia lamblia*
Hs_KIF9	9A	NP_071737	*Homo sapiens*
Hs_KIF6	9B	NP_001275949	*Homo sapiens*
Lm_Kinesin-9B	9B	XP_001687540	*Leishmania major*
Pt_Kinesin-9A	9A	XP_001455832	*Phaeodactylum tricornutum*
Pt_Kinesin-9B	9B	XP_001445877	*Phaeodactylum tricornutum*
Pt_Kinesin-9B	9B	XP_001430724	*Phaeodactylum tricornutum*
Pp_Kinesin-9A	9A	Phypa_425498	*Physcomitrella patens*
Pp_Kinesin-9A	9A	Phypa_458410	*Physcomitrella patens*
Pp_Kinesin-9B	9B	Phypa_428375	*Physcomitrella patens*
Tb_Kinesin-9A	9A	Tb927.7.6290	*Trypanosoma brucei*
Tb_Kinesin-9B	9B	Tb927.7.726	*Trypanosoma brucei*
Tt_Kinesin-9B	9B	XP_001025897	*Tetrahymena thermophila*
Tt_Kinesin-9B	9B	XP_00102480	*Tetrahymena thermophila*
Tv_Kinesin-9B	9B	XP_001325581	*Trichomonas vaginalis*

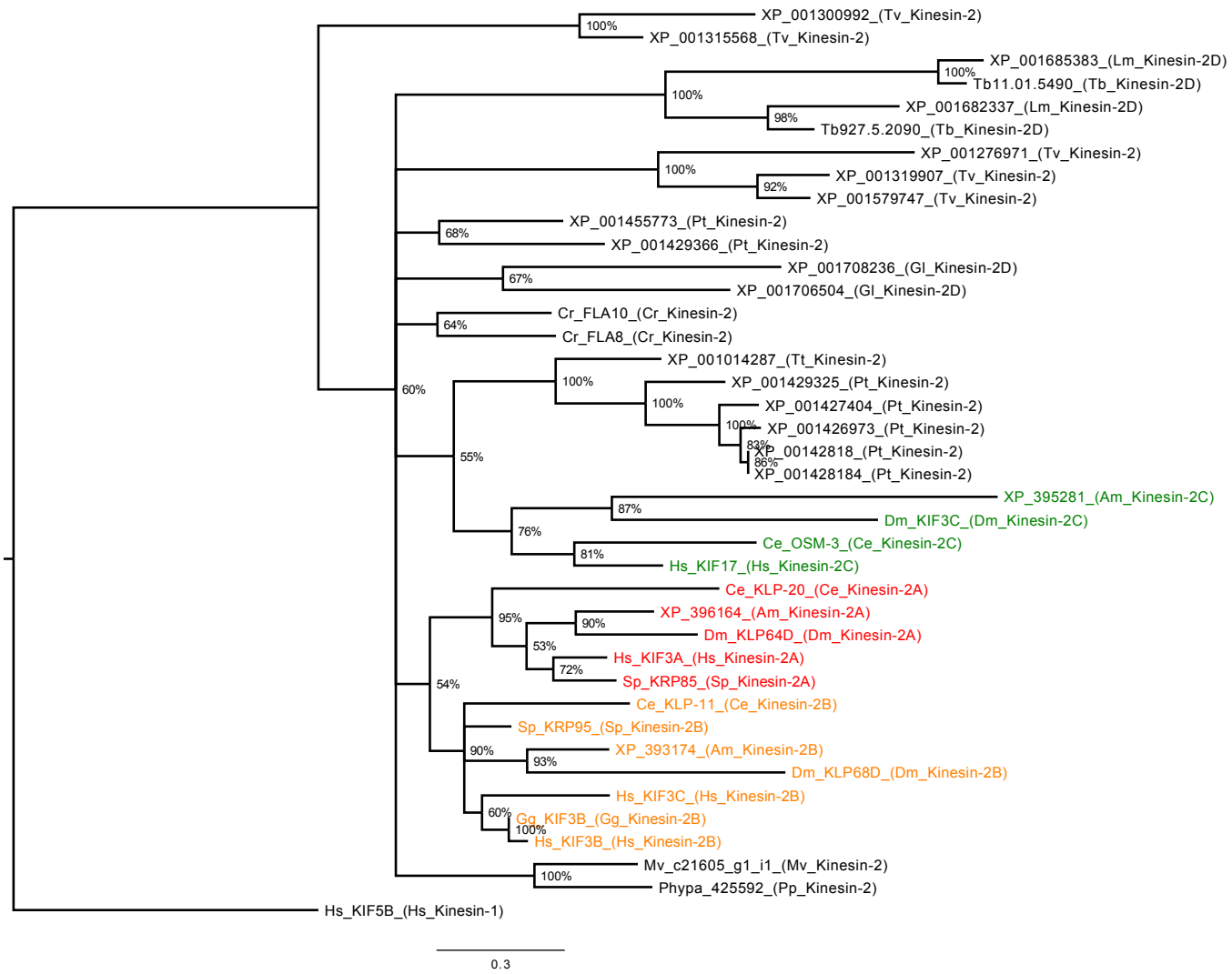


Figure 4-2. Characterization of kinesin-2 in *Marsilea*. A maximum likelihood (ML) phylogenetic tree constructed using kinesin-2 motor domains. Kinesin-1 was used as an out-group for analysis. Kinesin-2 motors can be separated into three subfamilies, kinesin-2A (red), -2B (orange), and -2C (green). There are also many kinesin-2 sequences that do not correspond to any well-supported subfamily (black). The kinesin-2 motor in *Marsilea* is most similar to the kinesin-2 in *Physcomitrella* and does not fall into a kinesin-2 subfamily.

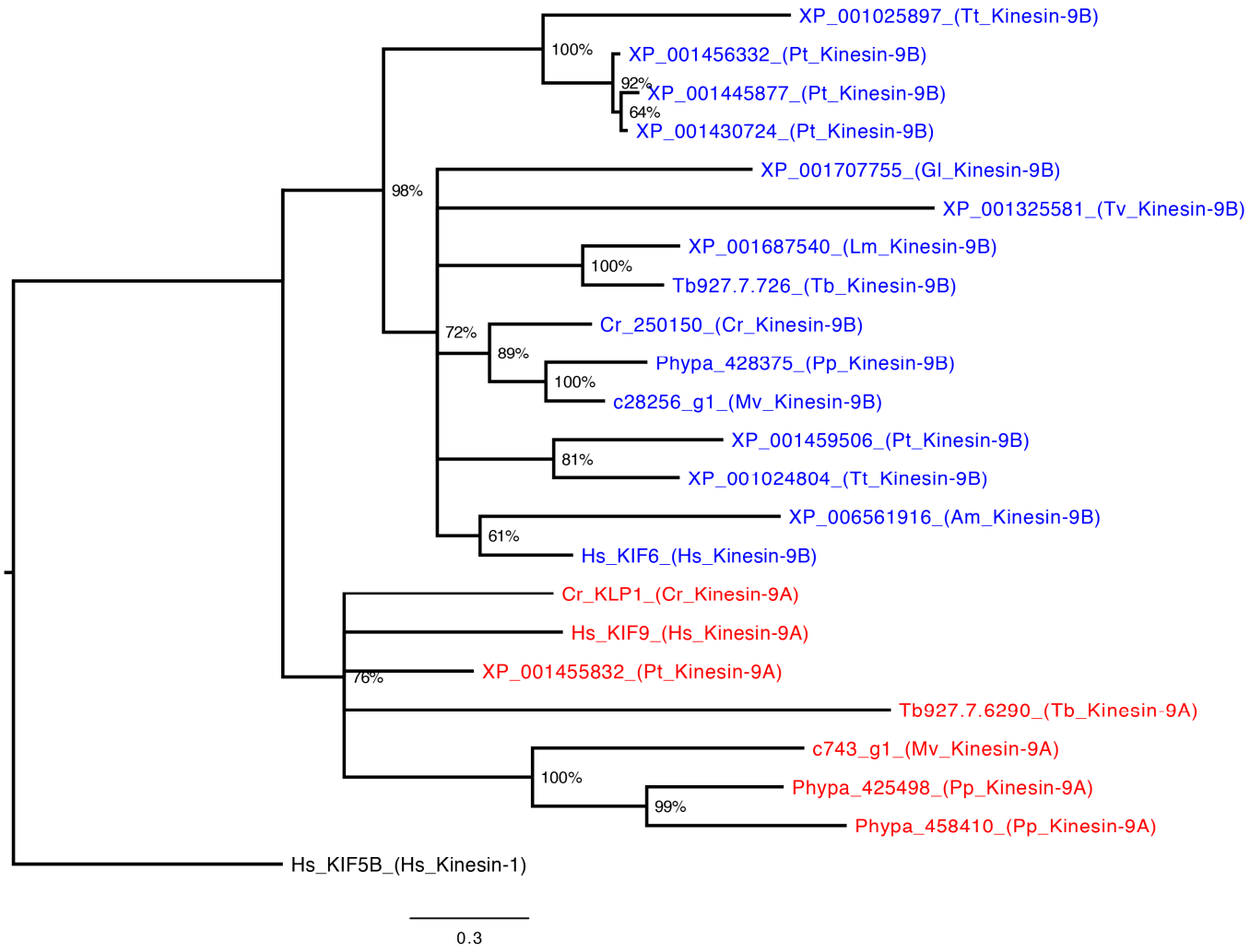


Figure 4-3. Characterization of the kinesin-9 family in *Marsilea*.
A ML phylogenetic tree constructed using kinesin-9 motor domain sequences. Kinesin-1 was used as an out-group for analysis. Kinesin-9 can be separated into two well-supported subfamilies, kinesin-9A (red) and kinesin-9B (blue). *Marsilea* has one kinesin-9A and one kinesin-9B.

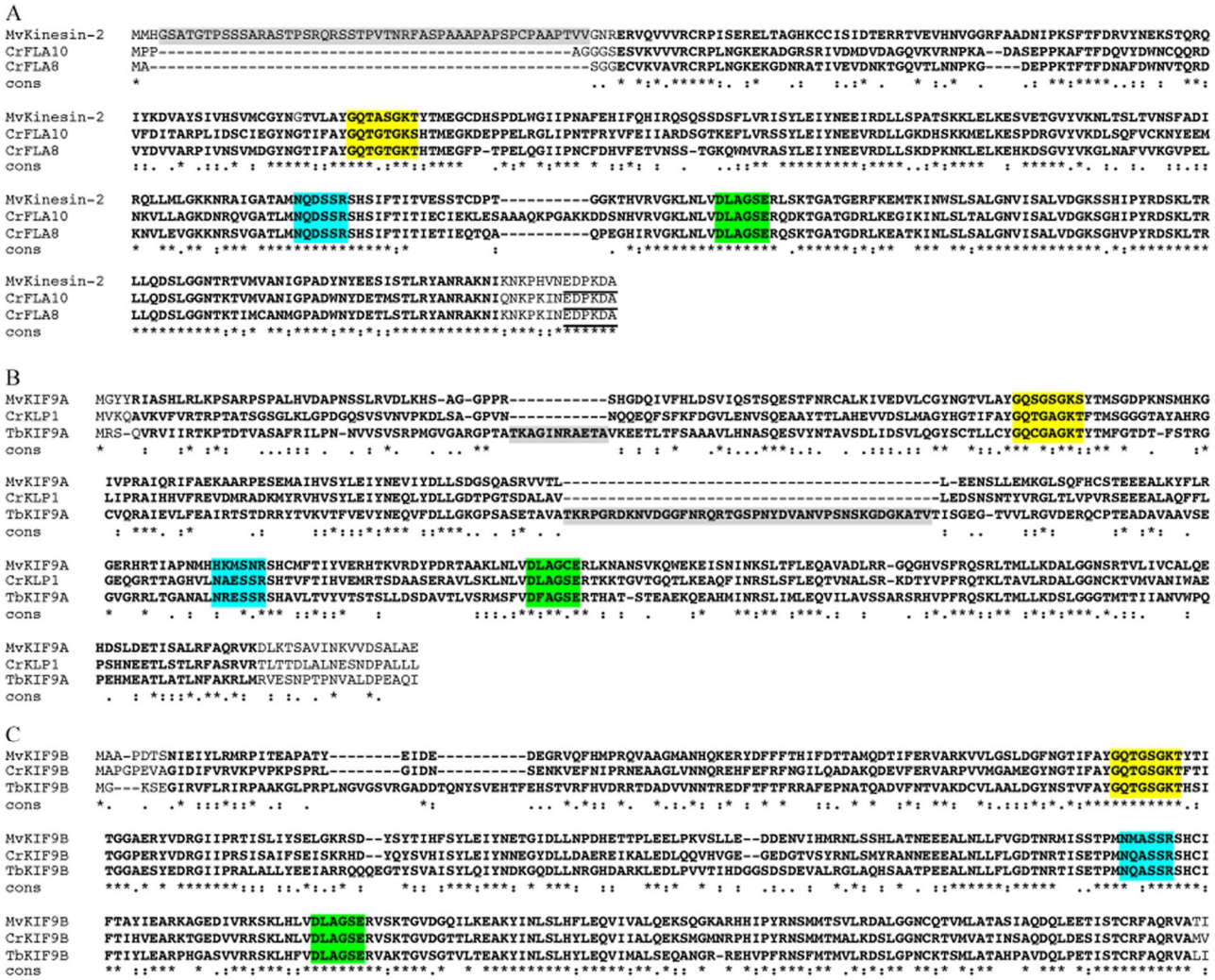


Figure 4-4. Domain architecture of *Marsilea* kinesin-2, kinesin-9A, and kinesin-9B. Multiple sequence alignments of (A) kinesin-2, (B) kinesin-9A, and (C) kinesin-9B motor domains (bold) highlighting important regions for motor domain function. P-loop (yellow), switch I (blue), and switch II (green) are represented in each motor. Alignments generated using T-coffee (Notredame et al., 2000; Di Tommasso et al., 2011).

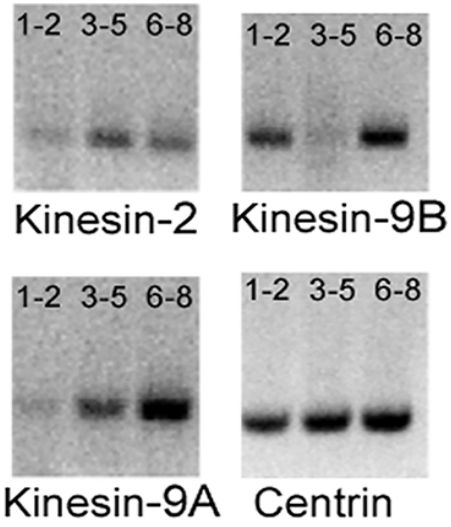


Figure 4-5. Kinesin-2 and kinesin-9 transcripts increase in abundance during gametophyte development in *Marsilea*. An increase in MvKinesin-2, -9A, and -9B mRNA abundance was detected using RT-PCR against poly(A+)-RNA isolated at 1-2, 3-5, and 6-8 hours of gametophyte development. The abundance of centrin mRNA does not change during development and was used as a control.

Kinesin-2 and kinesin-9 are involved in spermatogenesis

To determine the function of these kinesins during spermatogenesis, I performed RNAi knockdown experiments, treating gametophytes at the time of spore hydration with dsRNA. I transcribed and constructed dsRNA from unique regions of each kinesin (Figure 4-6A, B). It was important to use unique regions to make dsRNA in order to prevent broad silencing of off-target sequences and to ensure that only one kinesin was being silenced at a time. The effectiveness of RNAi was measured using RT-PCR on each transcript after knockdown. In each case, the presence of the transcript could not be detected after knockdown at 8 hours of development (Figure 4-6C, D, E). Microspores were grown for 8 hours, fixed, embedded in methacrylate, and sectioned. The sections were stained with TBO and examined with bright field microscopy to observe broad morphological changes in development.

At 8 hours of development, all cell divisions are complete and spermatids can be clearly distinguished from jacket cells by their size, location within the microspore, shape, and staining pattern. Jacket cells are located towards the periphery of the microspore near the microspore wall and contain starch filled plastids. Jacket cells do not contain as much mRNA or protein as the spermatogenous cells, and they exhibit weak staining with TBO. During this stage of development, each spermatid begins to differentiate into a motile spermatid by forming an elongated, coiled nucleus with its associated (forming) microtubule ribbon, creating a slightly boomerang shaped cell when observed in these sections (Figure 4-6F; Myles and Hepler, 1977).

Spermatogenous cells can be distinguished from jacket cells in MvKinesin-2 knockdowns; however, in the majority of gametophytes (79/107; 73.8%) spermatogenous cells are larger than in untreated gametophytes and they do not have a consistent size and shape compared to controls. This suggests that one or more cell division cycles have been skipped or failed to reach completion (Figure 4-6G; Klink and Wolniak, 2001). In a minority (28/107; 26.2%) of these knockdowns, spermatogenous cells are the correct size and shape and appear morphologically normal (Figure 4-6H).

In MvKinesin-9A knockdowns, spermatogenous cells are the same size and shape as controls suggesting normal progression through all of the cell division cycles (Figure 4-6I). However, a distinct phenocopy was observed with knockdowns of kinesin-9B. In these gametophytes, spermatogenous cells appear larger than controls and the cells are rounded suggesting that nuclear elongation has failed. Thus, while cell divisions have proceeded normally, differentiation is aberrant (Figure 4-6J).

Spermatid differentiation is incomplete without MvKinesin-2, MvKinesin-9, and MvKinesin-9B

In order to assess altered patterns of spermatid maturation and differentiation, I fixed and sectioned gametophytes after 8 hours of development. These gametophytes had undergone silencing of MvKinesin-2, MvKinesin-9A, or MvKinesin-9B, through the addition of dsRNAs at the time of spore rehydration. After fixation, embedding and sectioning, the gametophytes were stained with DAPI, to observe nuclear elongation, and with anti-centrin antibodies, to observe the

presence and distribution of basal bodies in the spermatids (Deeb et al., 2010). During normal spermatid differentiation, the basal bodies become situated at regular intervals along the microtubule ribbon and the nuclear coil. These prepositioned basal bodies later serve as templates for the growth of ciliary axonemes (Figure 4-7A). By observing the distribution centrin protein in kinesin knockdowns, I was able to make conclusions about the formation and localization of basal bodies in these cells.

In each knockdown, the appearance and placement of basal bodies is altered. At 8 hours of development, anti-centrin staining in the majority (85/114; 74.6%) of MvKinesin-2 knockdowns is diffuse and there are few, if any, aggregates of centrin that resemble typical basal body staining in spermatogenous cells. Some larger centrin aggregates that possibly resemble the blepharoplast can be found in spermatogenous cells (Figure 4-7B). DAPI staining in these cells is also distinctly different from controls and it is apparent that the nuclear coil is not properly formed at this stage (Figure 4-7B). Just as a small percentage of microspores treated with dsRNA derived from MvKinesin-2 appeared to proceed through development normally when observed with TBO, some gametophytes (29/114; 25.4%) exhibit normal centrin and DAPI staining (Figure 4-7C). These gametophytes were either unaffected by RNAi or it is possible that there are two separate phenocopies that result from the knockdown of MvKinesin-2 during spermatogenesis in *Marsilea*.

In MvKinesin-9A knockdowns, I observed aggregates of centrin staining that resemble basal bodies. The presumptive basal bodies become localized in areas of the spermatids where they normally are not observed. DAPI staining in these cells shows a normal, elongated nucleus (Figure 4-7D). Therefore, the elongation stage of

spermatid differentiation apparently occurs, but there are anomalies in the localization of basal bodies in these cells. MvKinesin-9B knockdowns show large aggregates of centrin protein (Figure 4-7E) that resemble blepharoplast particles. The blepharoplast is a cytoplasmic particle that forms during the last spermatogenous cell division (Sharp, 1914; Hepler, 1976). During the last mitotic division, the blepharoplast functions like a centrosome at the spindle pole, though it lacks any organized centrioles. As the spermatids are formed, the blepharoplast disappears and then reassembles to serve as a site for *de novo* basal body assembly (Hepler, 1976). In this knockdown, no further maturation of the blepharoplast (for basal body formation) was observed. Spermatogenous cells in MvKinesin-9B knockdowns exhibit nuclei that are round in shape (Figure 4-7E), while normal spermatids undergo nuclear elongation and coiling (Myles and Hepler, 1977). This pattern of centrin immunolabeling and DAPI nuclear staining is characteristic of arrest at a stage of development preceding basal body formation in spermatids, and suggests that either spermatid maturation has stopped early in gamete differentiation or that the differentiation process has been slowed substantially.

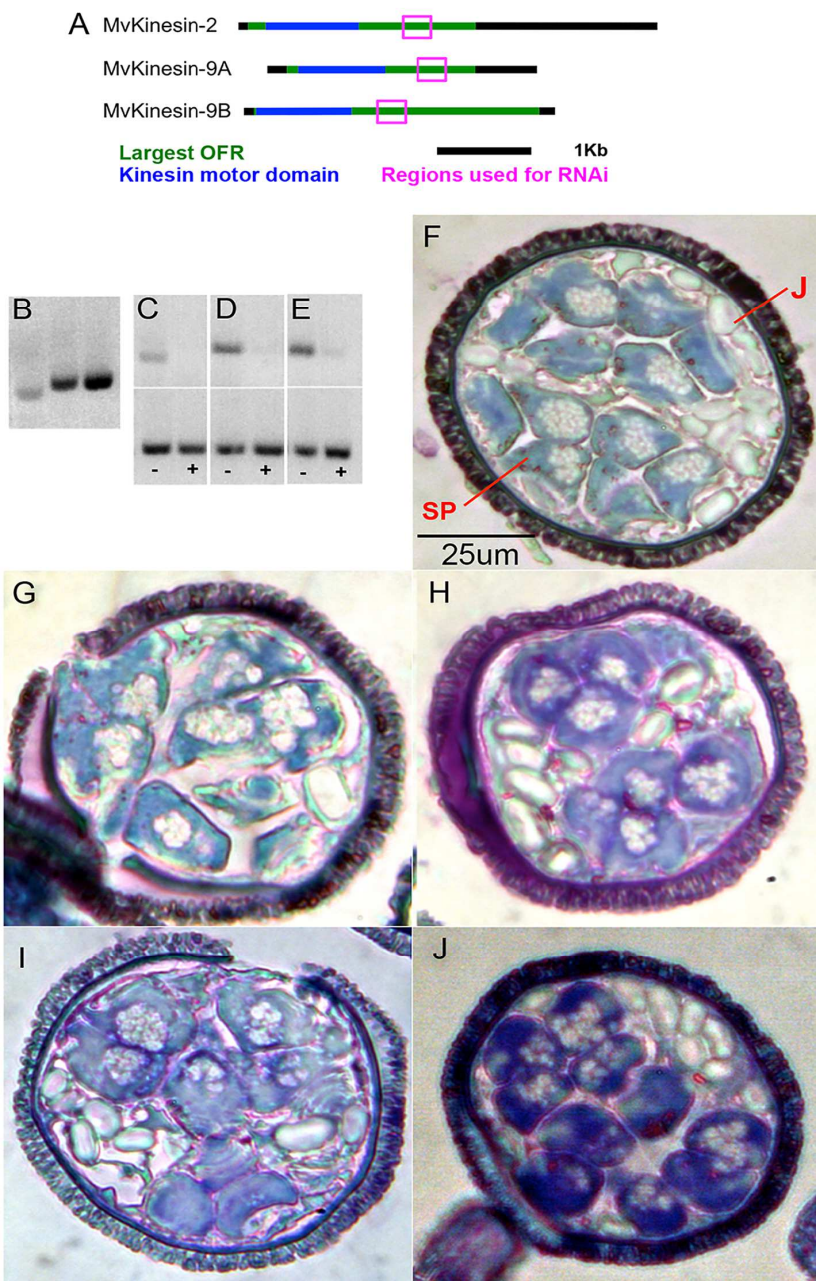


Figure 4-6. Kinesin-2 and kinesin-9 are involved in spermatogenesis.
(A) Unique 350-400nt regions used for constructing dsRNA. (B) dsRNA constructed from MvKinesin-2, -9A, and -9B, respectively, using poly(A+)-RNA isolated at 8h as a template. RT- PCR for (C) MvKinesin-2, (D) MvKinesin-9A, and (E) MvKinesin-9B before (-) and after (+) the addition of dsRNA for knockdown. The presence of each transcript cannot be detected after its knockdown. Centrin mRNA (bottom panel) does not change after the addition of various kinesin dsRNAs. (F) Untreated, control microspores developed for 8h, embedded in methacrylate, sectioned, and stained with TBO. Spermatogenous (SP) and jacket (J) cells can easily be distinguished from each other. Sectioned microspores treated with (G-H) MvKinesin-2, (I) MvKinesin-9A, and (J) MvKinesin-9B dsRNA and stained with TBO.

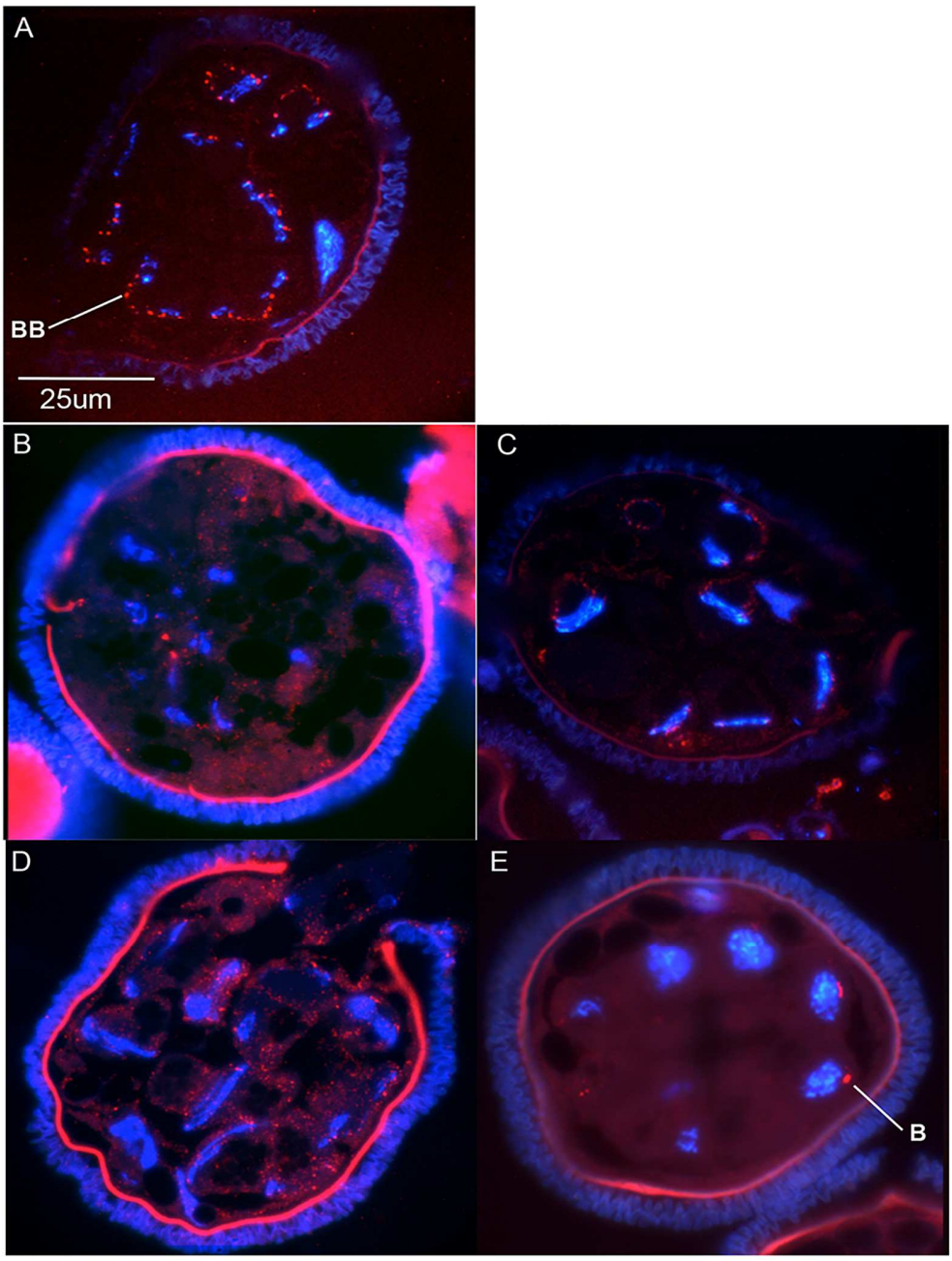


Figure 4-7. Spermatid differentiation is incomplete after silencing.
(A) Untreated sectioned microspores developed for 8h and labeled with anti-centrin Ab (red) and DAPI (blue). Sectioned 8h microspores treated with (B-E) MvKinesin-2, MvKinesin-9A, and MvKinesin-9B dsRNA. (B) Centrin staining is diffuse and there are very few centrin aggregates that resemble basal bodies. The nuclear coil is not properly formed. (C) A small percentage of MvKinesin-2 knockdowns have a normal pattern of anti-centrin and nuclear staining. (D) Centrin staining is found throughout each spermatid and aggregates that resemble basal bodies are not localized at regular intervals adjacent to the nuclear coil. Most cells have a nuclear coil that appears similar to controls. (E) The blepharoplast (B), a centrosome like particle, is visible. Nuclei in these spermatids have failed to coil and differentiation has not occurred.

Silencing of MvKinesin-2, MvKinesin-9A, and MvKinesin-9B cause defects in ciliogenesis and motility

To determine whether the abnormalities observed with these kinesin knockdowns during spermatogenesis had any impact on ciliogenesis or on motility in released gametes, I observed the kinesin knockdowns after 11 hours of development. At 11 hours, the normal process of spermatogenesis reaches completion, and 32 motile spermatozoids, each with ~140 cilia, break free from their enclosing microspore walls. First, the opaque outer exine wall of the microspore begins to breakdown revealing a much thinner and translucent intine wall (Figure 4-8A1). Then, two clusters of 16 spermatozoids emerge from the microspore (Figure 4-8A2). The gametes are each contained within a thin wall-like structure built from an extension of the cytoplasm that originally surrounded each spermatid. Each motile spermatozoid rapidly spins in place, presumably in order to break free from its surrounding wall (Appendix I-5). The shape of each spermatozoid resembles a corkscrew with cilia extending vertically from the edges of the microtubule and nuclear coil (Figure 4-8A3). Once released, the spermatozoids swim in a shallow helical path, rotating as they swim. Borrowing terms from aerodynamics, I called this rotational behavior 'rolling' (Figure 4-8B, Appendix I-6). This pattern is repeated until the spermatozoid dies or reaches the egg cell in the female gametophyte (produced by the megaspore) for fertilization.

The majority (20/27; 74.1%) of microspores analyzed that were treated with MvKinesin-2 dsRNA emerged much differently than controls. Although the exine wall thinned as usual (Figure 4-8C1), the spermatids exited from the microspore as

individuals, rather than as groups of 16 cells (Figure 4-8C2, Appendix I-7). The spermatozoids that emerged this way were much larger than controls and often contained two or more coiled microtubule ribbons with attached cilia. It is reasonable to suspect that these spermatozoids contain more than one nucleus, as the coil typically comprises the microtubule ribbon, the elongated nucleus, and associated mitochondria. Also, these cells lacked the characteristic extension of cytoplasm that unusually surrounds each spermatid (Figure 4-8C3). Due to their large size and multiple coils (Figure 4-8C3), these cells were unable to swim and could only quiver in place or yaw along their y-axes (Figure 4-8B, Appendix I-8). I call this phenocopy '*Monster*' because each cell has multiple coils and cilia. It is likely that these cells failed to complete cytokinesis during the last spermatogenous cell division, but nevertheless, they were able to form separate coils for each of their elongated nuclei, complete with microtubule ribbons and cilia.

In a minority (7/27; 25.9%) of MvKinesin-2 knockdowns, the spermatids emerged from the microspore as normal; however, these spermatozoids exhibited abnormally long ciliary axonemes (Figure 4-8D3). The spermatozoids with long cilia were unable to swim in a shallow helical path. These cells could only roll and essentially, swim in place (Figure 4-8B, Appendix I-9). I refer to this phenocopy as '*Rapunzel*' for its long cilia. *Monster* and *Rapunzel* represent two distinct phenocopies caused by MvKinesin-2 knockdowns. It is possible that MvKinesin-2 participates in two distinct processes during spermatogenesis; the first occurs during cytokinesis leading to spermatid formation, and the second occurs during ciliary axonemal formation and affects ciliary length control. Another explanation is that the

Monster and *Rapunzel* phenocopies are two manifestations of the same biological event, where the *Monster* phenocopy develops earlier during spermatogenesis and *Rapunzel* later during ciliogenesis. Because *Monster* spermatids were released from the microspores as a group of cells and *Rapunzel* spermatids as individuals, I suspect that each of these distinct phenocopies occurs in different microspores and that all the spermatozoids emerging from one microspore display the same phenotype.

The thinning of the exine wall and the emergence of the spermatids from the microspore is normal in kinesin-9A knockdowns (Figure 4-8E1, E2). Once the spermatozoids emerge (Figure 4-8E3), however, their swimming behavior is affected by the knockdown (Appendix I-10). These cells roll, yaw, and pitch in place (Figure 4-8B), effectively spinning without vectorial movement. Directional swimming for these cells occurs infrequently; they travel only short linear distances before switching back to the spinning behavior (Appendix I-10). Based on the movies, it is difficult to determine why the kinesin-9A knockdowns are unable to swim properly.

I have named our kinesin-9B knockdowns '*Late Bloomer*' because the cells emerge slowly after the seemingly normal thinning of the exine (Figure 4-8F1, F2). The cells finally emerge ~16 hours after microspore hydration, in contrast to 11 hours for controls, and for the knockdowns of MvKinesin-2, and MvKinesin-9A. The *Late Bloomer* spermatozoids that emerge are morphologically normal and have the same shape as control gametes (Figure 4-8F3). These cells also exhibit a helical swimming behavior similar to that of normal cells (Appendix I-11).

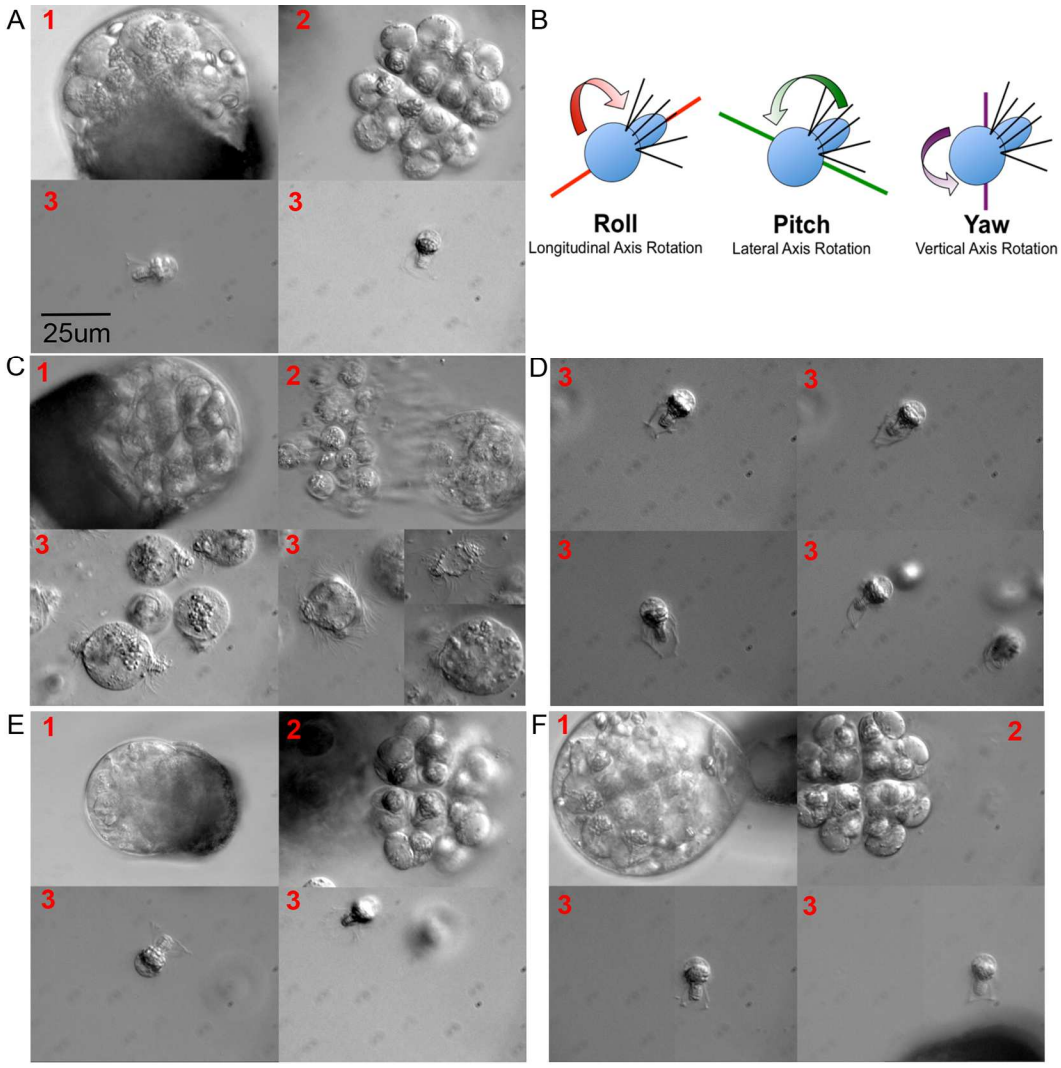


Figure 4-8. MvKinesin-2, MvKinesin-9A, and MvKinesin-9B have distinct roles in ciliogenesis and motility. (A) Untreated microspores during the final stages of spermatogenesis, visualized at 11h. 1-Shedding of exine and thinning of intine around the microspore. 2-Release of 32 spermatogenous cells from each microspore in groups of 16 cells. 3-Motile spermatozoids emerge. (B) Cartoon representing spermatozoid swimming patterns. (C) MvKinesin-2 knockdowns visualized at 11h. Thinning of the exine wall is normal, but cells emerge from the microspore as individuals. Spermatozoids have multiple sets of coils with attached cilia and are called Monster. (D) In a minority of MvKinesin-2 knockdowns spermatozoids appear to have longer cilia compared to controls and are called Rapunzel. MvKinesin-2 knockdowns quiver or roll (Monster) and swim in place (Rapunzel). (E) MvKinesin-9A knockdowns visualized at 11h. Spermatozoids emerge and appear normal. MvKinesin-9A knockdowns pitch and yaw in place. (F) The microspore cell wall thins and spermatozoids emerge normally at 16h of development in MvKinesin-9B knockdowns. Development and emergence is slowed relative to controls, and this phenocopy is termed Late Bloomer. Controls/MvKinesin-9B knockdowns roll in place and rapidly swim directionally.

MvKinesin-9A knockdowns have irregularly positioned cilia

To determine why kinesin-9A knockdowns were unable to swim normally when compared to controls, I used a variety of methods to observe the arrangement of cilia on the fully developed spermatozoids. Firstly, I fixed fully emerged spermatozoids quickly in 2% PFA on the surface of a microscope slide. I then observed these fixed cells with DIC microscopy. In controls, the corkscrew shape of the spermatozoid cell body, the nuclear coil, and the ciliary axonemes can clearly be seen (Figure 4-9A). Normal spermatozoids have the cilia placed in two regular rows along the dorsal flank of the anterior, coiled cell body (Myles and Hepler, 1977; 1982). Kinesin-9A knockdowns show cilia that are irregularly positioned on the spermatozoid cell body and are not restricted to the anterior portion of the cell (Figure 4-9B). I named this phenocopy '*Porcupine*' for the cilia that emerge from all points of the cell. None of the other kinesin silencing treatments exhibited irregularly placed cilia similar to *Porcupine* in spite of the fact that MvKinesin-2 knockdowns (*Monster*) generally have cell bodies that are misshapen and larger than normal (Figure 4-9C) and Kinesin-9B knockdowns (*Late Bloomer*) appear to be structurally normal even though they emerge at 16 hours instead of 11 hours after spore hydration (Figure 4-9D).

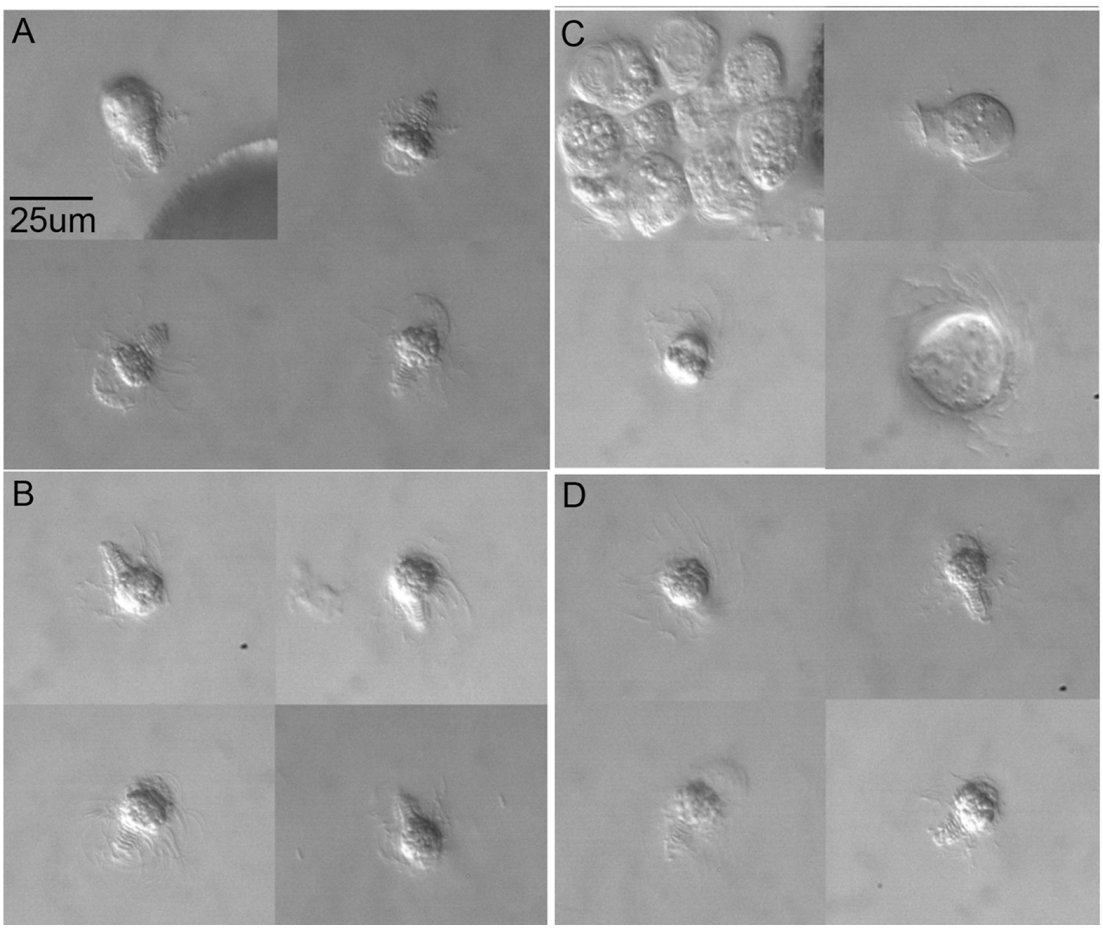


Figure 4-9. MvKinesin-9A knockdowns have irregularly positioned cilia. (A-D) After spermatozoids emerged from the microspore, they were fixed in 2% PFA on a coverslip and visualized with DIC microscopy. (A) Untreated cells have a characteristic corkscrew shape and cilia can been seen emerging from the microtubule ribbon and organelle coil. Developing microspores treated with (B) MvKinesin-9A, (C) MvKinesin-2, and (D) MvKinesin-9B dsRNA. (B) Cilia that appear to emerge from all parts of the spermatozoid cell body, not just the coil. (C) Spermatozoids are larger than controls and do not have the characteristic corkscrew shape. (D) Spermatozoids appear normal and look similar to controls.

Cilia are irregularly positioned in MvKinesin-9A knockdowns due to improper positioning of basal bodies

I hypothesized that the improper positioning or orientation of basal bodies along the microtubule ribbon caused the abnormal distribution of cilia in the Porcupine phenocopy. In order to test this hypothesis I sectioned fixed microspores at

10.5 hours after spore hydration and stained the sections with TBO, DAPI, and anti-centrin antibodies. Sections were visualized with phase contrast and fluorescent microscopy. At 10.5 hours of development, the normal spermatozoids are fully developed, but have yet to emerge entirely from the microspore wall. Using TBO staining at this time point, the organelle coil can easily be observed in each nearly mature spermatozoid. Cilia emerging along this coil are also visible. The cilia are wrapped around the cell body coil and are seen in transverse-section in some of the gametes as a white area adjacent to the organelle coil. I refer to this area as the ciliary band (Figure 4-10A). Anti-centrin staining in control sections form aggregates that resemble basal bodies. In control spermatids, the basal bodies are restricted to areas on the dorsal side of the coiled nucleus (Figure 4-10B). Overlaying the fluorescence images with phase contrast images of the same section, it appears that the centrin aggregates associate uniformly along the edges of the organelle coil and are often within areas of the ciliary band (Figure 4-10C).

In comparison with untreated gametophytes, in MvKinesin-9A knockdowns stained with TBO, it is difficult to visualize the ciliary band. In cells that possess a visible ciliary band, the band is irregularly shaped compared to controls (Figure 4-10D). This suggests anomalies in the orientation and localization of the basal bodies in the spermatid. Centrin staining confirms this, showing aggregates that resemble basal bodies present throughout the cytoplasm of the nearly mature spermatozoids. Aggregates of centrin are present at the dorsal face of the spermatozoid along the edges of the organelle coil as in controls, but they are also abundant along the posterior portion of many spermatozoids (Figure 4-10E, F). The failure for these cells

to position the axonemes correctly during differentiation is most likely the reason for the *Porcupine* phenocopy and the observed odd swimming patterns.

In contrast to this phenocopy, MvKinesin-2 knockdowns visualized with TBO have large, irregularly shaped cells. I suspect this is the consequence of arrested, aberrant or blocked cell division cycles. Coils are visible only in some of the maturing spermatids (Figure 4-10G). DAPI staining is reminiscent of the nuclear coil, however the coils are at odd angles to each other. This makes it difficult to determine if the large, irregular shaped cells contain more than one nuclear coil (Figure 4-10H, I). Anti-centrin antibody staining in these knockdowns mostly appears as a diffuse cloud of fluorescence in spermatogenous cells. Aggregates that resemble basal bodies are present in some cells but they do not appear localized in a regular pattern that resembles basal body distributions in normal cells (Figure 4-10H, I). This pattern is reminiscent of the *Monster* phenocopy where the released gametes are larger than normal and possess multiple coils.

In kinesin-9B knockdowns, the spermatogenous cells resemble control cells though the coil and cilia are not as apparent (Figure 4-10J). Anti-centrin antibody staining forms aggregates that resemble basal bodies that are positioned at regular intervals along the anterior portion of the nuclear coil (Figure 4-10K). These cells do not look like they have developed to the same stage as the controls, though they have they clearly proceeded into the later stages of differentiation. This provides further evidence that knockdown of kinesin-9B causes a general slowing of differentiation.

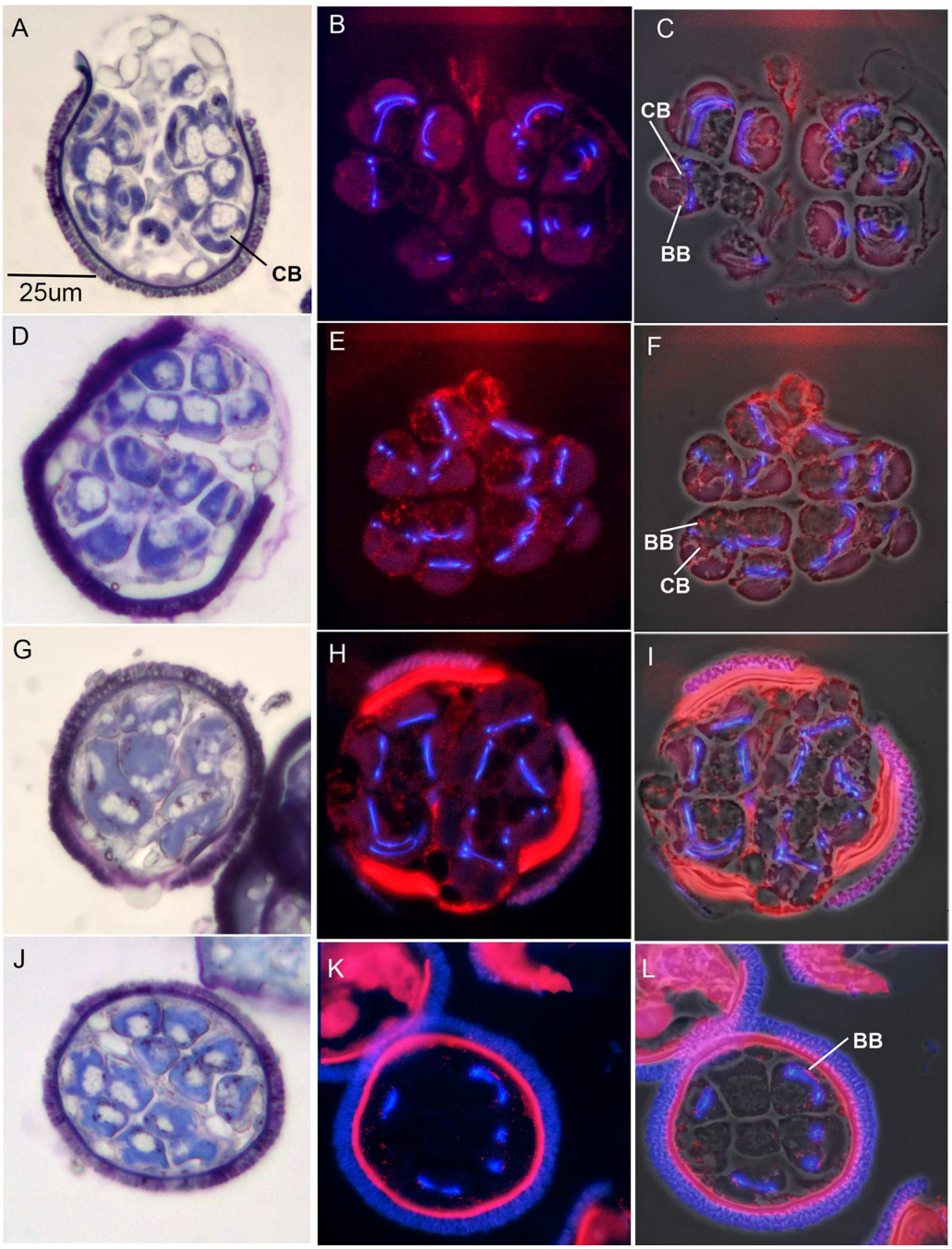


Figure 4-10. MvKinesin-9A is required to position of basal bodies during gametogenesis. (A-C) Controls visualized at 10.5h stained with (A) TBO, (B-C), centrin Ab (red), and DAPI (blue). (C) Fluorescence images overlaid on phase contrast images. The organelle coil and ciliary band (CB) is detectable in spermatogenous cells. Basal bodies (BB) are most prominent anterior to the coil in the area containing the CB. Microspores treated with (D-F) MvKinesin-9A, (G-I) -2, or (J-L) -9B dsRNA, visualized at 10.5h, and stained with (D, G, J) TBO or (E, F, H, I, K, L) centrin Ab and DAPI. (F, I, L) Fluorescence images overlaid on phase contrast images. (D-F) The CB is irregular and not clearly defined. Basal bodies are randomly localized. (G-I) Spermatogenous cells are large. Centrin staining is diffuse. (J-L) Nuclear coil and CB are not defined. Basal bodies appear similar to controls at 8h of development.

Discussion and Conclusions

MvKinesin-2 has atypical functions in cytokinesis and ciliogenesis during male gametophyte development in Marsilea

Phylogenetic analysis of MvKinesin-2 shows that this kinesin is atypical compared to other members of the kinesin-2 family. Firstly, MvKinesin-2 does not cluster with the typical kinesin-2α, β, or γ subgroups. This was not entirely unexpected as both FLA10 and FLA8 in *Chlamydomonas* (Scholey, 2013) and the kinesin-2 in *Physcomitrella* also group independently. Similarly many non-animal kinesin-2s also cannot be placed in the usual kinesin-2α, β, or γ subgroups (Figure 4-2). Secondly, I was only able to find one transcript that encodes a kinesin-2 in *Marsilea*. This was surprising because kinesin-2 is best known in to function in IFT as a heterotrimeric complex. A single kinesin-2 motor is also present in *Physcomitrella* (Wickstead et al., 2010b; Shen et al., 2012) and certain protists (Marande and Kohl, 2011). Although the reason for this is unclear, organisms that are only ciliated for a short time during the life cycle show a general reduction in cilia-associated proteins (Marande and Kohl, 2011). The single kinesin-2 motor found in *Marsilea* may function as a homodimer, similarly to *C. elegans* OSM-3 though evidence to support the existence of homodimeric kinesin-2 outside of sensory cilia is limited (Awan et al., 2004). I was unable to discern whether MvKinesin-2 gametophytes functions as a homodimer, with yet unidentified partners, or if it acts alone. It is possible that MvKinesin-2 and single kinesin-2 proteins found in other organisms function separately from the more well studied kinesin-2 motors. Because the dry spore contains large quantities of stored (pre) mRNAs and proteins (Hart and

Wolniak, 1998, 1999), it is also possible that some of the IFT components are translated during spore desiccation and stored as proteins during quiescence. The transcripts that encode these stored proteins would not necessarily be represented in the transcriptome obtained from gametophytes after spore rehydration.

Functional silencing of MvKinesin-2 through RNAi showed that MvKinesin-2 is required for cytokinesis during the mitotic cell divisions that ultimately produce 32 spermatids in the gametophyte (*Monster* phenocopy) and for regulating the length of cilia (*Rapunzel* phenocopy). However, I cannot eliminate the possibility that the *Monster* and *Rapunzel* phenocopies represent two different manifestations of the same biological event where the role for MvKinesin-2 in cytokinesis precedes its involvement in ciliogenesis. *Monster* spermatids are still able to produce cilia, but these abnormally large gametes containing multiple coils with attached cilia were only able to quiver in place (Appendix I-8). In addition, these cells lacked the extension of cytoplasm that typically surrounds the anterior portion of each spermatid (Figure 4-8C2). In *Tetrahymena* kinesin-2 similarly participates in cytokinesis and in ciliogenesis (Brown et al., 1999) and this motor has been implicated in mammalian cytokinesis through the transport of important regulators of cell division to the midbody (Fan and Beck, 2004; Haraguchi et al., 2006; Keil et al., 2009). Although, kinesin-2 localizes to the mitotic spindle in *Chlamydomonas* and sea urchin embryos, mutants are able to progress through mitosis normally. Thus, a clear role for this motor in mitosis has yet to be established (Henson et al., 1995; Vashishtha et al., 1996; Morris and Scholey, 1997; Matsuura et al., 2002; Miller et al., 2005).

IFT proteins are very similar to proteins involved in vesicle trafficking and it is currently thought that the proteins involved in IFT and vesicle trafficking are evolutionally connected (Jékely and Arendt, 2006; van Dam et al, 2013). In support of this idea, kinesin-2 has been implicated in the intracellular transport of Golgi-derived vesicles and in membrane dynamics (Le Bot et al., 1998; Fan and Beck, 2004; Stauber et al., 2006; Nekrasova et al., 2011). I suspect that the cytokinesis and membrane extension defects observed in the *Monster* phenocopy point to a function for kinesin-2 during spermatogenesis in intracellular transport. During plant cell cytokinesis, the coordinated efforts of a variety of motor proteins are necessary to deliver Golgi-derived vesicles along phragmoplast microtubules to build the cell plate (Lipka and Müller, 2012). Kinesin-2 has never been implicated in this process and a recent study in *Physcomitrella* showed that kinesin-2 was not expressed in caulonemal cells undergoing mitosis (Miki et al., 2014). Since both *Marsilea* and *Physcomitrella* only produce ciliated cells in their gametophytes during spermatogenesis, it is likely that the expression of kinesin-2 is restricted in land plants to male gametes (or the spermatids that mature into these motile cells). It is possible that this motor has evolved a role in cytokinesis and membrane dynamics specifically for spermatogenesis. Further comparisons between both *Physcomitrella* and *Marsilea* sporophyte and gametophyte kinesin-2 expression patterns are required to answer this question.

In a minority of cells, MvKinesin-2 knockdowns produced abnormally long ciliary axonemes (*Rapunzel* phenocopy) while maintaining normal cell size and shape. This result suggests a function for MvKinesin-2 during ciliogenesis. *Rapunzel*

spermatozoids were only able to swim and roll in place. In other organisms, kinesin-2 is responsible for anterograde transport during ciliogenesis. Mutations in this kinesin typically result in no change in ciliary length or the absence of full-length cilia due to suppressed IFT activity (Huang et al., 1977; Kozminski et al., 1995; Snow et al., 2004; Evans et al., 2006; Mukhopadhyay et al., 2007). Therefore, the production of long cilia was unexpected. One possible explanation is that other proteins are responsible for modulating IFT dynamics and ciliary length during male gametophyte development in *Marsilea*. Evidence in support of this hypothesis can be found from the cephalic male cilia (CEM) of *C. elegans* and from *Chlamydomonas*. After CEM loss of KLP-11 (a member of the heterotrimeric kinesin-2 complex) cells produce long cilia as a consequence of complex interactions of KLP-11, OSM-3, and the kinesin-3 motor, KLP-6, during IFT (Morsci and Barr, 2011). In *Chlamydomonas,* the plant specific kinesin-14, KCBP, localizes at basal bodies and in the ciliary membrane (Dymek et al., 2006). The exact function of this kinesin in cilia is unknown, but ability for this motor to direct retrograde transport (Jonsson et al., 2015) makes it an interesting option as an additional IFT motor. Similarly, KCBP and other plant-specific kinesins are abundant during ciliogenesis in *Marsilea* (Tomei and Wolniak, 2016). It is possible one or more of these motors have the ability to modulate kinesin-2 during ciliogenesis in *Marsilea*. Another explanation is that the two phenocopies observed after knockdown of MvKinesin-2 are the consequences of perturbing the same biological process and that these events are related during spermatid development.

Kinesin-13 and kinesin-8 are microtubule depolymerizers and modulate microtubule length during mitosis and ciliogenesis (Desai et al., 1999; Blaineau et al., 2007; Moores and Milligan, 2007; Mayr et al., 2007; Piao et al., 2009; Varga et al., 2009; Delgehyr et al., 2012; Niwa et al., 2012; Wang et al., 2013). Mutations in kinesin-13 produce cilia that are the incorrect length. Whether these cilia are long or short depends on the type of mutation and the organism used for analysis (Blaineau et al., 2007; Dawson et al., 2007; Piao et al., 2009; Chan and Ersfeld, 2010; Kobayashi et al., 2011; Delgehyr et al., 2012; Wang et al., 2013). More recently, kinesin-13 was identified as an axoneme-assembly promoting factor (Vasudevan et al., 2014). Exactly how kinesin-13 regulates the ciliary length is still unresolved. The mechanism by which kinesin-8 modulates the length of cilia is more clear. This kinesin depolymerizes microtubules at the tips of cilia and kinesin-8 mutants have elongated axonemes (Niwa et al., 2012). Kinesin-2 has never been shown to exhibit microtubule-depolymerizing activity, although the atypical structure of kinesin-2 in *Marsilea* does make this an intriguing option for further study.

MvKinesin-9A is required to orient basal bodies along the microtubule ribbon for spermatid differentiation during spermatogenesis

My analysis of the kinesin-9 family in *Marsilea* replicates previous findings (Wickstead and Gull, 2006; Demonchy et al., 2009) that show two kinesin-9 subgroups, kinesin-9A and kinesin-9B. In the *Marsilea* gametophyte transcriptome, a transcript that encodes one member of each kinesin-9 subgroup is present. Through kinesin-9 silencing experiments, I found that kinesin-9A is necessary for the organization and placement of basal bodies during spermatid differentiation. These

basal bodies are assembled *de novo* and become the sites of ciliogenesis during spermatid maturation. With the basal bodies not properly oriented along the microtubule ribbon, cilia are misplaced in the cell body, and the spermatozoids are unable to maintain normal directional swimming patterns and instead only spin and flip in place.

In both *Chlamydomonas* and *T. brucei*, kinesin-9A is also important for motility and this is achieved through the interaction of kinesin-9A with central pair microtubules (Bernstein et al., 1994; Yokoyama et al., 2004; Demonchy et al., 2009), and not through regulating the positioning and orientation of basal bodies. Hydin, a central pair protein required for motility, stabilizes the interaction of kinesin-9A with the central pair microtubules (Lechtreck et al., 2007; 2008). The central pair microtubules and proteins interact with the radial spokes to control the activity of dynein arms and regulate cilia beating and motility (Smith, 2002). The typical phenotype observed in kinesin-9A mutants of a reduction in ciliary beating (Yokoyama et al., 2004; Demonchy et al., 2009) was not observed in *Marsilea* kinesin-9A knockdowns. Instead cilia were unable to maintain normal swimming patterns and they beat in a disorganized, uncoordinated pattern.

This result does not mean that kinesin-9A lacks a conserved role in the control over ciliary assembly and beat. Axonemes of the *Marsilea* male gametophyte have the typical 9+2 microtubule organization, but it has long been known that outer dynein arms are extremely rare, or even absent, although the binding sites on the microtubules do exist (Wolniak and Cande, 1980; Hyams and Campbell, 1985; Hyams, 1985). A search of the gametophyte transcript did not result in any sequences

that resemble outer arm dynein (Tomei and Wolniak, 2016). In the relative absence of outer dynein arms, relatively slow beat frequencies are typically observed (Wolniak and Cande, 1980). Since there are significant structural and functional differences in the *Marsilea* gametophyte axoneme it is possible that the defects in basal body and cilia orientation observed in kinesin-9A knockdowns are a consequence of problems with central pair microtubules or radial spoke proteins. Further studies investigating the localization of kinesin-9A and detailed images of the axoneme after kinesin-9A knockdown in the *Marsilea* are needed.

Knockdowns of kinesin-9B produced a general slowing of differentiation as fully mature and functional spermatozoids were released five hours later than controls. Early stages of development were not altered after kinesin-9B knockdown. This result implies that kinesin-9B is needed for the differentiation of spermatids into motile cells. The specific process during differentiation is unknown, but is likely that there is sufficient redundancy among kinesin isoforms exists to allow development, albeit slow, to continue without this motor. Functional studies on kinesin-9B are few, but several have now appeared in the literature. In *T. brucei*, kinesin-9B is responsible for building the paraflagellar rod (PFR) (Demonchy et al., 2009). The PFR is unique to kinetoplastic protozoan like *T. brucei* so, in a fashion similar to the spermatozoid of *Marsilea*, it is not surprising in other eukaryotes that kinesin-9B would perform functions beyond PFR assembly during ciliogenesis.

Chapter 5: The *Marsilea* Male Gametophyte does not Require IFT Dynein, the BBsome, or Outer Arm Dynein for IFT-Dependent Ciliogenesis and Motility

Introduction

Male gametophyte development in the water fern *Marsilea vestita* is a rapid process that produces 32 motile gametes, each with ~140 cilia from a single undifferentiated cell. The process is initiated by placing dry microspores into water, and requires only eleven hours to reach completion (see Chapter 1; Wolniak et al., 2011, 2015). I am interested in the molecular processes that regulate rapid development, especially during the morphogenesis and differentiation of spermatids into motile spermatozoids. Eukaryotic ciliogenesis is a highly conserved process that is largely dependent upon intraflagellar transport (IFT) and the action of axonemal dynein for motility. IFT is the process by which proteins important for the assembly and function of cilia are transported to the distal ends of forming axonemes by members of the kinesin-2 family and transported proximally back to the cell body by IFT dynein (Kozminski et al., 1993; Rosenbaum and Witman, 2002). This process also involves IFT particles and BBsome proteins, which bind to IFT motors and form scaffolds that assist in the transport of cargo during IFT.

The study of ciliogenesis in green plants has largely been restricted to the green alga *Chlamydomonas* (Silflow and Lefebvre, 2001) and the processes that regulate ciliogenesis in land plants have mostly been ignored. In the previous chapters

of this dissertation I chose to address this problem by classifying the kinesin family of motor proteins that are present during gametophyte development in *Marsilea vestita* and I identified specific kinesins that are involved in differentiation and ciliogenesis. However, I did not examine any other of the numerous proteins that are known to play significant roles in building motile cilia. In this appendix chapter, I investigate the presence and abundance of transcripts that encode dynein and IFT proteins during male gametophyte development in *Marsilea*. This analysis contributes to the study of ciliogenesis in land plants and provides evidence for the existence of a basic protein complement required for IFT-dependent ciliogenesis and motility.

Dyneins are large molecular motors that hydrolyze ATP and bind to microtubules in order to drive intracellular transport and axoneme motility. Like kinesin-14, cytoplasmic dynein is conducts minus-end directed microtubule transport. Although apparently required for intracellular transport in most eukaryotes (Paschal and Vallee, 1987; Schroer et al., 1989), green plants lack cytoplasmic dynein. The prevailing hypothesis is that plants use an expanded and diversified group of kinesin-14s to compensate for the absence of cytoplasmic dynein (Lawrence et al., 2001), but more work is needed to determine exactly how plants drive intracellular transport without this important motor. Similar to cytoplasmic dynein, IFT dynein, also referred to as cytoplasmic dynein 2 or 1b, is responsible for retrograde transport during ciliogenesis (Pazour et al., 1999; Porter et al., 1999). Generally, the absence of IFT dynein is restricted to organisms that do not make cilia or use IFT, although there are some examples where the loss of IFT dynein precedes the disappearance of IFT-dependent ciliogenesis (Wickstead and Gull, 2007). A second type of dynein, known

as axonemal dynein, is responsible for generating the force required for axoneme motility. Axonemal dynein is present in organisms that make motile cilia, but in a similar fashion to cytoplasmic and IFT dynein, some notable exceptions exist (Wickstead and Gull, 2007).

Dynein proteins consist of monomers, dimers, or trimers of heavy chain (HC) subunits that contain a motor domain that belongs to the AAA^+ superfamily and an n-terminal tail domain responsible for oligomerization and subunit binding (Neuwald et al., 1999). Dynein HC molecules can be separated into nine major classes including cytoplasmic dynein, IFT dynein, and seven dynein that are associated with the axonemes of motile cilia (Asai and Wilkes, 2004; Morris et al., 2006; Wickstead and Gull, 2007; Wilkes et al., 2008; Yagi, 2009). The seven dynein HC classes found within motile axonemes can further be classified into two main groups, those that are localized to the outer portion of the axoneme (outer arm dynein) and those that are located more internally, closer to the central-apparatus and radial spokes (inner arm dynein). There are two types of outer arm dynein HCs (OADα and OADβ) (Höök and Valle, 2006; Morris et al., 2006; Wickstead and Gull, 2007; Wilkes et al., 2008) that are found as either two-headed (Nicastro et al., 2006), or as in *Chlamydomonas,* as three-headed species (Johnson and Wall, 1983; Goodenough and Heuser, 1984; Nicastro et al., 2005). The remaining five dynein HCs are all classified as inner arm dyneins. Inner arm dynein HCs either exist as doubled-headed heterodimeric complexes of IAD-1α and IAD-1β or as single-headed, IAD3, IAD4, IAD5, species (Smith and Sale, 1992; Myster et al., 1997; Wickstead and Gull, 2007).

Each of these seven classes of dynein HCs complex with different intermediate chains (IC), light intermediate chains (LIC), and light chains (LC) that are important for regulation and for cargo binding. There are six classes of dynein IC proteins that preferentially form a complex with a specific dynein HC. DYNC1I is found with cytoplasmic dynein, D2IC (FAP133) is present with IFT dynein, IC70 and IC78 associate with outer arm dynein, and IC138 and IC140 complex with inner arm dynein (Mitchell and Kang, 1991; Paschal et al., 1992; Takada and Kamiya, 1994; Ogawa et al., 1995; Wilkerson et al., 1995; Perrone et al., 1998; Yang et al., 1998; Hendrickson et al., 2004; Rompolas et al., 2007). In a similar fashion, there are three main groups dynein LIC proteins. DYNC1LI specifically associates with cytoplasmic dynein, DYNC2L1 with IFT dynein, and FAP146 with inner arm dynein (Yamamoto et al., 2006; 2008; Wilkes et al., 2009). Dynein LC proteins are slightly more difficult to classify and unlike dynein IC and LIC, they are able to complex with multiple classes of dynein HCs and other non-dynein molecules (King et al., 2002; Wilkes et al., 2007). LC8, for example, not only complexes with multiple classes of dynein HCs (King et al., 1996; Pazour et al., 1998), but also myosin motors (Espindola et al., 2000) and radial spoke proteins (Yang et al., 2001) and is thought to constitute a major dimerization hub for protein signaling networks (Barbar, 2008).

In addition to dynein molecules, IFT particles are also highly conserved and are required for building motile cilia. IFT particles can be separated into two main subcomplexes, IFT-A and IFT-B (Piperno and Mead, 1997; Cole et al., 1998; Ou et al., 2005). IFT-A is composed of six subunits, IFT144, 140, 139, 122, 121, and 43 that are conserved in eukaryotic organisms (Qin et al., 2001; Blacque et al., 2006;

Efimenko et al., 2006; Absalon et al., 2008; Tsao and Gorovsky, 2008). The IFT-B subcomplex is larger and consists of a core set of nine proteins, IFT88, 81, 74/72, 70, 52, 46, 27, 25 and 22, with an additional four peripheral proteins, IFT172, 80, 57/55, and 20 (Cole et al., 1998; Lucker et al., 2005; Wang et al., 2009; Fan et al., 2010; Lucker et al., 2010; Bhogaraju et al., 2011; Taschner et al., 2011). Functional analyses of IFT proteins revealed that the IFT-A and IFT-B subcomplex not only represent distinct entities, but also function in different aspects of IFT and ciliogenesis. IFT-A mutants produce cilia with a phenotype similar to that observed after the disruption of IFT dynein and display an accumulation of proteins at the ciliary tip. Thus, the IFT-A subcomplex appears to be involved in retrograde IFT (Pazour et al., 1998; Piperno et al., 1998; Pazour et al., 1999; Porter et al., 1999; Schafer et al., 2003; Iomini et al., 2009). In contrast, mutations in IFT-B proteins result in the shortening or absence of cilia, similar to the results seen after disruption of kinesin-2 motors, leading to the hypothesis that IFT-B is required for ciliogenesis and anterograde IFT (Walther et al., 1994; Pazour et al., 2000; Brazelton et al., 2001; Hou et al., 2007).

The BBsome is another large protein complex that is important for the construction of cilia and in IFT. The BBsome is mainly responsible for the trafficking of membrane proteins to the cilium and for the construction of a specialized ciliary membrane (Nachury et al., 2007; Jin et al., 2010). The BBsome is also implicated in IFT particle assembly and turnaround during IFT (Blacque et al., 2004; Ou et al., 2005). Mutations in BBsome proteins are responsible for Bardet-Biedl syndrome, which is a significant human ciliopathy characterized by a wide range of

physiological symptoms from vision impairment to obesity and polydactylism (Beales, 2005). However, the BBsome is not required for ciliogenesis or for IFT in most eukaryotes (Kulaga et al., 2004; Mykytyn et al., 2004; Yen et al., 2006; Nachury et al., 2007; Loktev et al., 2008; Mukhopadhyay et al., 2008; Lechtreck et al., 2009).

Results

Dynein heavy chain (DHC)

Using the conserved dynein heavy chain domain (PF03028), I searched our reference transcriptome and extracted seven sequences that encode a full-length dynein protein (Table 5-1). Since dynein proteins are very large, the motor domain itself is about 3000 amino acids, and the pfam model frequently used to identify dynein sequences only covers a 783 amino acid region in the c-terminus of the conserved motor domain, it is easy to under-represent the dynein family by using this type of search. To remedy this, I also searched the reference transcriptome against well-established dynein heavy chain sequences in *Chlamydomonas.* This resulted in the identification of several sequence fragments in the *Marsilea* transcriptome with low similarity to the IFT dynein (Table 5-2). I speculated that perhaps the IFT dynein fragments founds in *Marsilea* were part of a larger transcript and the result of an alignment problem in our transcriptome assembly. Blastn searches of the IFT dynein fragments against the transcriptome revealed that are unique sequences and do not show similarity to any of the contigs in the transcriptome except to themselves (Table 5-3).

Table 5-1. Transcripts that encode dynein HC proteins in the *Marsilea* male gametophyte.

Accession	Transcript ID	Len	PF03028	Identity by Blastx, Restricted to plants	Identity by Blastx, Restricted to Chlamydomonas	Identity (Figure 5-1)
KU666433	c1276_g1_i1	13192	0.00E+00	Inner arm dynein 3 [S. moellendorffii], 0.00E+00	Dynein heavy chain 9, 0.00E+00	IAD-3
KU666434	c27157_g1_i1	13166	0.00E+00	Inner arm dynein 3 [S. moellendorffii], 0.00E+00	Dynein heavy chain 6, 0.00E+00	IAD-3
KU666435	c27157_g2_i1	12441	0.00E+00	Dynein heavy chain 6 [S. moellendorffii], 0.00E+00	Dynein heavy chain 2, 0.00E+00	IAD-4
KU666436	c31020_g3_i1	8204	0.00E+00	Inner arm dynein 3-2 [S. moellendorffii], 0.00E+00	Dynein heavy chain 8, 0.00E+00	IAD-3
KU666437	c31426_g1_i1	12990	0.00E+00	Inner arm dynein, group 5 [S. moellendorffii], 0.00E+00	Dynein heavy chain 7, 0.00E+00	IAD-5
KU666438	c31683_g1_i2	16476	0.00E+00	Inner dynein arm 1-α [P. patens], 0.00E+00	Inner arm dynein 1 heavy chain β, 0.00E+00	IAD-1β
KU666439	c38124_g1_i1	14174	0.00E+00	Inner dynein arm 1-α [P. patens], 0.00E+00	Inner arm dynein 1 heavy chain α, 0.00E+00	IAD-1α

Table 5-2. The search for additional dynein sequences revealed sequence fragments with low similarity to IFT dynein.

Transcript ID	Length	Subject	E-value	Query length	Subject length
c36224_g1_i1	218	dynein heavy chain DHC1b (AAC99457)	4.00E-24	2-217	708-779
c43764_g1_i1	206	dynein heavy chain DHC1b (AAC99457)	1.00E-18	3-206	718-785
c53625_g1_i1	221	dynein heavy chain DHC1b (AAC99457)	1.00E-04	3-221	4093-4161
c75680_g1_i1	228	dynein heavy chain DHC1b (AAC99457)	7.00E-07	2-226	3090-3164
c80687_g1_i1	259	dynein heavy chain DHC1b (AAC99457)	4.00E-17	3-242	433-512

Table 5-3. IFT dynein fragments do not associate with any larger contigs in the transcriptome assembly.

Query	Subject	% Identical	# Mismatch	# Gaps	Q. Start	Q. End	S. Start	S. End	E-value
c36224_g1_i1	c36224_g1_i1	100	0	0	1	218	1	218	7E-117
c43764_g1_i1	c43764_g1_i1	100	0	0	1	206	1	206	3E-110
c53625_g1_i1	c53625_g1_i1	100	0	0	1	221	1	221	1E-118
c75680_g1_i1	c75680_g1_i1	100	0	0	1	228	1	228	2E-122
c80687_g1_i1	c80687_g1_i1	100	0	0	1	259	1	259	1E-139

Next, I constructed a maximum-likelihood phylogenetic tree to classify the dynein transcripts present in the *Marsilea* male gametophyte. I included dynein sequences from the biflagellate green alga *Chlamydomonas* for comparison (Appendix I-13), as this is the closest organism to *Marsilea* that has a fully classified dynein family (Wickstead and Gull, 2007). Dyneins have been identified in *Physcomitrella* and *Selaginella*, but the entire protein family has yet to be examined. I found that the of all the full-length dynein transcripts identified through PF03028 encode members of the inner arm dynein (IAD) family and that both double headed (IAD-1α and IAD-1β) and single headed (IAD-3, IAD-4, IAD-5) inner arm dynein species are present in the *Marsilea* male gametophyte. Conversely, transcripts that encode cytoplasmic dynein 1, cytoplasmic dynein 2 (IFT dynein), and outer arm dynein (OAD) are absent in the *Marsilea* gametophyte transcriptome (Figure 5-1). These results echo previous findings and provide further evidence suggesting that plants do not contain cytoplasmic dynein (Lawrence et al., 2001), and that land plants with ciliated gametophytes do not require outer dynein arms or IFT dynein for motility or ciliogenesis, respectively (Wickstead and Gull, 2007).

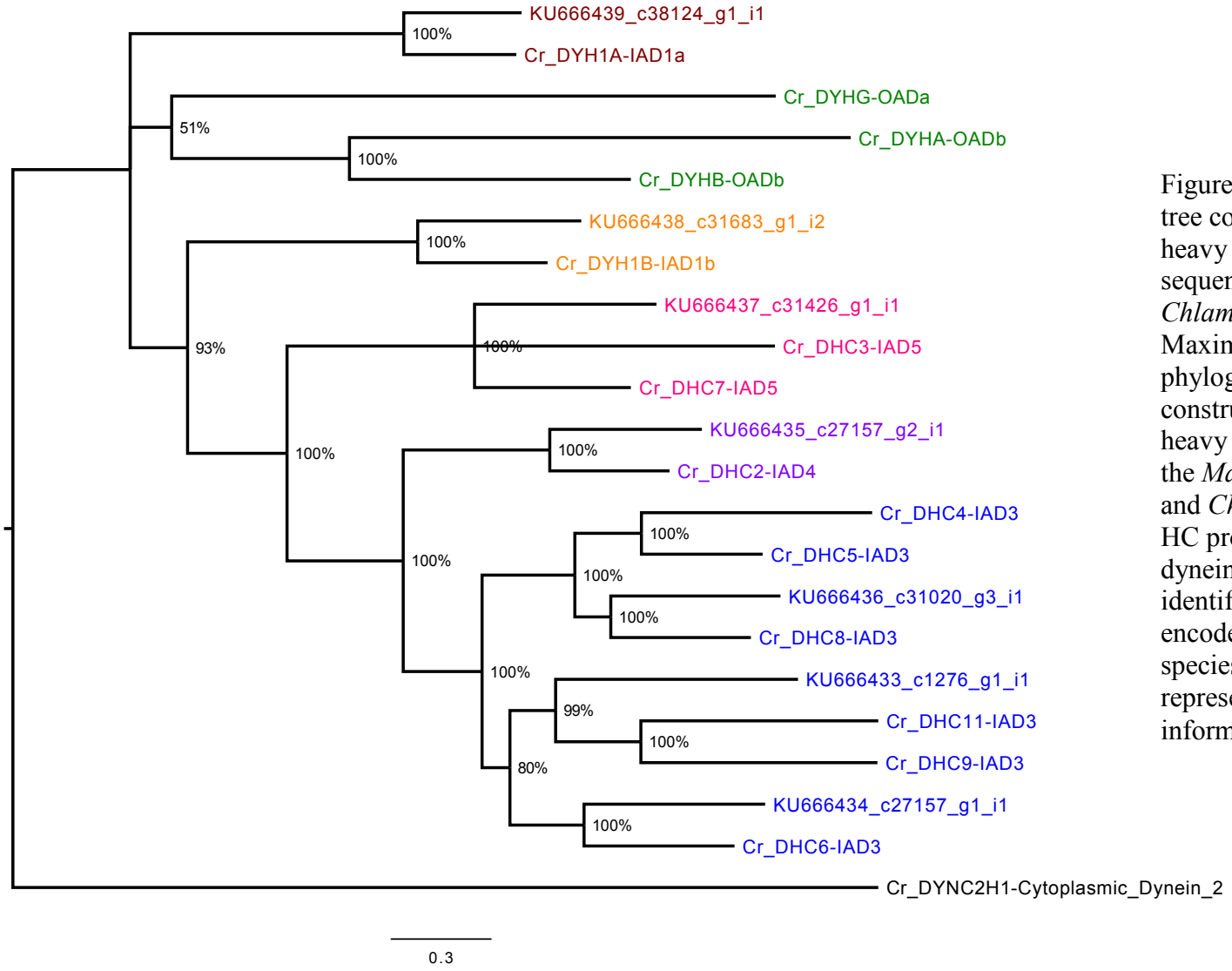


Figure 5-1. Phylogenetic tree comparing dynein heavy chain protein sequences in *Marsilea* and *Chlamydomonas*. Maximum likelihood phylogenetic tree constructed with dynein heavy chains identified in the *Marsilea* transcriptome and *Chlamydomonas* dynein HC protein sequences. All dynein HC sequences identified in *Marsilea* encode dynein inner arm species. Percent values represent bootstrap information for each node.

Dynein intermediate (IC), light intermediate (LIC), and light chains (LC)

To expand upon the dynein heavy chain analysis, I also searched for dynein intermediate (IC), light intermediate (LIC), and light chain (LC) transcripts. There are several conserved domains in dynein IC, LIC, and LC proteins present; however, none of these domains is unique or universally expressed in this class of proteins. For example, IC proteins typically contain a WD40 domain. While this domain is conserved in dynein IC proteins, many other eukaryotic proteins with a wide variety of adaptor and regulatory functions also contain WD40 domains, making it inappropriate to use this domain as the basis of a search. For the identification of dynein LC proteins it may be possible to use conserved domains, such as LC type 1 (PF01221), axonemal LC (PF10211), and Tctex (PF03645), but it is important to recognize that there are several key LCs in which these domains are absent. Therefore, instead of using conserved domains, blastx searches were employed to find transcripts that encode dynein IC, LIC, and LC in the *Marsilea* gametophyte using candidate sequences found in *Chlamydomonas* (Table 5-4). Using this approach it is possible to miss highly divergent members of the dynein complex, but this strategy provides a streamlined method for examining the dynein family in the *Marsilea* male gametophyte.

Chlamydomonas contains two IC proteins that associate with inner arm dynein, IC140 (IDA7) and IC138 (BOP5), two that associate with outer arm dynein, ODA5 and ODA6, and one that associates specifically with IFT dynein, DYNC2L1 (FAP133) (Takada et al., 1994; Ogawa et al., 1995; Wilkerson et al., 1995; Perrone et al., 1998; Yang and Sale, 1998; Hendrickson et al., 2004; Rompolas et al., 2007). I

was able to identify transcripts that encode homologs of IC140 (IDA7) and IC138 (BOP5) in *Marsilea*. No matches (e-value < 1e-10) were found for ODA5 or ODA6 (Table 5-5). A similar situation was also observed for dynein LIC. *Chlamydomonas* makes two LICs, FAP146 and D1bLIC. FAP146 associates with inner arm dynein and D1bLIC with IFT dynein (Yamamoto et al., 2006; 2008; Wilkes et al., 2009). Sequence homologs for FAP146 were identified in the *Marsilea* transcriptome, while D1bLIC homologs were conspicuously absent (Table 5-5).

Like dynein IC and LIC, many dynein LCs also specifically complex with axoneme (inner or outer arm) or cytoplasmic dynein; however the situation is a little more nuanced as many LC proteins associate with multiple dynein HCs classes plus additional proteins. Through blastx searches of the transcriptome, I surveyed *Marsilea* for transcripts that encode homologs of thirteen dynein LC founds in *Chlamydomonas* (Table 5-4). Good matches for IDA4, LC8, Tctex1, Tctex 2, and Roadblock/LC7 were identified in the transcriptome (Table 5-5). All of these LCs associate with inner arm dynein species. Several low similarity matches dynein outer arm LCs (LC1 and LC5) were also discovered (Table 5-5). The significance of these low similarity hits is questionable as the regions that show similarity are often restricted to conserved domains that are present in a wide variety of eukaryotic proteins (leucine repeats and thioredoxin domains, respectively). Transcripts that encode outer arm dynein LC6 (OAD13), LC9, LC2 (OAD12), LC3, and LC10 are completely absent from the *Marsilea* male gametophyte (Table A1-5).

Table 5-4. Complete list of *Chlamydomonas* dynein IC, LIC, and LCs used to identify homologous sequences in *Marsilea*.

	Chlamydomonas	**Top Blastx in Marsilea**	**PFAM**	**NCBI Accession**	**Notes**
ICs	IC140 (IDA7)	2.00E-112	pfam00400 (WD domain)	AAD45352	Associates with inner arm dynein
	IC138 (BOP5)	3.00E-93	pfam00400 (WD domain)	XP_001696921	Associates with inner arm dynein
	IC78 (IC1/ODA9)	No Hits	pfam00400 (WD domain)	Q39578	Associates with outer arm dynein
	IC70 (IC2/ODA6)	No Hits	pfam00400 (WD domain)	P27766	Associates with outer arm dynein
	FAP133	No Hits	No conserved domains	XM_001699649	Associates with IFT dynein
LICs	FAP146 (p38)	1E-70	No conserved domains	XP_001691840	Associates with inner arm species
	D1bLIC	No Hits	pfam08477 (Miro-like protein)	XP_001694720	Associates with IFT dynein
LCs	IDA4 (p28)	8E-103	pfam10211 (Axonemal dynein light chain)	Q39604	Associates with inner arm dynein

	Tctex1	4.00E-43	pfam03645 (Tctex-1 family)	AAC18035	Associates with inner arm dynein
	Tctex2	5.00E-25	pfam03645 (Tctex-1 family)	DAA05278	Associates with inner arm dynein
	LC7 (ODA15, Roadblock)	1.00E-19	pfam03259 (Roadblock/LC7 domain)	AAD45881	Associates with outer and inner arm dynein heavy chains
	LC8 (FLA14)	3.00E-55	pfam01221 (Dynein light chain type 1)	Q39580	Associates with outer, inner I1/f, and IFT dynein heavy chains
	LC5 (LC14, DLC5)	4.00E-11	pfam00085 (Thioredoxin)	Q39591	Associates with outer arm dynein heavy chain-α
	LC1 (DCL1)	4.00E-16	pfam12799 (Leucine Rich repeats, 2)	AAD41040	Associates with outer arm dynein heavy chain-γ
	LC4 (LC18, DLC4)	No Hits	pfam13405 (EF-hand domain)	Q39584	Associates with outer arm dynein heavy chain. Binds Ca^{2+}
	LC3 (LC16, DLC3)	No Hits	pfam00085 (Thioredoxin)	Q39592	Associates with outer arm dynein heavy chain-β
	LC10 (MOT24)	No Hits	pfam01221 (Dynein light chain type 1)	EDP00562	Associates with outer arm dynein heavy chain
	LC9	No Hits	pfam03645	AAZ95589	Associates with outer arm dynein

			(Tctex-1 family, dynein LC)		heavy chain
	LC11 (ODA13)	No Hits	pfam01221 (Dynein light chain type 1)	Q39579	Associates with outer arm dynein heavy chain
	LC19 (ODA12)	No Hits	pfam03645 (Tctex-1 family, dynein LC)	AAB58383	Associates with outer arm dynein heavy chain

Table 5-5. Transcripts that encode dynein IC, LIC, and LCs found in *Marsilea.*

Transcript Query	PFAM	Prot. Cords.	Chlamy. Subject	% Identical	# Mismatch	# Gaps	Q. Start	Q. End	S. Start	S. End	E-value
c22966_g1	pfam00400 (WD40 domain)	453-2567[-]	IC140(IDA7)	32.15	370	13	2390	627	293	933	2E-112
c32037_g1	pfam00400 (WD40 domain)	273-3200[+]	IC138(BOP5)	46.13	163	3	2226	3182	712	1046	3E-93
c38355_g1		140-1324[+]	FAP146(p38)	37.76	196	5	161	1174	10	334	1E-70
c28706_g1	pfam10211 (Ax. dynein LC)	430-1179[-]	IDA4(p28)	64.83	79	1	1140	445	4	239	8E-103
c17162_g1	pfam03645 (Tctex-1 family)	369-713[+]	Tctex1a	56.14	47	1	369	710	4	114	4E-43
c48532_g1	pfam03645 (Tctex-1 family)	481-918[+]	Tctex2b	45.22	61	1	577	915	6	120	5E-25
c26568_g1	pfam03259 (Roadblock/LC7)	185-553[+]	LC7	40.51	47	0	269	505	7	85	1E-19
c16094_g1	pfam03259 (Roadblock/LC7)	1101-1649[-]	LC7	34.04	57	2	1436	1167	7	99	8E-15
c23739_g1	pfam01221 (Dynein LC type 1)	134-433[-]	LC8	87.06	11	0	391	137	7	91	3E-55

c23739_g2	pfam01221 (Dynein LC type 1)	589-888[-]	LC8	87.06	11	0	846	592	7	91	3E-53
c17481_g1	pfam01221 (Dynein LC type 1)	398-700[+]	LC8	85.88	12	0	443	697	7	91	1E-53
c206_g1	pfam01221 (Dynein LC type 1)	357-1550[-]	LC8	47.78	45	1	653	384	3	90	5E-22
c49898_g1	pfam01221 (Dynein LC type 1)	556-1497[+]	LC8	48.89	44	1	1204	1473	3	90	3E-22
c15093_g1	pfam00085 (Thioredoxin)	281-667[-]	LC5	30.68	57	3	601	341	26	110	4E-11
c8277_g1	pfam12799 (Leucine repeats)	668-4153[-]	LC1	32.86	86	2	2260	1865	53	192	2E-11
c70353_g1	pfam12799 (Leucine repeats)	318-1571[+]	LC1	30.57	80	5	414	800	37	192	8E-11
c38732_g1	pfam12799 (Leucine repeats)	557-3889[+]	LC1	33.57	86	2	1103	1510	40	180	4E-16
c30653_g1	pfam12799 (Leucine repeats)	348-1385[-]	LC1	42.55	47	3	668	390	51	138	2E-11

The intraflagellar transport machinery

The absence of IFT dynein machinery (Table 5-1-3) led me to question if other components involved in IFT were present in the *Marsilea* male gametophyte. Specifically, I searched for transcripts that encode the kinesin-2 associated protein FLA3, IFT particles, and for the BBsome. All of these proteins have important roles in eukaryotic IFT and are required for building motile cilia. As with dynein IC, LIC, and LC, there are no specific domain signatures that are unique to these integral IFT proteins. Therefore, a similar blastx based approach was used to identify transcripts that encode members of the IFT transport machinery in the male gametophyte of *Marsilea* (Table 5-6). I found one transcript that encodes a *Marsilea* homolog to FLA3, which is the kinesin-2-associated protein in *Chlamydomonas* (Table 5-7). This suggests that single kinesin-2 motor found in *Marsilea* (Chapter 2, Chapter 4) may associate with this FLA3 homolog and form complexes similar to heterotrimeric kinesin-2.

IFT particles are highly conserved in ciliated organisms and can be separated into two main subcomplexes termed IFT-A and IFT-B. IFT-A is composed of six subunits, IFT144, 140, 139, 122, 121, and 43. Blastx searches of the *Marsilea* transcriptome against IFT protein sequences from *Chlamydomonas* revealed that *Marsilea* male gametophyte makes transcripts that encode homologs of IFT144, IFT140, IFT122, and IFT121 with a high degree of similarity (e-value = 0.0). However, transcripts that encode IFT139 and IFT43 were undetectable in the *Marsilea* gametophyte transcriptome (Table 5-7). The IFT-B subcomplex consists of a core set of nine proteins, IFT88, 81, 74, 70, 52, 46, 27, 22 and 25, with an additional

four peripheral proteins, IFT172, 80, 57, and 20 (Lucker et al., 2005; Wang et al., 2009; Fan et al., 2010; Lucker et al., 2010; Bhogaraju et al., 2011). Similar to the results for IFT-A subcomplex proteins, I was only able to identify transcripts that encode an incomplete set of both the IFT-B core and peripheral subcomplex proteins in the *Marsilea* male gametophyte. Transcripts that encode IFT70, 52, 46 core proteins and IFT172, 80, and 57 peripheral proteins were found in the gametophyte and show a high degree of similarity to counterpart *Chlamydomonas* sequences (Table 5-7).

The BBSome is a complex of proteins that are transported into the cilium through IFT and are important for ciliary membrane biogenesis, IFT assembly, and turnaround (Nachury et al., 2007; Berbari et al., 2008; Shah et al., 2008; Lechtreck et al., 2009; Wei et al., 2012). Blastx searches of the *Marsilea* transcriptome against BBsome proteins in *Chlamydomonas* did not identify any BBsome homologs in *Marsilea* (Table 5-7). This was not entirely surprising as the BBsome is not required for ciliary assembly in most cell types (Blacque et al., 2004; Kulaga et al., 2004; Mykytyn et al., 2004; Yen et al., 2006; Nachury et al., 2007; Loktev et al., 2008; Mukhopadhyay et al., 2008) and is frequently absent in organisms that have a reduced ciliary stage during the life cycle (van Dam et al., 2013).

Table 5-6. Complete list of *Chlamydomonas* IFT-associated proteins used identify homologous sequences in *Marsilea*.

	Chlamydomonas	**Top Blastx in Marsilea**	**PFAM**	**Accession**
IFT-A	IFT144	0.00E+00	WD40, SNAP, TPR repeat	ABU95019
	IFT140	0.00E+00	pfam04053 (Coatomer WD associated region)	XP_001696098
	IFT122	0.00E+00	pfam00400 (WD domain)	XP_001700201
	IFT121	0.00E+00	pfam00400 (WD domain)	XP_001702021
	IFT139	No Hits	pfam13432 (Tetratricopeptide repeat)	ABU95018
	IFT43	No Hits	pfam15305 (Intraflagellar transport protein 43)	XP_001696653
IFT-B Core	IFT88	3.00E-179	pfam13424 (Tetratricopeptide repeat)	XP_001700100
	IFT70	0.00E+00	pfam14559 (Tetratricopeptide repeat)	XP_001692406
	IFT52	1.00E-84	pfam09822 (ABC-type uncharacterized transport)	XP_001692161
	IFT46	5.00E-61	pfam12317 (Intraflagellar transport protein 46)	A2T2X4
	IFT27	No Hits	pfam00071 (Ras family)	A8HN58
	IFT81	No Hits	pfam12128 (DUF3584)	XP_001697224
	IFT74/72	No Hits	pfam05557 (Mitotic checkpoint protein)	XP_001689563
	IFT25	No Hits	pfam00754 (F5/8 type C domain)	B8LIX8
	IFT22	No Hits	pfam08477 (Miro-like protein)	XP_001689669
IFT-B	IFT172	0.00E+00	WD40	XP_001691740

Periphery	IFT80	2.00E-179	pfam00400 (WD domain)	XP_001693341
	IFT57	6.00E-77	pfam10498 (Intraflagellar transport protein 57)	XP_001698648
	IFT20	No Hits	pfam14931 (Intraflagellar transport protein 20)	XP_001701966
BBsome	BBS1	No Hits	pfam14779 (Ciliary BBSome 1)	XP_001701093
	BBS2	No Hits	pfam14782 (Ciliary BBSome 2, C-terminal), pfam14781 (Ciliary BBSome 2, N-terminal)	XP_001696363
	BBS3	No Hits	pfam00025 (ADP-ribosylation factor family)	XP_001701479
	BBS4	No Hits	pfam00515 (Tetratricopeptide repeat)	XP_001693990
	BBS5	No Hits	pfam07289 (DUF1448)	XP_001696253
	BBS7	No Hits	No conserved domains	XP_001689707
	BBS9	No Hits	pfam14727 (PTHB1 N-terminus)	XP_001692266
Kinesin-2 Associated	FLA3	1E-77	pfam05804 (Kinesin-associated protein (KAP))	XP_001698323

Table 5-7. Transcripts that encode FLA3 and IFT particles found in *Marsilea.*

Transcript Query	PFAM	Prot. Cords.	Chlamy. Subject	% Identical	# Mismatch	# Gaps	Q. Start	Q. End	S. Start	S. End	E-value
c38876_g1		931-3759[-]	IFT144	38.84	516	6	3510	946	488	1349	00E+0
c59356_g1		1155-4103[-]	IFT140	33.58	612	11	3980	1170	399	1350	0E+00
c20399_g1	pfam00400 (WD40 domain)	512-4063[-]	IFT122	43.74	612	19	3979	545	13	1168	0E+00
c26615_g1	pfam13181 (Tetratricopeptide)	298-3870[+]	IFT121	38.72	675	14	298	3801	1	1198	0E+00
c24856_g1	pfam13424 (Tetratricopeptide)	634-3093[-]	IFT88	45.43	331	4	2709	853	74	707	3E-179
c29554_g1	pfam13414 (Tetratricopeptide)	43-1989[-]	IFT70	51.45	307	2	2010	46	4	647	0E+00
c161_g1	pfam09822 (ABC-transport)	134-1543[+]	IFT52	41.06	236	11	179	1528	11	435	1E-84
c10572_g1	pfam12317 (IFT protein 46)	150-1169[+]	IFT46	46.15	116	3	495	1151	90	309	5E-61

c21562_g2		247-5469[+]	IFT172	38.27	1042	20	247	5433	1	1741	0
c29908_g1	pfam00400 (WD40 domain)	192-2471[+]	IFT80	38.52	442	7	192	2447	1	740	2E-179
c9621_g1	pfam10498 (IFT protein 57)	98-1246[+]	IFT57	35.96	199	4	140	1189	4	370	6E-77

Transcripts that encode cilia-associated proteins increase in abundance during spermatogenesis

In order to predict if transcripts that encode dynein and IFT machinery proteins present in the *Marsilea* gametophyte transcriptome are functional during ciliogenesis, I tracked the abundance of each transcript during development. As shown in Chapter 3, I calculated changes in transcript abundance between each time interval of development (1-2h vs. 3-5h, 3-5h vs. 6-8h, and 1-2h vs. 6-8h) from RNA-seq counts in replicate samples using EdgeR (Robinson et al., 2010). During each of these stages, progress through different developmental landmarks occurs. The first five hours of development are marked by a series of mitotic divisions that produce 32 spermatids and seven sterile cells. The final stage of development occurs 6-8 hours post hydration when each spermatid differentiates into a motile spermatozoid with about 140 cilia (for review, see Chapter 1; Wolniak et al., 2011; 2015). Transcripts that encode proteins with established roles in ciliogenesis tend increase in abundance during development, while transcripts that encode proteins involved in mitosis decrease in abundance (Boothby, 2013; Wolniak et al., 2015; Tomei and Wolniak, 2016). Therefore, I suspected that transcripts that encode inner arm dynein HCs, ICs, LICs and LCs, and IFT proteins would increase in abundance during gamete maturation as these proteins have important roles in ciliogenesis and motility in other organisms.

I first generalized changes in abundance into three main categories, as a) transcripts that increase, b) those that decrease, or c) those that did not change significantly during the entire course of development. In order for changes to be

classified as significant, transcripts must exhibit at least a two-fold change in abundance between the 1-2h and the 6-8h time interval (-1.0 < logFC > 1.0) and have an FDR<0.05. I found that the majority, ~79% (30/38), of these transcripts increased in abundance during development, while only ~8% (3/38) decreased in abundance. The remaining transcripts, ~13% (5/38), did not change significantly during male gametophyte development in *Marsilea* (Figure 5-2).

In order to determine the identity of transcripts that increase, decrease, or do not change in abundance during development, I separated the transcripts into eight groups based on predicted identity. These groups include dynein HC, dynein IC, dynein LIC, dynein LC, the kinesin-2 associated protein FLA3, IFT-A subcomplex, IFT-B subcomplex core, and IFT-B subcomplex periphery proteins. Of these groups, all transcripts that encode dynein HC, IC, LIC, FLA3, IFT-A, and IFT-B core proteins increase in abundance. In addition, the majority of transcripts that encode LC proteins also increase in abundance (Figure 5-2). Only three transcripts decrease significantly during development in *Marsilea* and all encode dynein LC proteins (Figure 5-2). None of the transcripts that encode IFT-B subcomplex periphery proteins change significantly in abundance (Figure 5-2). Normalized FPKM values for these transcripts and two others, both dynein LCs, that do not change significantly in abundance remain consistently low throughout development (Table 5-8).

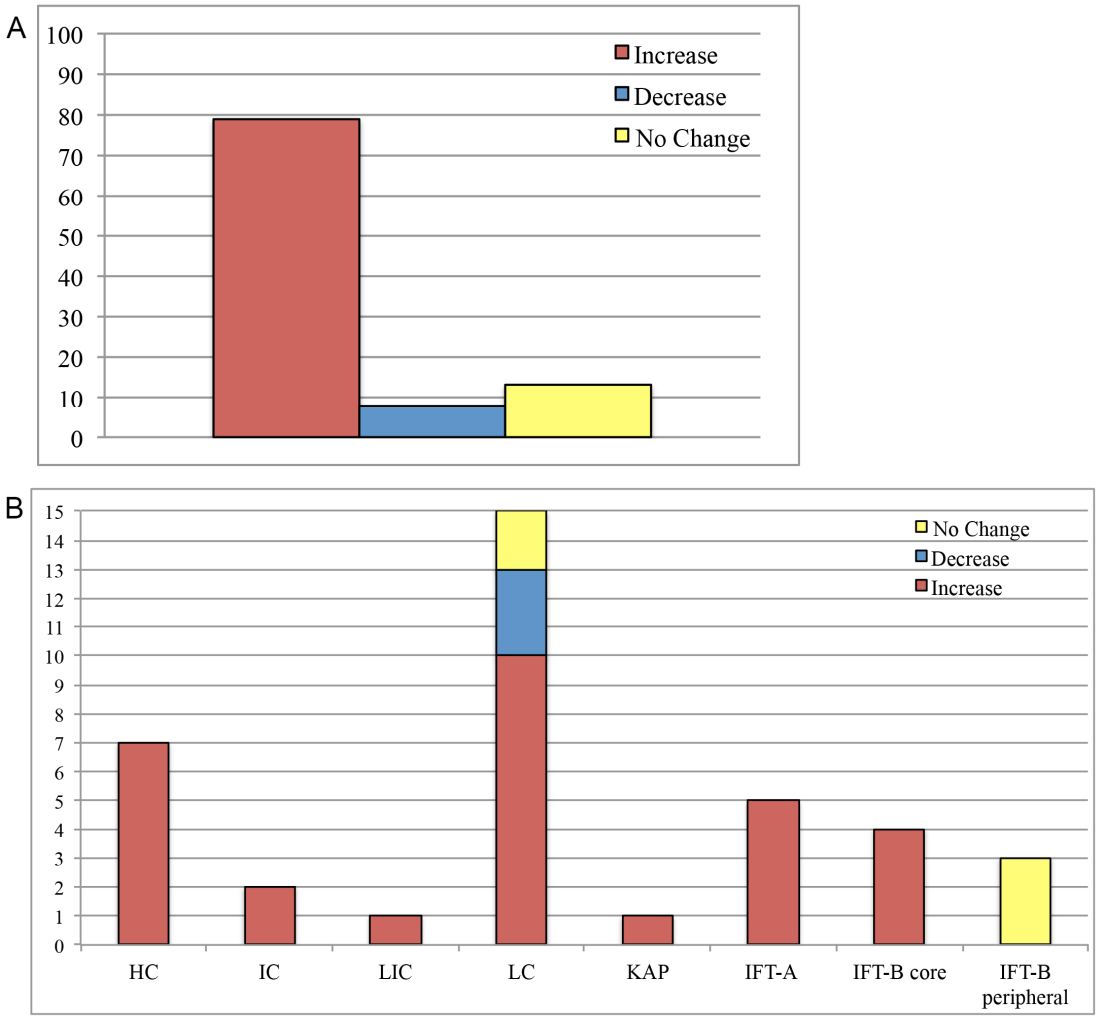


Figure 5-2. Cilia-associated transcripts change in abundance during spermatogenesis. (A) Percentage of cilia-associated transcripts that increase (red), decrease (blue), and do not change (yellow) in abundance. 79% (30/38) of kinesins increase and 8% (3/38) decrease in abundance. (B) Transcripts that encode cilia-associated proteins grouped by changes in abundance. Most groups have transcripts that only increase in abundance. All IFT-B subcomplex peripheral transcripts do not change in abundance during spermatogenesis.

Table 5-8. Normalized FPKM and logFC values for cilia-associated transcripts.

Transcript	Identity	1-2h FPKM	3-5h FPKM	6-8h FPKM	1-2 v 3-5 logFC	1-2 v 3-5 FDR	3-5 v 6-8 logFC	3-5 v 6-8 FDR	1-2 v 6-8 logFC	1-2 v 6-8 FDR
c38124_g1	IAD1a	0.1027	5.3545	53.068	5.75150033	8.44E-17	2.74985222	0.0117564	8.58393554	1.17E-58
c31683_g1	IAD1b	4.6563	5.289	72.239	2.47351911	1.80E-03	3.29909008	1.33E-03	5.84949957	4.52E-32
c31020_g3	IAD3	0.0127	1.7755	82.048	7.7673557	4.59E-16	4.19643192	2.30E-04	12.0733320	2.20E-81
c1276_g1	IAD3	0.1097	2.438	75.7	5.17866250	3.76E-09	3.77302363	1.26E-03	9.02874170	2.18E-55
c27157_g1	IAD3	0.157	2.5635	69.594	4.71914225	2.51E-07	3.58484925	1.87E-03	8.37687678	5.33E-47
c27157_g2	IAD4	0.0387	6.7205	222.46	7.5501641	3.19E-23	4.44597123	6.79E-06	12.0707258	4.28E-83
c31426_g1	IAD5	0.183	2.477	70.141	4.46898456	3.30E-07	3.63660404	1.68E-03	8.17295401	8.40E-54
c22966_g1	IC140(IDA7)	0.5463	2.784	24.073	3.18650857	0.0178210	1.69726066	0.1292824	4.9867506	8.31E-14
c32037_g1	IC138(BOP5)	0.1037	0.9905	75.533	4.17074004	7.06E-07	4.80670312	2.20E-04	9.09367489	8.17E-63
c38355_g1	FAP146	0.0027	0.2955	54.493	7.0717665	1.70E-05	6.04254541	6.30E-04	13.2545795	7.46E-70
c28706_g1	IDA4(p38)	0.083	5.3625	486.10	6.8535906	3.47E-14	5.09444087	2.63E-05	12.0555124	2.77E-88
c17162_g1	Tctex1a	0.0077	3.4195	46.192	9.4105737	2.05E-12	2.30765548	0.0611235	11.8579831	1.33E-59
c48532_g1	Tctex2b	0.014	2.408	32.712	8.1194259	5.83E-12	2.30601305	0.0532020	10.56	5.16E-64
c26568_g	LC7	0.0193	1.468	54.388	6.9775686	1.71E-07	3.7312277	0.0114984	10.8407036	1.43E-57
c16094_g1	LC7	0.048	8.1915	51.496	8.18919046	6.52E-16	1.30438986	0.2746425	9.6042645	5.36E-53
c23739_g1	LC8	0.0857	7.144	742.45	7.1815346	5.34E-12	5.24516757	1.68E-04	12.5512309	6.52E-74
c23739_g2	LC8	0.0653	8.761	495.67	7.8692756	5.15E-16	4.43593952	1.67E-04	12.4286379	7.31E-87

c17481_g1	LC8	8.175	29.51	1613.5	2.58576747	0.0452549	4.51101208	4.25E-05	7.19915650	4.61E-47
c206_g1	LC8	3.0707	0.728	1.4687	-1.1453248	0.3831992	-0.4737942	1	-1.4850657	1.91E-03
c49898_g1	LC8	3.1763	0.752	0.7123	-1.1427211	0.462056	-1.5503566	0.7092158	-2.5497445	4.36E-07
c15093_g1	LC5	0.1497	0.0475	0.3107	-2.397005	1	1.82130547	1	-0.5591619	0.921856
c8277_g1	LC1	0.1603	1.0085	36.415	3.54243384	6.94e-05	3.74210787	2.61E-03	7.42981894	4.59E-55
c70353_g1	LC1	0.887	4.467	10.818	3.16902296	0.0171523	-0.1378134	1	3.13615760	1.22E-06
c38732_g1	LC1	3.63	1.507	1.1987	-0.3870807	0.9309766	-1.7534477	0.4740072	-2.0088918	1.19E-07
c30653_g1	LC1	0.7183	0.2475	0.2147	-0.8390535	0.7197615	-1.6939716	0.6108124	-2.3970743	2.79E-05
c38876_g1	IFT144	0.0077	0.6655	10.761	7.54409876	4.97E-11	2.54931695	0.0358702	10.2481960	2.18E-63
c59356_g1	IFT140	0.0703	0.409	10.399	3.42207181	3.48E-04	3.18387750	0.0147626	6.78663303	5.98E-43
c20399_g1	IFT122	2.8113	2.232	14.599	0.52387393	1	1.31687441	0.2474089	1.95461374	1.68E-07
c26615_g1	IFT121	0.0783	1.7965	6.921	5.34745300	1.12E-07	0.53392618	0.7694026	5.98205775	4.75E-20
c24856_g1	IFT88	0.078	1.695	23.294	5.28224113	1.69E-09	2.35603857	0.0427786	7.78712969	3.55E-53
c29554_g1	IFT70	0.48	34.698	13.35	3.80538603	3.51E-03	0.8401831	1	4.31400934	1.46E-09
c161_g1	IFT52	0.4777	1.647	7.6817	2.66055192	0.2391705	0.7527033	0.6055739	3.51212698	5.22E-06
c10572_g1	IFT46	0.607	4.348	36.789	3.69991485	3.43E-05	1.68764779	0.1323177	5.49180203	2.28E-34
c21562_g2	IFT172	1.032	0.4925	0.4863	-0.1635930	1	-1.4673320	0.810042	-1.4951510	1.37E-03
c29908_g1	IFT80	0.4543	0.343	0.536	0.5143729	1	-0.8299689	1	-0.1372877	0.999987
c9621_g1	IFT57	3.0117	1.904	1.755	0.25739928	1	-1.5795973	0.7012015	-1.1900219	0.016557
c21294_g1	FLA3	0.007	0.3105	39.266	6.0398653	9.03E-06	5.40762827	1.79E-03	11.5835920	5.84E-60

In order to examine patterns of transcript abundance and how they correlate with each distant state of development more specifically, I generated a heatmap that compares changes in abundance that occur during the mitotic stage of development (1-2h vs. 3-5h), during differentiation (the 3-5h vs. 6-8h), and over the entire course of development (1-2h vs. 6-8h). Negative values for logFC represent decreases (shown in blue) in abundance, while positive values represent increases (shown in red). Unchanging and non-significant changes in abundance are depicted in yellow. Similar to what was observed for transcripts that encode kinesin proteins (see Chapter 3), transcripts that decrease in abundance appear to do so over the entire course of development (between the 1-2h and 6-8h time intervals) rather that during a specific stage (Figure 5-3). Transcripts that increase show more diverse patterns of abundance. The majority, 75% (21/28), of the transcripts that increase in abundance do so over the entire course of development; however, 25% (7/28) increase specifically between the 1-2h and 3-5h time interval (Figure 5-3). These increases suggest that these transcripts are important for events that occur during the later stages of development that are associated with basal body formation, cellular morphogenesis, differentiation, ciliogenesis, and motility.

Figure 5-3. Heatmap depicting changes in abundance for cilia-associated transcripts. Increases in abundance (LogFC > 1.0) are shown in increasing shades of red and decreases (LogFC < -1.0) are shown in increasing shades of blue. Non-significant changes in abundance are shown in yellow. Changes were calculated between the 1-2h and 3-5h time intervals, 3-5h and 6-8h time intervals, and over the entire course of development (between the 1-2h and 6-8h time intervals). Transcripts are grouped with the largest increases in abundance between the 1-2h and 6-8h time interval at the

Discussion and Conclusion

Loss of cytoplasmic dynein, IFT dynein, and outer arm dynein in Marsilea

Using the conserved dynein heavy chain domain (PF03028), I found seven dynein HC transcripts in the *Marsilea* male gametophyte (Table 5-1). Phylogenetic analysis of these dynein HC transcripts revealed that they all encode inner arm dynein HCs and that *Marsilea* contains both single headed (IAD-3, IAD-4, IAD-5) and double headed (IAD-1α and IAD-1β) inner arm dynein species (Figure 5-1). Transcripts that encode outer arm dynein, cytoplasmic dynein, and IFT dynein are all undetectable in the *Marsilea* male gametophyte transcriptome (Table 5-2, 3). Consistent with the loss of all dynein HCs except inner arm dynein, transcripts that encode IC and LIC proteins that associate with cytoplasmic, IFT, and outer arm dynein HCs are also absent from this transcriptome. The only IC and LICs present are those that associate with inner arm dynein HC, namely IC138, IC140, and FAP146 (Table 5-4, 5). Dynein LC proteins are a bit more promiscuous and have the ability to associate with multiple classes of dynein HCs, plus additional proteins (King et al., 1996; Pazour et al., 1998; Espindola et al., 2000; Yang et al., 2001; Barbar, 2008). I found transcripts that encode homologs of five different classes of LC proteins in the *Marsilea* transcriptome, IAD4, LC8, Tctex1, Tctex2, and LC7 (Table 5-4, 5). All of these LCs associate with inner arm dynein, although LC8 and LC7 additionally associate with cytoplasmic and outer arm dynein (King et al., 1996; Pazour et al., 1998; Koonin and Aravind, 2000; DiBella et al., 2004).

This absence of cytoplasmic, IFT, and outer arm dynein HCs, ICs, LICs, and LCs in the *Marsilea* transcriptome was not entirely surprising as the evolutionary history of dynein in eukaryotes is largely a history of loss. There is substantial evidence to support that the last common ancestor to all eukaryotes already possessed all nine classes of dynein HCs seen in modern cells and the loss of specific dynein HC classes is typically associated with major morphological events in evolutionary history, such as the loss of cilia or motility (Wickstead and Gull, 2007). Cytoplasmic dynein is absent in all plants; including green algae (Lawrence et al., 2001; Yu et al., 2002; Tuskan et al., 2006; Jaillon et al., 2007; Wickstead and Gull, 2007; Schnable et al., 2009), therefore the absence of cytoplasmic dynein in *Marsilea* was not unexpected. There is speculation that additional minus-end kinesin motors are necessary to compensate for the loss of cytoplasmic dynein in green plants (see Chapter 1), although more work is required to test this hypothesis. More intriguing is the absence of transcripts that encode homologs of IFT and outer arm dynein in the *Marsilea* transcriptome. Like cytoplasmic dynein, IFT dynein and outer arm dynein have been lost in several instances over the course of evolutionary history; however unlike cytoplasmic dynein, the loss of these dynein classes is typically restricted to organisms that do not use or require IFT to assemble their axonemes (Briggs et al., 2004) or organisms that simply lack ciliary axonemes altogether (Wickstead and Gull, 2007), respectively. This makes the loss of these specific dynein classes in *Marsilea* of particular interest.

The IFT fingerprint in Marsilea

The absence of transcripts that encode IFT dynein suggests that *Marsilea* uses IFT-independent mechanisms to build cilia, though several lines of evidence point to the inaccuracy of this assumption. Firstly, although ciliogenesis can occur independently of IFT (Han et al., 2003; Sarpal et al., 2003; Briggs et al., 2004), these situations are rare and require the assembly of cilia in the cytoplasm, rather than as membrane extensions as they are constructed in *Marsilea* (Chapter 1). Secondly, kinesin-2, the anterograde motor associated with IFT, is required for ciliogenesis in the *Marsilea* male gametophyte (Chapter 4). Thirdly, a transcript that encodes a FLA3 homolog was identified in *Marsilea.* FLA3 is an adaptor protein required for the function heterotrimeric kinesin-2 during IFT. With the addition of FLA3 it is possible that the single kinesin-2 motor identified in *Marsilea* (Tomei and Wolniak, 2016) forms traditional heterotrimeric complexes with a yet undetected or highly divergent partner or that *Marsilea* kinesin-2 functions in a unique kinesin/adaptor protein arrangement. More functional studies on the role of FLA3 in *Marsilea* are required to answer this question. Fourthly, and perhaps most importantly, searches for IFT machinery in *Marsilea* revealed the presence transcripts that encode homologs of IFT-A and IFT-B subcomplex proteins, although important IFT proteins are absent (Table 5-5, 6). An incomplete set of IFT subcomplex proteins has also been observed in other ciliated land plants, like *Physcomitrella* and *Selaginella* (Wickstead and Gull, 2007; Hodges et al., 2010; Desai et al., 2015). Finally, the loss of IFT dynein appears to pre-date the loss of IFT in some organisms. Like in *Marsilea,* IFT dynein is also

absent in *Physcomitrella* and *Thalassiosira (*Wickstead and Gull, 2006; Desai et al., 2015), yet these organisms use IFT to build motile cilia.

The absence of IFT dynein, the only known retrograde IFT motor, coupled with the requirement of IFT for ciliogenesis in *Marsilea* raises some important and unresolved questions. How does retrograde IFT occur without IFT dynein or is retrograde transport necessary for ciliogenesis? Although never investigated, it is possible that minus-end directed kinesin motors replace the action of IFT dynein in *Marsilea* and other ciliated plants. During gametophyte development in *Marsilea,* various kinesin-14s increase in abundance during the time interval of development associated with ciliogenesis and may have important roles in this process (Chapter 3, Tomei and Wolniak, 2016). Or maybe retrograde IFT is not required for ciliogenesis and a less specific transport mechanisms, such as diffusion, is sufficient to return ciliary particles to the cell body. The microtubule plus end tracking protein, EB1, accumulates at the tips of cilia independently of IFT and instead uses diffusion and capture for localization (Harris et al., 2016). A similar process may be involved in retrograde IFT in *Marsilea.*

Although transcripts that encode IFT subcomplex proteins are present in the *Marsilea* gametophyte transcriptome, transcripts that encode BBsome proteins are absent (Table 5-6). The BBSome is a protein complex that is transported into cilia through IFT. While implicated in the biogenesis of the ciliary membrane, IFT assembly, and turnaround (Nachury et al., 2007; Berbari et al., 2008; Shah et al., 2008; Lechtreck et al., 2009; Wei et al., 2012), the BBsome is not specifically required for ciliogenesis. BBsome mutants produce fully functional cilia in a variety

of organisms and the BBsome is frequency absent in organisms that have a reduced ciliary stage (Blacque et al., 2004; Kulaga et al., 2004; Mykytyn et al., 2004; Yen et al., 2006; Nachury et al., 2007; Loktev et al., 2008; Mukhopadhyay et al., 2008; van Dam et al., 2013). Therefore, the absence of BBsome homologs in *Marsilea* was not particularly surprising.

Motility without outer arm dynein

In addition to the absence of IFT dynein while still maintaining IFT, *Marsilea* also makes motile cilia without outer arm dynein (Wolniak and Cande, 1980; Hyams, 1985; Hyams and Campbell, 1985). This situation is not unique to *Marsilea,* as both outer and inner arm dynein have independently been lost in organisms that are able to produce motile cilia. Like *Marsilea, Physcomitrella* has also lost all components of the outer arm dynein complex (HCs, ICs, and LICs), but has retained the inner arm dynein (Wickstead and Gull, 2007; Desai et al., 2015). In *Chlamydomonas,* mutants that are unable to make outer arm dynein are still capable of building motile cilia (Kamiya and Okamoto, 1985), although mutations in inner arm dynein contribute to a loss of motility (Kamiya et al., 2000). In both *Marsilea* and *Physcomitrella*, motile axonemes without outer arm dynein are produced in organisms are only ciliated as gametes. The reason for this is unknown, although a general reduction in cilia-associated proteins has been observed in organisms that are only ciliated for a short period of time (Marande et al., 2011).

Transcripts that are involved in IFT and motility increase in abundance

Previous work from our lab has shown that patterns of transcript abundance

and availability in are important for regulating rapid development in the *Marsilea* male gametophyte. pre-mRNAs are stored as intron-containing, masked transcripts until spore rehydration, when they are unmasked, processed, and subsequently translated in the developing gametophyte (Tsai et al., 2004; Boothby et al., 2013; Wolniak et al., 2015). My analysis of the dynein family and IFT particles in *Marsilea* shows that the majority, ~79% (30/38), of these transcripts increased in abundance during development (Figure 5-2). Transcripts that increase in abundance encode all inner arm dynein HCs, ICs, and LICs, FLA3, IFT-A (IFT144, 140, 122, and 121), and IFT-B core (IFT88, 70, 52, and 46) proteins. Many of these transcripts exhibit large changes in abundance. A *Marsilea* homolog of FAP146, a dynein LIC that associates with inner arm dynein, has a logFC of over 13, which translates to about a 9,000 fold-change in abundance, between the 1-2h and 6-8h time intervals of development. Other transcripts show similarly large increases (Table 5-8). Past analyses have shown that transcripts that increase in abundance during development become detectable by unmasking, splicing, and polyadenylation at a specific time intervals after spore hydration (Deeb et al., 2010; Boothby and Wolniak, 2011; Boothby et al., 2013; Wolniak et al., 2015) and that patterns of transcript abundance directly correlate with protein function (Chapter 3; Tomei and Wolniak, 2016). Based on the observed patterns of transcript abundance, it is likely that *Marsilea* homologs of inner arm dynein, FLA3, IFT-A, and IFT-B core proteins are involved in spermatid ciliogenesis and motility. Functional analysis of these proteins during spermatid differentiation is required to make conclusions about dynein and IFT particles in *Marsilea.*

In contrast ~8% (3/38) of dynein and IFT transcripts decrease in abundance, while the remaining ~13% (5/38) do not change significantly during male gametophyte development in *Marsilea* (Figure 5-2). The three transcripts that decrease in abundance all encode two dynein LC1 proteins and LC8 (Figure 5-2, Table 5-8). Unlike dynein ICs and LICs, dynein LCs have the ability to complex with other proteins in addition to dynein and LC8 are thought to serve as an important dimerization and signaling hub (King et al., 1996; Pazour et al., 1998; Espindola et al., 2000; Yang et al., 2001; Barbar, 2008). It is possible that these dynein LCs are involved in processes that are unrelated to axonemal dynein and are functional during earlier stages of development. Transcripts that do not change in abundance during gametophyte development in *Marsilea* encode two dynein LCs (an LC5 and an LC8 protein) and the IFT-B periphery subcomplex (IFT172, 80, and 57) (Figure 5-2, Table 5-8). Kinesin transcripts that do not change in abundance during development have high FPKM values and are required for the successful passage through the early stages of gametogenesis (Chapter 3; Tomei and Wolniak, 2016). However, FPKM values for transcripts that encode LC and IFT-B proteins that do not change in abundance remain low throughout development. The exact meaning of this is unclear, but it is possible that these transcripts are not functional or are never translated into functional proteins during gametophyte development. Further analysis of these transcripts and others with consistently low FPKM values is required to assess their purpose and scope during gametophyte development.

It appears that spermatids in *Marsilea* only produce transcripts that encode cilia-associated proteins that are absolutely required for the construction of motile

cilia. A similar pattern of protein loss is observed in *Physcomitrella* and *Selaginella*. This could be in response to the restriction of motile cilia in *Marsilea* and other ciliated land plants to the male gametophyte. *Marsilea* spermatozoids are produced very quickly and only eleven hours from start to finish is required to produce 32 spermatozoids from one single undifferentiated cell. It is possible that evolution has selected for the fastest possible way to produce functional motility, while ignoring some of the finer points of ciliogenesis (IFT dynein, outer arm dynein, a full set of IFT subcomplex proteins, BBsome proteins) seen in organisms that require motility throughout the life cycle. I hypothesize that the complement of motility and IFT-associated proteins identified in the *Marsilea* male gametophyte represents a basic protein network necessary for the IFT-dependent construction of motile cilia.

Chapter 6: Conclusions and Perspectives

Final Conclusions

The results presented in this dissertation describe how kinesin motor proteins are required to regulate important events and key developmental stages that occur during spermatogenesis and spermiogenesis in *Marsilea vestita*. This work started with a few experiments designed to test how a single kinesin-13 motor was involved in establishing cell fate and polarity during gametophyte development and expanded to encompass a transcriptome-based investigation of the entire kinesin family and some of the first studies that address the mechanisms that control ciliogenesis in land plants. Presented in this chapter are my summarized conclusions and some suggestions for the future of this work.

There are three main developmental stages required for successful spermatogenesis *Marsilea*. The first is the establishment of polarity and cell fate within the gametophyte through a series of asymmetric divisions that separate sterile cells from spermatogenous initials. Next, symmetric divisions are responsible for creating 32 spermatids from the spermatogenous initials, which are surrounded by six sterile jacket cells and one prothallial cell. The last stage is marked by morphogenesis and requires the differentiation of each spermatid into a motile spermatozoid containing a coiled nucleus and microtubule array that is studded by the regular distribution of basal bodies that form *de novo* and then become the sites of ciliogenesis for about 140 axonemes. One of my first observations upon examining

the processes that regulate gametophyte development was that although each one of these stages is unique in biological application and consequence, at their core, each stage is regulated by the cytoskeleton. I hypothesized that cytoskeletal dynamics and the coordinated events of proteins that regulate cytoskeletal arrays must be required for development. Work from our lab previously established that both the cytoskeleton (Wolniak and Swamy, 2004) and kinesin motor proteins (Deeb, 2009) are important for in gametophyte development. Therefore, I decided to investigate these dynamics further by determining how the kinesin family contributes to the processes that regulate gametophyte maturation, differentiation, and ciliogenesis.

To begin, I performed a transcriptome-based search to establish the identity of every member of the kinesin family that is present during gametophyte development. Chapter 2 outlines this work and concludes that the male gametophyte of *Marsilea* makes at least 56 distinct kinesin transcripts. These transcripts encode members of the kinesin-2, -4, -5, -7, -8, -9, -10, -12, and -13 families, plus a diverse group of kinesin-14s, several plant specific, and 'orphaned' kinesins. This complement of kinesin motors closely resembles that of other plants that produce ciliated spermatozoids, such as *Physcomitrella patens.* The absence of kinesin-3, -6, and -11 and the expansion of the kinesin-14 family was not surprising in *Marsilea* as this is a typical feature found in the kinesin family of many land plants. Transcripts that encode kinesin-1 are also absent in *Marsilea*, yet members of the kinesin-1 family can be identified in *Arabidopsis*, *Physcomitrella*, and *Chlamydomonas*. The *Marsilea* male gametophyte makes transcripts that encode members of the kinesin-2, -9, and -'orphan' III families. These kinesins are only found in organisms that are ciliated. In

addition, my analysis of the kinesin family in *Chlamydomonas*, *Physcomitrella*, and *Marsilea* adds kinesin-4 II and ARK-LIKE to the group of motors that are restricted to ciliated plants. Overall, the kinesin family in *Marsilea* appears to be an intermediate between those of *Physcomitrella* and *Arabidopsis*, sharing many features with *Physcomitrella* (kinesin-2, -4 II, -9, ARK-LIKE, -'orphan' I, and -'orphan' III) and many with *Arabidopsis* (kinesin-10, kinesin-'orphan' IV, a relatively reduced group of kinesin-12s, and an expanded group of kinesin-7s and kinesin-14s). This is consistent with the evolutionary relationship among these three plants.

Chapter 3 continues with the global examination of the kinesin family in *Marsilea* and establishes the functional significance many kinesins during gametophyte development. Development in *Marsilea* is post-transcriptionally regulated with transcripts stored in the quiescent spore becoming unmasked, spliced, and polyadenylated in a precise pattern necessary for spermatogenesis. I found that most kinesin transcripts change in abundance during development. Transcripts that increase in abundance later in development encode kinesins with conserved roles in ciliogenesis and motility. Transcripts that decrease during development encode many kinesins with established roles in plant mitosis. Functional analysis of nine kinesins with different patterns of transcript abundance was investigated through the RNAi knockdowns of each kinesin during development. Kinesin-4 Ic and kinesin-13a both decrease in abundance and are required for mitosis during development. Kinesin-13a is needed very early in development for events that occur one to two hours post hydration, such as the establishment of cell fate and patterning of asymmetric divisions. Kinesin-4 Ic is required for the successful completion of the later

symmetric divisions that occur within spermatogenous cells. Kinesin-13b, which does not change in abundance and knockdowns, had a similar, yet more severe, phenocopy to kinesin-13a knockdowns where gametogenesis was unorganized and development stopped prior to the establishment of cell fate. Kinesin-14 VI and ARK-LIKE both increase in abundance and are necessary differentiation events that regulate the transformation of each spermatid into a corkscrew-shaped motile spermatozoid. Knockdowns of kinesin-12 II, ARK, and -'orphan' III did not show any discernable defects in development when visualized at eight hours. Overall, I found that the temporal regulation of kinesin transcripts directly correlates with protein function and overarching cellular processes that are occurring at different phases of gametophyte development. These conclusions support the hypothesis that kinesin motor proteins participate in rate-limiting roles during development, especially during the formation of the ciliary axonemes in motile spermatozoids.

Chapter 4 further investigates the processes that regulate spermatid differentiation, specifically focusing on how kinesin-2 and kinesin-9 contribute to ciliogenesis during spermatid morphogenesis. In Chapter 2, I found that the male gametophyte of *Marsilea* produces transcripts that encode a single kinesin-2 motor, kinesin-9A, and kinesin-9B. mRNAs that encode these kinesins increase in abundance and presumably become available during the stage of development associated with spermatid differentiation and ciliogenesis. Functional analyses showed that kinesin-2 is involved in cell division (Monster phenocopy) and in regulating the length of motile cilia (Rapunzel phenocopy). Knockdown experiments showed that kinesin-9A is required for the proper positioning of basal bodies

(Porcupine phenocopy) during spermatid differentiation leading to spermatozoids that are unable to power directional swimming. In contrast, kinesin-9B (Late Bloomer phenocopy) does not appear to be required for the construction of motile spermatozoids in *Marsilea*.

Heterotrimeric kinesin-2 is the main motor associated with anterograde IFT in motile cilia and mutations typically produce cells that lack cilia due to resultant problems with IFT (Huang et al., 1977; Walther et al., 1994; Kozminski et al., 1995; Cole et al., 1998). Therefore, the involvement of kinesin-2 in cytokinesis and in regulating ciliary length in the *Marsilea* male gametophyte is atypical and was slightly unexpected. However, there are several explanations for this seemly odd combination of phenocopies. I only identified a single transcript that encodes a kinesin-2 motor in *Marsilea*. Kinesin-2 normally functions as heterotrimeric complexes in organisms that make motile cilia. This same situation has been noted in other ciliated land plants like *Physcomitrella* (Wickstead et al., 2010b; Shen et al., 2012). It is therefore likely that the single kinesin-2 motor found in *Marsilea* and other ciliated land plants functions differently from its better-characterized heterotrimeric counterpart. Although best known for its functions during ciliogenesis, kinesin-2 has also been implicated in mitosis and in intracellular membrane trafficking in a number of cell types (Le Bot et al., 1998; Fan and Beck, 2004; Stauber et al., 2006; Nekrasova et al., 2011) thus potentially explaining the *Monster* phenocopy observed during gametophyte development. Also kinesin-2 is not the only motor implicated in anterograde IFT. In the *C. elegans* cephalic male cilia (CEM) KLP-6, a member of the kinesin-3 family, works together with kinesin-2 during IFT

to regulate ciliary length (Morsci and Barr, 2011). It is possible that the *Marsilea* kinesin-2 interacts with other motors during ciliogenesis and in IFT thus explaining the *Rapunzel* phenocopy.

The *Porcupine* phenocopy observed in *Marsilea* after knockdown of kinesin-9A was also different than currently established roles for this motor. The typical phenotype observed in kinesin-9A mutants of a reduction in ciliary beating due to the interaction of kinesin-9A with central pair microtubules (Bernstein et al., 1994; Yokoyama et al., 2004; Demonchy et al., 2009). In *Marsilea,* I attribute disorganized ciliary beating and the inability to maintain normal swimming patterns on the mis-localization of basal bodies in mature spermatozoids. The reason for these contradictions is not currently understood; however, significant differences in the structure of axoneme in fern spermatozoids including those from *Marsilea* (Wolniak and Cande, 1980; Hyams, 1985; Hyams and Campbell, 1985) may be to blame.

In Chapter 5 I complemented this analysis but searching the transcriptome for other important players in ciliogenesis and motility, such as dynein and the IFT machinery. I found that the male gametophyte of *Marsilea* makes transcripts that encode both single headed (IAD-3, IAD-4, IAD-5) and double headed (IAD-1α and IAD-1β) inner arm dynein heavy chain proteins. Intermediate chain (IC138 and IC140) and light intermediate chain (FAP146) proteins that associate with inner arm dynein are also present. All of these transcripts increase in abundance and have predicted functions during the stage of development that is associated with differentiation, ciliogenesis, and motility. Transcripts that encode cytoplasmic dynein, IFT dynein, and outer arm dynein HC, IC, and LIC proteins are absent. This

complement of dyneins closely resembles that of other plants, especially those that produce ciliated spermatozoids. Like *Marsilea*, motile axonemes in *Physcomitrella* and *Selaginella* are produced without IFT dynein and outer arm dynein. This proves that IFT dynein and outer arm dynein are not absolutely required for motility; however, relatively slow beat frequencies are typically observed in fern spermatozoids (Wolniak and Cande, 1980). Although transcripts that encode IFT dynein are absent in the *Marsilea* male gametophyte, IFT is still required for ciliogenesis (see Chapter 4) and IFT subcomplex proteins are present in the gametophyte transcriptome. Many of these IFT subcomplex proteins increase in abundance during development and are therefore likely to be required for ciliogenesis in *Marsilea*. It is unclear how retrograde IFT occurs in ciliated land plants without IFT dynein, but it is possible either minus-end directed kinesins or IFT-independent transport mechanisms are used instead.

The *Marsilea* male gametophyte makes what appears to be the basic complement of proteins required for the construction of motile cilia through IFT. Transcripts that encode these proteins all increase in abundance and include inner arm dynein HCs, ICs, LICs, and LCs, kinesin-2 (see Chapter 4) and its adaptor protein FLA3, IFT144, 140, and 121 of the IFT-A subcomplex, and IFT88, 70, 52, and 46 of the IFT-B subcomplex.

Future Experiments

The data presented in this dissertation not only significantly enhance our understanding of the role of kinesin motor proteins during rapid development in the

Marsilea male gametophyte, but also address substantial gaps in the study of ciliogenesis in the spermatozoids of embryophyte plants. However, important questions regarding how kinesin motors control development processes remain unanswered. The *Marsilea* male gametophyte is an excellent system for the study of ciliogenesis in land plants since the whole purpose of development is to produce motile spermatozoids in a stunningly short period of time. Studies on how kinesins regulate spermatid differentiation and ciliogenesis in *Marsilea* will not only significantly add to the body of research on plant kinesins, which is largely restricted to mitosis, but will help place the mechanisms that regulate ciliogenesis in land plants into evolutionary context.

In chapter 2, I used the gametophyte transcriptome to study the kinesin family in *Marsilea* on a global scale. This analysis is important for understanding how kinesins contribute to gametophyte development, but a more complete analysis of the kinesin family requires genomic, not transcriptomic data. A genomic examination of the kinesin family in *Marsilea* is beyond the scope of this dissertation as my focus was mainly on gametophyte development, but this type of analysis would be useful to the plant cell biologist. One area where genomic analysis will be helpful is in determining if *Marsilea* produces kinesin-1. In my analysis, I was not able to detect any transcripts that encode members of the kinesin-1 family though kinesin-1s have been identified in *Chlamydomonas*, *Physcomitrella*, and *Arabidopsis.* It is possible that the *Marsilea* genome contains kinesin-1, but that kinesin-1 is not required for the production of male gametes. In support of this hypothesis, *Arabidopsis* kinesin-1 has been shown to be important for female gametophyte development (Zhou et al., 2011).

Further analyses are required to answer this question and determine if kinesin-1 is truly absent in *Marsilea* or if it is only absent in male gametophytes.

In addition to genomic analyses, an RT-PCR based approach could be employed to compare the kinesin family in the *Marsilea* gametophyte to the sporophyte. If a specific kinesin is made in the male gametophyte but not in the sporophyte of *Marsilea*, then it is possible to conclude that the kinesin is necessary for events that are restricted to the gametophyte, such as ciliogenesis. Important conclusions can also be made about kinesins that are expressed in both the gametophyte and sporophyte. This type of analysis would be useful in addressing functional questions about many kinesins in *Marsilea*. For example, comparisons of the kinesin family in the *Marsilea* male gametophyte to *Chlamydomonas*, *Physcomitrella*, and *Arabidopsis* added kinesin-4 II and ARK-LIKE to the group of kinesins that is restricted to ciliated plants. Heretofore, neither of these kinesins has ever been implicated in ciliogenesis. An analysis of the expression of kinesin-4 II and ARK-LIKE in *Marsilea* gametophytes versus sporophytes could provide further evidence for the involvement of these kinesins during ciliogenesis.

Similar to kinesin-4 II and ARK-LIKE, kinesin-‘orphan’ III is only found in ciliated organisms (Wickstead and Gull, 2006), but roles for these kinesins during ciliogenesis have not been established. In Chapter 3 I show that the knockdown of kinesin-‘orphan’ III had little to no effect on the first eight hours of male gametophyte development in *Marsilea* and that ARK-LIKE is required for spermatid differentiation. To determine if kinesin-4 II, ARK-LIKE, or -‘orphan’ III are truly required for ciliogenesis, spermatozoids must not only be examined during each stage

of development as they are in Chapter 3, but also after they emerge as mature gametes from the microspore wall.

Chapter 3 examines patterns of kinesin transcript abundance and shows how changes in abundance correlate with the function of kinesin motor proteins during gametophyte development; however, significant questions remain. Why do some kinesins have patterns of abundance that are in contrast to their established roles in other plants? 50% of the transcripts that encode kinesin-7 I decrease in abundance, a pattern reminiscent of kinesins that are involved in mitosis, yet this kinesin has never been implicated in mitosis. Instead, in other plants, kinesin-7 I is expressed in mitochondria where its function is unknown (Itoh et al., 2001). If kinesin-7 I is also expressed in mitochondria in *Marsilea,* why does this kinesin change in abundance during development? Are members of this family involved in the coiling of the mitochondria that occurs during spermatid differentiation? More analysis of this kinesin and mitochondrial dynamics during gametophyte development is necessary to answer these questions.

Another kinesin cluster worth further study during male gametophyte development in *Marsilea* is the ARK family. ARKs are plant-specific and participate in positioning asymmetric division planes through guiding the localization of the nucleus (Malcos and Cyr, 2011; Miki et al., 2015). In *Marsilea,* transcripts that encode ARKa and ARKb increase in abundance during development while ARKc transcripts decrease. Knockdowns of ARKc did not produce any discernable defects during the first eight hours of development. None of this evidence suggests a conserved function for ARKs in positioning asymmetric divisions. Instead, it is

possible that other motors work together with ARK during this process, potentially providing clues about additional motor involvement in division control. Why do two of the three ARKs increase in abundance when the only known role for this kinesin is during mitosis? Are ARKs also important for the morphological events of differentiation and ciliogenesis in *Marsilea?* It is intriguing to speculate that the role of ARKs in nuclear positioning has been shifted to nuclear coil formation during the differentiation of spermatids in *Marsilea*. This would be in line with the increases in abundance observed for transcripts that encode ARKa and ARKb. Additional knockdown studies are needed to make definitive conclusions.

The transcript that encodes kinesin-14 VI increases in abundance during development and knockdown experiments suggest a role for this kinesin during spermatid differentiation. Why is it then that in *Arabidopsis* kinesin-14 VI is involved in PPB formation and memory (Buschmann et al., 2015) during mitosis? This is in complete contrast to lack of evidence supporting a mitotic role for kinesin-14 VI in *Physcomitrella* (Miki et al., 2014) and the observed function of kinesin-14 VI during spermatid differentiation in *Marsilea.* Perhaps the answer can be found in *Chlamydomonas* where kinesin-14 VI has dual roles during mitosis and in the flagellum, although its exact function is unknown (Dymek et al., 2006). In any case, further analysis of kinesin-14 VI in *Marsilea* is required to reconcile the contrasting evidence. Maybe kinesin-14 VI has additional functions in ciliated versus non-ciliated organisms? Perhaps the recently discovered ability for kinesin-14 VIb to power minus-end microtubule transport in *Physcomitrella* (Jonsson et al., 2015) is important for the function of this motor in ciliated plants. The absence of IFT dynein and

questions about retrograde transport during ciliogenesis in land plants (see Chapter 5 for details) makes this interesting and a potentially fruitful area of future study.

Chapter 4 outlines the function of kinesin-2 and kinesin-9 during ciliogenesis in *Marsilea* and contributes to some of only studies investigating ciliogenesis in land plants. I found that kinesin-2 and kinesin-9A have atypical roles in ciliogenesis and motility and that kineins-9B is ultimately not required for the formation of motile spermatozoids. Many questions remain about why these kinesins with established roles in IFT and motility appear to function atypically in *Marsilea.* Why does *Marsilea,* like *Physcomitrella*, only produce one kinesin-2? Does this kinesin function as a heterotrimer with undetected and possibly divergent partners? Or maybe kinesin-2 functions in homodimeric complexes, similar to what has been observed in sensory cilia. The identification of a *Marsilea* transcript that encodes a FLA3-like protein, an important kinesin-2 associated protein (see Chapter 5 for further details), adds further confusion. Homodimeric kinesin-2 does not require an associated protein for IFT. Perhaps *Marsilea* kinesin-2 functions in unique homodimeric complexes that associates with the FLA3-homolog identified in the transcriptome. Is this unique kinesin-2 complex responsible for the atypical function of kinesin-2 during ciliogenesis in *Marsilea*? Are there other motors associated with anterograde IFT in *Marsilea*? Further biochemical analysis of kinesin-2 and potential binding partners plus a more comprehensive study of kinesins with potential roles in ciliogenesis is needed. Specifically an investigation of kinesin-13 and kinesin-8 during ciliogenesis may be beneficial, as both of these kinesins are important for regulating ciliary length in a variety of systems (Niwa et al., 2012; Vasudevan et al., 2014).

In *Marsilea,* knockdowns of kinesin-9A produced gametophytes with disorganized and mis-localized basal bodies leading to spermatozoids that were unable to maintain vectoral swimming patterns. How does kinesin-9A regulate basal body positioning? What are the mechanisms that control the localization of basal bodies during gametophyte development? In *Marsilea* basal bodies are formed *de novo* from an expansion of the blepharoplast, a specialized centrosome-like organelle (Hepler, 1976; Myles and Hepler, 1977). Further studies investigating the localization of kinesin-9A and detailed images of basal bodies and axonemes after kinesin-9A knockdown in the *Marsilea* are needed.

In Chapter 5 I investigate the presence of dynein and the IFT machinery in the transcriptome from the male gametophyte of *Marsilea*. I found that these cells make motile cilia without IFT dynein, outer arm dynein, or the BBsome, but that ciliogenesis likely is dependent on IFT due to the presence of IFT subcomplex proteins. How this occurs is not understood. Functional studies on dynein and IFT subcompex proteins in *Marsilea* are required to determine how these proteins regulate motility and ciliogenesis in male gametophytes. Investigations on how IFT occurs without IFT dynein and motility without outer arm dynein are necessary to more completely understand how *de novo* ciliogenesis occurs in the specialized cells of an otherwise non-motile organism.

Chapter 7: Methods

Microspore harvesting and isolation

Dry sporocarps were collected from Marsilea vestita sporophytes raised in artificial ponds (University of Maryland, Research Greenhouse Complex). Microspores were isolated by grinding dry sporocarps in a coffee grinder (Braun) for two to three short pulses and separated from smaller and larger debris by passing the dry material through a series of calibrated screen- sieves (Hepler, 1976; Deeb et al., 2010, Van der Weele et al., 2007).

Culturing gametophytes

Four milligrams of microspores were cultured in 1ml commercial spring water (Deer Park) for one hour at 20°C with rotational shaking in 2ml tubes. A pushpin was used to make 5 small holes in the top and 3 small holes on the side of the tube. Gametophytes were then transferred to 50ml Erlenmeyer flasks containing an additional 9ml of spring water, bringing the total to 10ml spring water. Flasks were covered with aluminum foil and cultured with rotational shaking at 20°C (Hepler 1976; Hart and Wolniak, 1999; Klink and Wolniak, 2001; Tsai and Wolniak, 2001; Deeb et al., 2010) for the desired amount of time, typically for 1-2, 3-5, or 6-8 hours.

Poly(A+)-RNA isolation

Microspores were isolated from the 50ml flasks (as described above) by transferring the contents to 50ml centrifuge tubes and spun in a clinical centrifuge

tube for 3-5 minutes to pellet the microspores. As much spring water as possible was removed carefully with a micropipette. Poly(A+)-RNA was isolated using the magnetic bead kit (New England BioLabs magnetic mRNA Isolation Kit cat # S1550S) according to protocols defined by the manufacturer. Isolated RNA was used immediately in downstream applications or flash-frozen with liquid nitrogen in 6ul aliquots and stored at -80°C for future use.

Transcriptome assembly

A reference transcriptome was assembled for the gametophyte using poly(A+)-RNA that was isolated from gametophytes that developed for 1-2, 3-5, or 6-8 h. Poly(A+)-RNA isolates were prepared for sequencing with the Illumina TruSeq RNA Sample Preparation Kit, using the manufacturer's low-throughput protocols. The fragments were sequenced with an Illumina HiSeq 2000 instrument that was set up for paired-end, 100-base sequencing of multiplexed samples. Sequencing was conducted by the IBBR sequencing core (www.ibbr.umd.edu/facilities/sequencing). Deconvolved RNAseq read fastq files were filtered for quality prior to transcriptome assembly. The transcriptome was assembled *de novo*, using several successive versions of Trinity (Grabherr et al., 2011). Later versions of the application produced a database with lower noise. The reference was annotated using Trinotate (http://trinotate.github.io), which employs BLAST with the swiss-prot/uniprot, uniref90, and pfam databases. Sequences were mapped from each time-isolate (three replicates) to the combined reference using the Tuxedo suite (Tophat, Bowtie and Cufflinks: Trapnell et al., 2009; 2010), which provided FPKM measures of relative abundance within each of the samples. EdgeR analysis (Robinson et al., 2010)

enabled abundance comparisons between time intervals (see below). Gene Ontology analyses (Blast2GO: Ashburner et al., 2010) show patterns of transcript enrichment for cell components and functions during different phases of development.

Identifying kinesin-like sequences from the transcriptome

127 kinesin-like sequences were identified in the assembled transcriptome using RPS-BLAST against the conserved kinesin motor domain (PF00225) (Table 2-1). Blastn was then used to compare the remaining sequences to each other. Many of the remaining sequences were identical except for small regions and are likely different isoforms of the same transcript (example c26633_g1_i1 and c26633_g1_i2). After eliminating all sequences with partial motor domains, there were 56 unique kinesin transcripts remaining (Table 2-2).

Phylogenetic analysis of the kinesin family in Marsilea

For phylogenetic comparison all kinesin sequences in *Marsilea*, *Arabidopsis* (http://arabidopsis.org), *Physcomitrella* (www.cosmoss.org, version 1.6) and from representative members of each kinesin family in Humans (www.ncbi.nlm.nih.gov) were collected. Accession numbers for these sequences can be found in Chapter 1. Each kinesin sequence was translated using the ExPASy Translate Tool (http://web.expasy.org/translate/) and the largest continuous open reading was used for analysis. Kinesin motor domains were extracted through comparison to the conserved kinesin motor domain (PF00225) and by using the NCBI Conserved Domain Search engine (hwww.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Motor domain sequences were then imported into MEGA (www.megasoftware.net/, Tamura

et al., 2013) and a multiple sequence alignment was generated using the ClustalW default parameters (Appendix I-1). Maximum likelihood (ML) phylogenetic trees were built using the Genetic Algorithm for Rapid Likelihood Inference (GARLI) web service (www.molecularevolution.org, Bazinet et al., 2014). Trees were generated using a fast ML stepwise-addition algorithm and 430 attachment branches were evaluated for each taxon. The parameters used were as follows: rate matrix-LG, state frequencies-estimate, proportion of invariable sites-estimate, rate heterogeneity model-gamma distribution, number of rate categories-4. Bootstrap analysis was done at 1000 replicates and bootstrap values are shown at each node. Phylogenetic trees were then viewed using FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and colored manually according to family with midpoint rooting.

Kinesin-1 phylogeny

Sequences for previously identified kinesin-1, ARK, ARK-LIKE, and 'orphan' kinesins were obtained using NCBI, Phytozome, and Cosmoss databases from *Marsilea, Chlamydomonas*, *Physcomitrella*, and *Arabidopsis*. The motor domain from each sequence was identified using the NCBI Conserved Domain Database and isolated for analysis. Kinesin motor domain alignments (Appendix I-2) and the corresponding phylogenetic tree was built as previously described above with 50 attachment branches evaluated for each taxon.

Transcript abundance

The Bioconductor package edgeR (Robinson et al., 2010) was used to calculate transcript abundance from RNAseq counts in our assembled transcriptome.

Changes in transcript abundance were calculated between the 1-2 and 3-5h, 3-5 and 6-8h, and 1-2 to 6-8h time interval samples. Changes in abundance are represented as the $\log_2$ of the fold-change (logFC) between two time intervals. Significant changes in abundance were determined by considering transcripts with a false discovery rate (FDR) value of less than 0.05 that exhibited more than a two fold change in abundance ($-1.0 < \text{logFC} > 1.0$) between two time intervals. Transcripts that encode kinesin-like proteins were then isolated from this dataset and compared.

Identifying kinesin-2 and kinesin-9 sequences in Marsilea

Kinesin-2 and kinesin-9 sequences were identified by searching the gametophyte transcriptome with kinesin-2 and kinesin-9 sequences from *Physcomitrella* (Phypa_425592, Phypa_425498, Phypa_458410, Phypa_428375) and *Chlamydomonas* (CrFLA8, CrFLA10, CrKLP1, CrKIF9b). Stand-alone BLAST against the transcriptome database was used for the analysis. A maximum e-value of $1E^{-100}$ was used to yield the best possible matches and avoid identifying kinesins unrelated to kinesin-2 and -9 in the search.

MvKinesin-2 and MvKinesin-9 phylogeny

Sequences for previously analyzed kinesin-2 and kinesin-9 family members were obtained using NCBI, Phytozome, and Cosmoss databases. The motor domain from each sequence was identified using the NCBI Conserved Domain Database and isolated for analysis. Kinesin-2 (Appendix I-3) and kinesin-9 motor domain alignments (Appendix I-4) and the corresponding phylogenetic trees were built as

previously described above with 50 attachment branches evaluated for each taxon. For each tree, kinesin-1 was used as an out-group for comparative analysis.

Primers

Primers for these studies were generated using the gametophyte transcriptome in order to perform RT-PCR and to make dsRNA (Appendix I-12). Unique sequences in each kinesins were chosen.

Reverse transcription polymerase chain reaction (RT-PCR)

Reverse transcription reactions were conducted using isolated polyA+RNA and AMV RT enzyme (New England Biolabs) according to manufacture instructions. PCR was carried out using 10ul containing 1ng of cDNA from each RT reaction and amplified using Taq polymerase (New England Biolabs) for 30 cycles. RT-PCR products were run on a 1.5% TAE agarose gel and visualized using GRgreen (BioLabo Scientific Instruments) and UV light.

RNA interference (RNAi)

dsRNA was generated and RNAi was performed as previously described (Deeb et al., 2010). Starting material for dsRNA construct generation was made by RT-PCR amplification of specific targets using gene specific primers with T7 promoter sequences at their extreme 5' ends. After RT-PCR dsRNA constructs were made as in Klink and Wolniak, 2001. The quality and quantity of dsRNA was analyzed by spectrophotometry and gel electrophoresis. RNAi was performed by adding 50-100ug dsRNA to the microspores upon hydration with 1ml of spring water in a 2ml microcentrifuge tube (Hart and Wolniak, 1999, Deeb et al., 2010). After one

hour, gametophytes were transferred to 50ml flasks containing a total of 10ml spring water and cultured with rotational shaking at 20°C.

Fixing, embedding, and sectioning microspores

At the time of analysis, gametophytes were fixed with 4% paraformaldehyde, embedded in methacrylate, and sectioned as previously described (Hepler, 1976; Hart and Wolniak, 1999).

Gametophyte staining with toluidine blue o (TBO)

Toluidine Blue O (TBO) staining was performed on sectioned material and observed with bright field microscopy as previously described (O'Brien and McCully, 1981, Baskin and Wilson, 1997; van der Weele et al., 2007). For all images, thousands of microspores are treated, sectioned, and viewed. About 100 microspores were imaged for each treatment and dominant defects in development were determined by counting the incidence of each phenocopy after knockdown.

Immunofluorescence

Primary antibodies used in this study were as follows: mouse anti-alpha-tubulin (Calbiochem Cat# CP06) used diluted 1:100 and mouse anti-Centrin clone 20H5 (Millipore 04-1624) diluted 1:200. Secondary antibodies used in this study were Alexa Fluor goat anti-rabbit 594 and Alexa Fluor goat anti-mouse 594 (Molecular Probes cat# A11012 and A11005 respectively) both diluted 1:1000 in PBST. DAPI was diluted in PBS and used at a final concentration of 2.5 µg/ml. Samples were placed in a humid chamber and incubated with DAPI for the final 10 minutes of staining. Approximately 100-200 microspores were photographed for each

treatment. Each experiments was conducted at minimum in duplicate.

Fluorescence microscopy

All widefield fluorescence microscopy was performed with a Zeiss Axioscope equipped with standard Fluorescein, TexasRed and UV filter sets. Confocal microscopy was performed on a Zeiss LSM700 using Zen 2009 software. Subsequently, .lsm stacks were exported into ImageJ 1.44k and rendered as 3D models using the ImageJ 3D Viewer plugin.

Analyzing Spermatozoid Swimming Behavior

Gametophytes were allowed to develop for 10.5 – 16 hours and viewed by placing several drops of culture media containing emerging and fully developed spermatozoids in a shallow dish with a coverslip for a bottom. Spermatozoids were analyzed using differential interference contrast (DIC) microscopy with a Zeiss Axiovert 135 TV microscope. Movies were taken with a PIKE F032B monochrome camera (Allied Vision Technology) using StreamPix software at 30 frames per second. Movie files were converted to .mov format. Still frames from movies were captured and converted into tiff format for analysis.

Visualizing Whole Spermatozoids

Spermatozoids were viewed by transferring a drop of the culture media at 11 hours to a glass microscope slide. A drop of 4% PFA was added to fix the spermatozoids rapidly. A coverslip was then placed on top of the drop containing spermatozoids, culture media, and PFA. To prevent crushing fully developed spermatozoids on the slide, the edges of the coverslip were coated in a thin layer of

Vaseline. Spermatozoids were then visualized using DIC microscopy.

Identifying dynein heavy chain sequences in the transcriptome

Seven full-length dynein heavy chain sequences were identified in the assembled transcriptome using RPS-BLAST against the conserved kinesin motor domain (PF03028). Blastn comparisons of the sequences to each other showed that all sequences were unique.

Identifying additional dynein heavy chain sequences

To find additional dynein-like sequences, stand-alone blast was used to search the *Marsilea* transcriptome against well-represented dynein sequences in *Chlamydomonas*. Several sequence fragments with low similarly to IFT dynein were found. Using blastn, these sequences were then compared to the entire transcriptome to search for the presence of larger contigs with similarity to the identified fragments. This search proved that all the sequence fragments were unique and that transcripts that encode IFT dynein and unlikely to exist in *Marsilea.*

Phylogenetic analysis of dyneins in Marsilea compared to Chlamydomonas

Sequences for *Chlamydomonas* dynein heavy chains were obtained using NCBI and Phytozome databases. The phylogenetic trees for dynein heavy chains were built by importing the sequence files into MEGA (Tamura et al., 2013) and multiple sequence alignments were generated using the ClustalW default parameters along the length of each dynein (Appendix I-13). The phylogenetic trees as built as previously described above with 50 attachment branches evaluated for each taxon. As

previously described, the phylogenetic tree was viewed using FigTree and colored manually according to family

Searching for IFT protein homologs in Marsilea

To find homologs of IFT proteins (FLA3, IFT-A subcomplex, IFT-B subcomplex, and BBsome) in *Marsilea*, stand-alone blast was used to search the *Marsilea* transcriptome against well-represented members of these protein families in *Chlamydomonas*. A maximum e-value of $1E^{-10}$ was used to yield the best possible matches, while still isolating divergent homologs.

Sequence information

Accession numbers for all sequences used for phylogenetic analysis and searches are included within this dissertation. The accession number for MvCentrin is U92973 and sequence information is available from the National Center for Biotechnology Information (NCBI) repository.

Appendix I: Multiple Sequence Alignments, Videos, and Large Data Sets

Appendix I-1

Multiple sequence alignment (MSA) of kinesin motor domains used to construct a phylogenetic tree and identify kinesin family members in *Marsilea.*

```
>AT3G63480-AtKinesin1
---------SNVTVCARFRPRSSKEMRDP------------------------------
SRDGVCARPIDAETFVFQ-------------------DDKEDEFTFSLDRVFYEDS------------
---------------TQAAVYEFLALPIMRDAVNGIN---------GTIITYGQ--------------
--------TGAGKTYSMEGPGIQDCD------EHNKGLLPRVVHGMFEQIS-S----SND---------
-------------------------------------------------IARYTVKLSMVEIYM-----
-EKVRDLLDL--S-------------------------------------------------------
-----------------------------------KANIQIKEN-----------------
KTQGILLSGVT----EVPVSDSVEALQHLCTGLANRAVGETQMNMSSSRS-HCAY--LFTIQQ-----
D-------------SVKD--KRVKTGKLILVD-----------------------------LAGSEK-
ADKTGAEG---RVLEEA-------------KTINKSLSA------LGNVINALTSGPS--------
SKGNHIPYRDSKLTRILQ-----DALGG-NSRMALLCCCSPSTLNASETLSTLRFGMRAKHI-------
--
>AT5G47820-AtKinesin4_FRA1
---------CSVKVAVHIRPLIGDERIQG------------------------------
CQDCVTVVTGKPQVQIG------------------------SHSFTFDHVYGSSG------------
---------------SPSTEMYEECAAPLVDGLFQGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTG-CGDSSQT--------GIIPQVMNALFTKIETLKQQ-------------
-------------------------------------------------IEFQIHVSFIEIHK-----
-EEVQDLLDP----CTVNKSDTNN-------------------------------------------
--------------------------TGHVGKVAHVPGKPPIQIRET-----------------
SNGVITLAGST----EVSVSTLKEMAACLDQGSVSRATGSTNMNNQSSRS-HAIFTITVEQMRKINTD-
------SPEN--GAYNGSLKEEYLCAKLHLVD-----------------------------LAGSER-
AKRTGSDG---LRFKEG-------------VHINKGLLA------LGNVISALGDEKKRK-------
DGAHVPYRDSKLTRLLQ-----DSLGG-NSRTVMIACISPADINAEETLNTLKYANRARNIR-------
-
>AT3G50240-AtKinesin4_KICP-02
---------CCVKVAVNVRPLIGDEVTQG------------------------------
CRECVSVSPVTPQVQMG------------------------THPFTFDHVYGSNG------------
---------------SPSSLMFEECVAPLVDGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTG-IKDGTKN--------GLIPQVMSALFNKIDSVKHQ-------------
-------------------------------------------------MGFQLHVSFIEILK-----
-EEVLDLLDS----SVPFNRLAN-------------------------------------------
--------------------------GTPGKVVLS--KSPVQIRES-----------------
PNGVITLSGAT----EVPIATKEEMASCLEQGSLTRATGSTNMNNESSRS-HAIFTITLEQMRKISSI-
------SVVK--DTVDEDMGEEYCCAKLHLVD-----------------------------LAGSER-
AKRTGSGG---VRLKEG-------------IHINRGLLA------LGNVISALGDEKRRK-------
EGAHVPYRDSKLTRLLQ-----DSLGG-NSKTVMIACISPADINAEETLNTLKYANRARNIQ-------
-
>AT5G60930-AtKinesin4
---------ECVRVAVNIRPLITPELLNG------------------------------
CTDCITVAPKEPQVHIG------------------------SHTFTYDFVYGNGG------------
---------------YPCSEIYNHCVAPLVDALFKGYN---------ATVLAYGQ--------------
```

```
--------TGSGKTYTMGTNYSGDCTNG--------GVIPNVMEDIFRRVETTKDS------------
-------------------------------------------------SELLIRVSFIEIFK-----
-EEVFDLLDS----NSSALLKNDS-----------------------------------------------
---------------------------GVQAKHTALSRAPIQIRET-----------------
ASGGITLAGVT----EAEVKTKEEMGSFLARGSLSRATGSTNMNSQSSRS-HAIFTITLEQKK----I-
------AGGS--CTTTEDGGEDILCAKLHLVD----------------------------LAGSER-
AKRTGADG---MRLKEG-------------IHINKGLLA------LGNVISALGDEKKRK-------
EGGHVPYRDSKLTRLLQ-----DSLGG-NSKTVMIACVSPADTNAEETLNTLKYANRARNIQ-------
-
>AT2G36200-AtKinesin5_AtKRP125c
--------GVNVQVLLRCRPFSDDELRSN------------------------------
APQVLTCNDLQREVAVSQN----------------IAGKHIDRVFTFDKVFGPSA------------
---------------QQKDLYDQAVVPIVNEVLEGFN---------CTIFAYGQ--------------
--------TGTGKTYTMEGECRRSKSAPCGGLPAEAGVIPRAVKQIFDTLE------GQQ---------
-------------------------------------------------AEYSVKVTFLELYN-----
-EEITDLLAPEDLSR-VAAEEKQ-----------------------------------------------
-------------------------------------KKPLPLMED----------------
GKGGVLVRGLE----EEIVTSANEIFTLLERGSSKRRTAETFLNKQSSRS-HSLFSITIHIKE------
-------------ATPEGEELIKCGKLNLVD----------------------------LAGSEN-
ISRSGARD---GRAREA-------------GEINKSLLT------LGRVISALVEH------------
LGHVPYRDSKLTRLLR-----DSLGG-RTKTCIIATVSPAVHCLEETLSTLDYAHRAKNIRNKPEVNQK
>AT3G45850-AtKinesin5
--------GVNVQVILRCRPLSEDEARIH------------------------------
TPVVISCNENRREVAATQS----------------IAGKHIDRHFAFDKVFGPAS------------
---------------QQKDLYDQAICPIVFEVLEGYN---------CTIFAYGQ--------------
--------TGTGKTYTMEG-GARKKN---GEFPSDAGVIPRAVKQIFDILE------AQG---------
-------------------------------------------------AEYSMKVTFLELYN-----
-EEISDLLAPEETIK---FVDEKS-----------------------------------------------
-------------------------------------KKSIALMED----------------
GKGSVFVRGLE----EEIVSTANEIYKILEKGSAKRRTAETLLNKQSSRS-HSIFSITIHIKE------
-------------NTPEGEEMIKCGKLNLVD----------------------------LAGSEN-
ISRSGARE---GRAREA-------------GEINKSLLT------LGRVINALVEH------------
SGHIPYRDSKLTRLLR-----ESLGG-KTKTCVIATISPSIHCLEETLSTLDYAHRAKNIKNKPEINQK
>AT2G28620-AtKinesin5
--------GVNIQVIVRCRPFNSEETRLQ------------------------------
TPAVLTCNDRKKEVAVAQN----------------IAGKQIDKTFLFDKVFGPTS------------
---------------QQKDLYHQAVSPIVFEVLDGYN---------CTIFAYGQ--------------
--------TGTGKTYTMEG-GARKKN---GEIPSDAGVIPRAVKQIFDILE------AQS---------
-------------------------------------------------AAEYSLKVSFLELYN-----
-EELTDLLAPEET-K---FADDKS-----------------------------------------------
-------------------------------------KKPLALMED----------------
GKGGVFVRGLE----EEIVSTADEIYKVLEKGSAKRRTAETLLNKQSSRS-HSIFSVTIHIKE------
-------------CTPEGEEIVKSGKLNLVD----------------------------LAGSEN-
ISRSGARE---GRAREA-------------GEINKSLLT------LGRVINALVEH------------
SGHIPYRESKLTRLLR-----DSLGG-KTKTCVIATVSPSVHCLEETLSTLDYAHRAKHIKNKPEVNQK
>AT2G37420-AtKinesin5
--------EVNVQVILRCKPLSEEEQKSS------------------------------
VPRVISCNEMRREVNVLHT----------------IANKQVDRLFNFDKVFGPKS------------
---------------QQRSIYDQAIAPIVHEVLEGFS---------CTVFAYGQ--------------
--------TGTGKTYTMEG-GMRKKG---GDLPAEAGVIPRAVRHIFDTLE------AQN---------
-------------------------------------------------ADYSMKVTFLELYN-----
-EEVTDLLAQDDSSR---SSEDKQ-----------------------------------------------
-------------------------------------RKPISLMED----------------
GKGSVVLRGLE----EEVVYSANDIYALLERGSSKRRTADTLLNKRSSRS-HSVFTITVHIKE------
-------------ESMGDEELIKCGKLNLVD----------------------------LAGSEN-
ILRSGARD---GRAREA-------------GEINKSLLT------LGRVINALVEH------------
SSHVPYRDSKLTRLLR-----DSLGG-KTKTCIIATISPSAHSLEETLSTLDYAYRAKNIKNKPEANQK
>AT1G21730-AtKinesin7-I_MKRP1
----------NITVTIRFRPLSPREVN------------------------------
NGDEIAWYADGDYTIRNEY----------------NPSLC-----YGFDRVFGPPT------------
---------------TTRRVYDIAAQQVVSGAMSGIN---------GTVFAYGV--------------
--------TSSGKTHTMHGE-----------QRSPGIIPLAVKDVFSIIQE-----TP----------
-------------------------------------------------EREFLLRVSYLEIYN-----
-EVINDLLDPTG-----------------------------------------------------------
```

```
----------------------------------------QNLRIRED----------------SQ-
GTYVEGIK----DEVVLSPAHALSLIASGEEHRHVGSNNVNLFSSRS-HTMFTLTIESSPH-GKGDD--
--------------G-EDVSLSQLHLID----------------------------LAGSE--
SSKTEITG---QRRKEG-------------SSINKSLLT------LGTVISKLT----------
DTKAAHIPYRDSKLTRLLQ-----STLSG-HGRVSLICTITPASSTSEETHNTLKFAQRCKHV------
---
>AT4G39050-AtKinesin7-I_MKRP2
----------SISVTVRFRPLSDREYQ--------------------------------
RGDEVAWYPDGDTLVRHEY---------------NPLTA-----YAFDKVFGPQA------------
---------------TTIDVYDVAARPVVKAAMEGVN---------GTVFAYGV--------------
--------TSSGKTHTMHGD-----------QESPGIIPLAIKDVFSIIQD-----TP----------
-------------------------------------------------GREFLLRVSYLEIYN-----
-EVINDLLDPTG-----------------------------------------------------------
----------------------------------------QNLRVRED----------------SQ-
GTYVEGIK----EEVVLSPGHALSFIAAGEEHRHVGSNNFNLLSSRS-HTIFTLMVESSAT-GDEY---
-----------------DGVIFSQLNLID----------------------------LAGSE--
SSKTETTG---LRRKEG-------------SYINKSLLT------LGTVIGKLS----------
EGKATHIPYRDSKLTRLLQ-----SSLSG-HGHVSLICTITPASSSSEETHNTLKFASRAKSI------
---
>AT2G21380-AtKinesin7-I
----------SISVTVRFRPMSEREYQ--------------------------------
RGDEIVWYPDADKMVRNEY----------------NPLTA-----YAFDKVFGPQS------------
---------------TTPEVYDVAAKPVVKAAMEGVN---------GTVFAYGV--------------
--------TSSGKTHTMHGD-----------QDFPGIIPLAIKDVFSIIQE-----TT----------
-------------------------------------------------GREFLLRVSYLEIYN-----
-EVINDLLDPTG-----------------------------------------------------------
----------------------------------------QNLRIRED----------------SQ-
GTYVEGIK----EEVVLSPGHALSFIAAGEEHRHVGSNNFNLMSSRS-HTIFTLMIESSAH-GDQY---
-----------------DGVIFSQLNLID----------------------------LAGSE--
SSKTETTG---LRRKEG-------------AYINKSLLT------LGTVIGKLT----------
EGKTTHVPFRDSKLTRLLQ-----SSLSG-HGHVSLICTVTPASSSTEETHNTLKFASRAKRI------
---
>AT3G12020-AtKinesin7-I
----------NVTVTVRFRPLSPREIR--------------------------------
QGEEVAWYADGETIVRNEH----------------NPTIA-----YAYDRVFGPTT------------
---------------TTRNVYDIAAHHVVNGAMEGIN---------GTIFAYGV--------------
--------TSSGKTHTMHGD-----------QRSPGIIPLAVKDAFSIIQE-----TP----------
-------------------------------------------------NREFLLRISYMEIYN-----
-EVVNDLLNPAG-----------------------------------------------------------
----------------------------------------HNLRIRED----------------KQ-
GTFVEGIK----EEVVLSPAHALSLIAAGEEQRHVGSTNFNLLSSRS-HTIFTLTIESSPL-GDKSK--
--------------G-EAVHLSQLNLVD----------------------------LAGSE--
SSKVETSG---VRRKEG-------------SYINKSLLT------LGTVISKLT----------
DVRASHVPYRDSKLTRILQ-----SSLSG-HDRVSLICTVTPASSSSEETHNTLKFAHRAKHI------
---
>AT5G06670-AtKinesin7-I
----------NVTVTVRFRPLSPREIR--------------------------------
KGEEIAWYADGETIVRNEN----------------NQSIA-----YAYDRVFGPTT------------
---------------TTRNVYDVAAQHVVNGAMAGVNVTLSVNSTTGTIFAYGV--------------
--------TSSGKTHTMHGN-----------QRSPGIIPLAVKDAFSIIQE-----TP----------
-------------------------------------------------RREFLLRVSYFEIYN-----
-EVVNDLLNPAG-----------------------------------------------------------
----------------------------------------QNLRIRED----------------EQ-
GTYIEGIK----EEVVLSPAHVLSLIAAGEEHRHIGSTSFNLLSSRS-HTMFTLTIESSPL-GDNNE--
--------------G-GAVHLSQLNLID----------------------------LAGSE--
SSKAETSG---LRRKEG-------------SYINKSLLT------LGTVISKLT----------
DRRASHVPYRDSKLTRLLE-----SSLSG-HGRVSLICTVTPASSNSEETHNTLKFAHRAKHI------
---
>AT1G18370-AtKinesin7-II_NACK1/HINKEL
----------KIVVTVRLRPMNKRELL--------------------------------
AKDQVAWECVNDHTIVSKP----QVQERL------HHQSS-----FTFDKVFGPES------------
---------------LTENVYEDGVKNVALSALMGIN---------ATIFAYGQ--------------
--------TSSGKTYTMRG-----------------VTEKAVNDIYNHIIK-----TP----------
-------------------------------------------------ERDFTIKISGLEIYN-----
```

```
-ENVRDLLNSDS-----------------------------------------------------------
-------------------------------------GRALKLLDD----------------
PEKGTVVEKLV----EETANNDNHLRHLISICEAQRQVGETALNDTSSRS-
HQIIRLTIQSTHRENSDC-------------------VRSYMASLNFVD------------------
----------LAGSER-ASQSQADG---TRLREG-------------CHINLSLMT------
LTTVIRKLS--V--------GKRSGHIPYRDSKLTRILQ-----HSLGG-
NARTAIICTLSPALAHVEQSRNTLYFANRAKEV---------
>AT3G43210-AtKinesin7-II_NACK2
----------KILVTVRMRPLNWREHA--------------------------------
KYDLIAWECPDDETIVFKN----PNPD--------KAPTK-----YSFDKVFEPTC-------------
---------------ATQEVYEGGSRDVALSALAGTN---------ATIFAYGQ--------------
--------TSSGKTFTMRG-----------------VTESVVKDIYEHIRK-----TQ----------
-------------------------------------------------ERSFVLKVSALEIYN-----
-ETVVDLLNRDT-----------------------------------------------------------
-------------------------------------G-PLRLLDD----------------
PEKGTIVENLV----EEVVESRQHLQHLISICEDQRQVGETALNDKSSRS-
HQIIRLTIHSSLREIAGC-------------------VQSFMATLNLVD------------------
----------LAGSER-AFQTNADG---LRLKEG-------------SHINRSLLT------
LTTVIRKLS--S--------GRKRDHVPYRDSKLTRILQ-----NSLGG-
NARTAIICTISPALSHVEQTKKTLSFAMSAKEV---------
>AT4G38950-AtKinesin7-II
----------KILVLVRLRPLNQKEIA--------------------------------
ANEAADWECINDTTILYRN----TLREGS------NFPSA-----YSFDKVYRGEC-------------
---------------PTRQVYEDGTKEIALSVVKGIN---------CSIFAYGQ--------------
--------TSSGKTYTMTG-----------------ITEFAVADIFDYIFQ-----HE----------
-------------------------------------------------ERAFSVKFSAIEIYN-----
-EAIRDLLSSDG-----------------------------------------------------------
-------------------------------------TS-LRLRDD----------------
PEKGTVVEKAT----EETLRDWNHLKELLSICEAQRKIGETSLNERSSRS-
HQMIRLTVESSAREFLGK-----------------ENSTTLMASVNFID------------------
----------LAGSER-ASQAMSAG---TRLKEG-------------CHINRSLLT------
LGTVIRKLS-----------KGRQGHINFRDSKLTRILQ-----PCLGG-
NARTAIICTLSPARSHVELTKNTLLFACCAKEV---------
>AT3G51150-AtKinesin7-II
----------KIFVSVRLRPLNVRERA--------------------------------
RNDVADWECINDETVIYRSH--LSISERS------MYPTA-----YTFDRVFGPEC-------------
---------------STREVYDQGAKEVALSVVSGVH---------ASVFAYGQ--------------
--------TSSGKTYTMIG-----------------ITDYALADIYDYIEK-----HN----------
-------------------------------------------------EREFILKFSAMEIYN-----
-ESVRDLLSTDI-----------------------------------------------------------
-------------------------------------SP-LRVLDD----------------
PEKGTVVEKLT----EETLRDWNHFKELLSICIAQRQIGETALNEVSSRS-
HQILRLTVESTAREYLAK-----------------DKFSTLTATVNFID------------------
----------LAGSER-ASQSLSAG---TRLKEG-------------GHINRSLLT------
LGTVIRKLS-----------KGKNGHIPFRDSKLTRILQ-----TSLGG-
NARTSIICTLSPARVHVEQSRNTLLFASCAKEV---------
>AT5G66310-AtKinesin7-II
----------KIYVSVRMRPLNDKEKF--------------------------------
RNDVPDWECINNTTIIYRSH--LSISERS------MYPSA-----YTFDRVFSPEC-------------
---------------CTRQVYEQGAKEVAFSVVSGVN---------ASVFAYGQ--------------
--------TSSGKTYTMSG-----------------ITDCALVDIYGYIDK-----HK----------
-------------------------------------------------EREFILKFSAMEIYN-----
-ESVRDLLSTDT-----------------------------------------------------------
-------------------------------------SP-LRLLDD----------------
PEKGTVVEKLT----EETLRDWNHFKELLSVCKAQRQIGETALNEVSSRS-
HQILRLTVESIAREFSTN-----------------DKFSTLTATVNFID------------------
----------LAGSER-ASQSLSAG---TRLKEG-------------CHINRSLLT------
LGTVIRKLS-----------KEKTGHIPFRDSKLTRILQ-----SSLGG-
NARTAIICTMSPARIHVEQSRNTLLFASCAKEV---------
>AT4G24170-AtKinesin7-II
----------KILVSVRVRPLNEKEKT--------------------------------
RNDRCDWECINDTTIICKF---HNLPDK----------SS-----YTFDKVFGFEC-------------
---------------PTKQVYDDGAKEVALCVLSGIN---------SSIFAYGQ--------------
--------TSSGKTYTMSG-----------------ITEFAMDDIFAYIDK-----HKQ---------
```

```
-------------------------------------------------ERKFTLKFSAMEIYN-----
-EAVRDLLCEDS-----------------------------------------------------
-------------------------------------STPLRLLDD----------------
PERGTVVEKLR----EETLRDRSHLEELLSICETQRKIGETSLNEISSRS-HQILRLTIESSSQQFSP-
------------------ESSATLAASVCFVD----------------------------LAGSER-
ASQTLSAG---SRLKEG-------------CHINRSLLT------LGTVIRKLS-----------
KGKNGHIPYRDSKLTRILQ-----NSLGG-NARTAIICTMSPARSHLEQSRNTLLFATCAKEV------
---
>AT5G42490-AtKinesin7-II
----------KILVSVRVRPQNEKEKA--------------------------------
RNDICDWECVNNTTIVCN----NNLPERS------LFPST-----YTFDKVFGFDS-------------
----------------PTKQVYEDGAKEVALCVLGGIN---------SSIFAYGQ--------------
--------TSSGKTYTMCG-----------------ITKFAMDDIFCYIQK-----HT----------
-------------------------------------------------DRKFTLKFSAIEIYN-----
-EAVRDLLSGDN-----------------------------------------------------
-------------------------------------NQ-RRLLDD----------------
PERGTVVEKLI----EETIQDRTHLEELLTVCETQRKIGETSLNEVSSRS-HQILRLTIESTGREYSP-
------------------DSSSTLAASVCFID----------------------------LAGSER-
ASQTLSAG---TRLKEG-------------CHINRSLLT------LGTVIRKL--------------
-----------------------------------------------------------------
>AT2G21300-AtKinesin7-II_CENPE
----------KILVLVRLRPLNEKEIL---------------------------------
ANEAADWECINDTTVLYRN----TLREGS------TFPSA-----YSFDRVYRGEC-------------
----------------PTRQVYEDGPKEVALSVVKGIN---------SSIFAYGQ--------------
--------TSSGKTYTMSG-----------------ITEFAVADIFDYIFK-----HE----------
-------------------------------------------------DRAFVVKFSAIEIYN-----
-EAIRDLLSPDS-----------------------------------------------------
-------------------------------------TP-LRLRDD----------------
PEKGAAVEKAT----EETLRDWNHLKELISVCEAQRKIGETSLNERSSRS-
HQIIKLTVESSAREFLGK-----------------ENSTTLMASVNFID------------------
----------LAGSER-ASQALSAG---ARLKEG-------------CHINRSLLT------
LGTVIRKLS-----------NGRQGHINYRDSKLTRILQ-----PCLGG-
NARTAIVCTLSPARSHVEQTRNTLLFACCAKEV---------
>AT3G10180-AtKinesin7-III
-----------IHVSVRARPLS-SED---------------------------------
AKTSPWKISSDSIFMPNHS----------------------SLAFEFDRIFREDC-------------
---------------KTVQVYEARTKEIVSAAVRGFN---------GTVFAYGQ--------------
-------TNSGKTHTMRGS-----------PIEPGVIPLAVHDLFDTIYQ-----DA-----------
-------------------------------------------------SREFLLRMSYLEIYN------
EDINDLLAPEH------------------------------------------------------
-------------------------------------RKLQIHEN----------------
LEKGIFVAGLR----EEIVASPQQVLEMMEFGESHRHIGETNMNLYSSRS-
HTIFRMIIESRQKMQDEGVG--------------NSCDAVRVSVLNLVD------------------
----------LAGSER-AAKTGAEG---VRLKEG-------------SHINKSLMT------
LGTVIKKLSEGV--------ETQGGHVPYRDSKLTRILQ-----PALGG-
NANTAIICNITLAPIHADETKSSLQFASRALRV---------
>AT1G59540-AtKinesin7-IV_ZCF125
----------KICVAVRVRPPAPEN-----------------------------------
GASLWKVEDNRISLHKSLD----------------TPITTASHAFDHVFDESS--------------
-------------TNASVYELLTKDIIHAAVEGFN---------GTAFAYGQ---------------
------TSSGKTFTMTGS-----------ETDPGIIRRSVRDVFERIHM-----IS-----------
-------------------------------------------------DREFLIRVSYMEIYN------
EEINDLLAVEN------------------------------------------------------
-------------------------------------QRLQIHEH----------------
LERGVFVAGLK----EEIVSDAEQILKLIDSGEVNRHFGETNMNVHSSRS-HTIFRMVIESRGKDNS--
------------------SSDAIRVSVLNLVD----------------------------LAGSER-
IAKTGAGG---VRLQEG-------------KYINKSLMI------LGNVINKLSDS---------
TKLRAHIPYRDSKLTRILQ-----PALGG-NAKTCIICTIAPEEHHIEESKGTLQFASRAKRI------
---
>AT3G49650-AtKinesin8-I
----------TLTVAVKCRPLMEKE---R------------------------------
GRDIVRVNNSKEVVVLDPDLSKDYLDRI---------QNRTKEKKYCFDHAFGPE-------------
--------------STNKNVY-RSMSSVISSVVHGLN---------ATVFAYGS--------------
--------TGSGKTYTMVG------TRS------DPGLMVLSLNTIFDMIKS-DKSSD-----------
```

```
--------------------------------------------------EFEVTCSYLEVYN-----
-EVIYDLLEKSSG-------------------------------------------------------
-----------------------------------------HLELRED-----------------
PEQGIVVAGLR----SIKVHSADRILELLNLGNSRRKTESTEMNGTSSRS-HAVLEIAVKRRQKNQN--
--------------------QVMRGKLALVD-----------------------------LAGSER-
AAETNNGG---QKLRDG-------------ANINRSLLA------LANCINALGKQHKK--------
GLAYVPYRNSKLTRILK-----DGLSG-NSQTVMVATISPADSQYHHTVNTLKYADRAKEI--------
-
>AT1G18550-AtKinesin8-II
----------RILVFVRLRPMGKKERENG------------------------------
SRCCVKVLNKRDVYLTEFTNE-NDYLR----------LKRLRVRHFTFDSSFPET--------------
---------------TTQQEVYSTTTGDLVEAVLEGRN---------GSVFCYGA--------------
--------TGAGKTYTMLG------TME------NPGVMVLAIKDLFAKVRQ-RSLDG-----------
-------------------------------------------------NHVVHLSYLEVYN-----
-ETVRDLLSPGR-------------------------------------------------------
-----------------------------------------PLILRED-----------------
KQGIVAAGLT----QYRAYSTDEVMALLQRGNQNRTTEPTRCNETSSRS-HAILQVIVEYKTRDASMN-
-------------------IISRVGKLSLID-----------------------------LAGSER-
ALATDQRT---LRSLEG-------------ANINRSLLA------LSSCINALVE------------
GKKHIPYRNSKLTQLLK-----DSLGG-SCNTVMIANISPSSQSFGETQNTLHWADRAKEI--------
-
>AT3G16060-AtKinesin13
----------KIKVVVRKRPLNKKESTKN------------------------------
EEDIVDTHAN--CLTVHETKLKVDLTA------------YVEKHEFVFDAVLDEE--------------
---------------VSNDEVYRETVEPVVPLIFQRIK---------ATCFAYGQ--------------
--------TGSGKTYTM-----------------KPLPLKASRDILRLMHH-TYRNQ-----------
-------------------------------------------------GFQLFVSFFEIYG-----
-GKLYDLLSERK-------------------------------------------------------
-----------------------------------------KLCMRED-----------------
GKQQVCIVGLQ----EYRVSDTDAIMELIERGSATRSTGTTGANEESSRS-
HAILQLAIKKSVEGNQSK--------------------PPRLVGKLSFID-------------------
----------LAGSERGADTTDNDK---QTRLEG-------------AEINKSLLA------
LKECIRALDN------------DQGHIPFRGSKLTEVLR-----DSFMG-
NSRTVMISCISPSSGSCEHTLNTLRYADRVK-----------
>AT3G16630-AtKinesin13
----------KIKVVVRKRPLNKKETAKK------------------------------
EEDVVTVSDN--SLTVHEPRVKVDLTA------------YVEKHEFCFDAVLDED--------------
---------------VSNDEVYRATIEPIIPIIFQRTK---------ATCFAYGQ--------------
--------TGSGKTFTM-----------------KPLPIRAVEDLMRLLRQPVYSNQ-----------
-------------------------------------------------RFKLWLSYFEIYG-----
-GKLFDLLSERK-------------------------------------------------------
-----------------------------------------KLCMRED-----------------
GRQQVCIVGLQ----EYEVSDVQIVKDFIEKGNAERSTGSTGANEESSRS-
HAILQLVVKKHVEVKDTRRR-----------NNDSNELPGKVVGKISFID-------------------
----------LAGSERGADTTDNDR---QTRIEG-------------AEINKSLLA------
LKECIRALDN------------DQLHIPFRGSKLTEVLR-----DSFVG-
NSRTVMISCISPNAGSCEHTLNTLRYADRVK-----------
>AT4G21270-AtKinesin14-I_ATK1/KATA
--------KGNIRVFCRVRPLLPDDGG------------------------------
RHEATVIAYPTSTEAQGRGVDLVQ---------------SGN--KHPFTFDKVFNHEASQ---------
--------------------EEVFFEISQLVQSALDGYK---------VCIFAYGQ------------
----------TGSGKTYTMMGRPE---------APDQKGLIPRSLEQIFQASQSLG-----AQG-----
-----------------------------------------------------WKYKMQVSMLEIYN---
---ETIRDLLSTNRTTSMD-------------------------------------------------
LVRADSGTSGKQ-----------------------------------YTITHD--------------
-VNGHTHVSDLT----IFDVCSVGKISSLLQQAAQSRSVGKTQMNEQSSRS-
HFVFTMRISGVNESTEQQ----------------------VQGVLNLID-------------------
----------LAGSERLSKSGATG----DRLKET-------------QAINKSLS------
ALSDVIFALAK------------KEDHVPFRNSKLTYLLQ-----PCLGGDS-
KTLMFVNISPDPTSAGESLCSLRFAARVNAx---------
>AT4G27180-AtKinesin14_I_ATK2/KATB
--------KGNIRVFCRVRPLLSGENS-------------------------------
SEEAKTISYPTSLEALGRGIDLLQ---------------NGQ--SHCFTFDKVFVPSASQ---------
--------------------EDVFVEISQLVQSALDGYK---------VCIFAYGQ------------
```

```
----------TGSGKTYTMMGRPG---------NPDEKGLIPRCLEQIFQTRQSLR-----SQG-----
-------------------------------------------------WKYELQVSMLEIYN---
---ETIRDLLSTN----KE-----------------------------------------------
AVRADNGVSPQK-----------------------------------YAIKHD--------------
-ASGNTHVVELT----VVDVRSSKQVSFLLDHAARNRSVGKTAMNEQSSRS-
HFVFTLKISGFNESTEQQ----------------------VQGVLNLID------------------
----------LAGSERLSKSGSTG----DRLKET-------------QAINKSLS------
SLGDVIFALAK-----------KEDHVPFRNSKLTYLLQ-----PCLGGDS-
KTLMFVNITPEPSSTGESLCSLRFAARVNACEIG------
>AT5G54670-AtKinesin14-I_ATK3/KATC
--------KGNIRVFCRVRPLLPGENN-----------------------------
GDEGKTISYPTSLEALGRGIDLMQ---------------NAQ--KHAFTFDKVFAPTASQ---------
--------------------EDVFTEISQLVQSALDGYK---------VCIFAYGQ------------
----------TGSGKTYTMMGRPG---------NVEEKGLIPRCLEQIFETRQSLR-----SQG-----
-------------------------------------------------WKYELQVSMLEIYN---
---ETIRDLLSTN----KE-----------------------------------------------
AVRTDSGVSPQK-----------------------------------HAIKHD--------------
-ASGNTHVAELT----ILDVKSSREVSFLLDHAARNRSVGKTQMNEQSSRS-
HFVFTLRISGVNESTEQQ----------------------VQGVLNLID------------------
----------LAGSERLSKSGSTG----DRLKET-------------QAINKSLS------
SLGDVIFALAK-----------KEDHVPFRNSKLTYLLQ-----PCLGGDA-
KTLMFVNIAPESSSTGESLCSLRFAARVNACEIG------
>AT4G05190-AtKinesin14-I_ATK5
--------KGNIRVFCRVRPLLPDDGG-----------------------------
RQEASVIAYPTSTESLGRGIDVVQ---------------SGN--KHPFTFDKVFDHGASQ---------
--------------------EEVFFEISQLVQSALDGYK---------VCIFAYGQ------------
----------TGSGKTYTMMGRPE---------TPEQKGLIPRSLEQIFKTSQSLS-----TQG-----
-------------------------------------------------WKYKMQVSMLEIYN---
---ESIRDLLSTSRTIAIE-----------------------------------------------
SVRADSSTSGRQ-----------------------------------YTITHD--------------
-VNGNTHVSDLT----IVDVCSIGQISSLLQQAAQSRSVGKTHMNEQSSRS-
HFVFTLRISGVNESTEQQ----------------------VQGVLNLID------------------
----------LAGSERLSRSGATG----DRLKET-------------QAINKSLS------
ALSDVIFALAK-----------KEDHVPFRNSKLTYLLQ-----PCLGGDS-
KTLMFVNISPDPSSTGESLCSLRFAARVNACEIG------
>AT5G41310-AtKinesin14-II
--------KGNIRVYCRIRPFLQGQNKK-------------------------------
QTSIEYTGENGE-LVVANPLK---------------QGKDTYRLFKFNKVFGPESTQ----------
------------------EEVFLDTRPMIRSILDGYN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPSIT--------SEEDRGVNYRALNDLFHLTQSRQ-----NS--------
-------------------------------------------------VMYEVGVQMVEIYN-----
-EQVRDLLSQD------------------------------------------------------------
------------------------------------------------------------------------
--VPDAS----MHSVRSTEDVLELMNIGLMNRTVGATTLNEKSSRS-HSVLSVHVRGVDVKTESV----
-------------------LRGSLHLVD-----------------------------
LAGSERVGRSEVTG----ERLKEA-------------QHINKSLS------ALGDVIFALAH------
------KNPHVPYRNSKLTQVLQ-----NSLGGQA-
KTLMFVQINPDEDSYAETVSTLKFAERVSGVEL-------
>AT1G63640-AtKinesin14-II
--------KGNIRVYCRIRPFLPGQNSR-------------------------------
QTTIEYIGETGE-LVVANPFK---------------QGKDTHRLFKFNKVFDQAATQ----------
------------------EEVFLDTRPLIRSILDGYN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPSIT--------SKEDWGVNYRALNDLFLLTQSRQ-----NT--------
-------------------------------------------------VMYEVGVQMVEIYN-----
-EQVRDILSDGGS----------------------------------------------------------
-SRR-LG-----------------------------------IWNTAL--------------PNG-
LAVPDAS----MHCVRSTEDVLELMNIGLMNRTVGATALNERSSRS-HCVLSVHVRGVDVETDSI----
-------------------LRGSLHLVD-----------------------------
LAGSERVDRSEATG----ERLKEA-------------QHINKSLS------ALGDVIFALAH------
------KNPHVPYRNSKLTQVLQ-----SSLGGQA-
KTLMFVQVNPDGDSYAETVSTLKFAERVSGVELG------
>AT1G18410-AtKinesin14-II
--------KGNIRVYCRVRPFLRGQGAS-------------------------------
KTVVEHIGDHGE-LVVLNPTK---------------PGKDAHRKFRFNKVYSPASTQ----------
```

```
------------------AEVFSDIKPLIRSVLDGYN---------VCIFAYGQ--------------
--------TGSGKTYTMTGPDGA--------SEEEWGVNYRALNDLFRISQSRK-----SN--------
-------------------------------------------------IAYEVGVQMVEIYN-----
-EQVRDLLS-------------------------------------------------------------
------G-----------------------------------ILSTTQ--------------QNG-
LAVPDAS----MYPVTSTSDVLELMSIGLQNRVVSSTALNERSSRS-HSIVTVHVRGKDLKTGSA----
------------------LYGNLHLVD----------------------------
LAGSERVDRSEVTG----DRLKEA--------------QHINKSLS------ALGDVIFSLAS------
------KSSHVPYRNSKLTQLLQ-----SSLGGRA-
KTLMFVQLNPDITSYSESMSTLKFAERVSGVELG------
>AT1G73860-AtKinesin14-II
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-------------------------------------------K-----GN-----------------
---------------------------------------ISYEVGVQMVEIYN------
EQVLDLLSDDNS----------------------------------------------------------
QKKTLG-----------------------------------ILSTTQ--------------QNG-
LAVPDAS----MYPVTSTSDVITLMDIGLQNRAVGSTALNERSSRS-HSIVTVHVRGKDLKTGSV----
------------------LYGNLHLVD----------------------------
LAGSERVDRSEVTG----DRLREA--------------QHINKSLS------SLGDVIFSLAS------
------KSSHVPYRNSKLTQLLQ-----TSLGGRA-
KTLMFVQLNPDATSYSESMSTLKFAERVSGVELG------
>AT3G44730-AtKinesin14-II_KP1
--------KGTIRVYCRVRPFFQEQKDM-------------------------------
QSTVDYIGENGN-IIINNPFK---------------QEKDARKIFSFNKVFGQTVSQ-----------
------------------EQIYIDTQPVIRSVLDGFN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPDLM--------TETTWGVNYRALRDLFQLSNART-----HV--------
-------------------------------------------------VTYEIGVQMIEIYN-----
-EQVRDLLVSDG----------------------------------------------------------
-SSRRLD-----------------------------------IRNNSQ--------------LNG-
LNVPDAN----LIPVSNTRDVLDLMRIGQKNRAVGATALNERSSRS-HSVLTVHVQGKELASGSI----
------------------LRGCLHLVD----------------------------
LAGSERVEKSEAVG----ERLKEA--------------QHINKSLS------ALGDVIYALAQ------
------KSSHVPYRNSKLTQVLQ-----DSLGGQA-
KTLMFVHINPEVNAVGETISTLKFAQRVASIELG------
>AT1G09170-AtKinesin14-II
--------KGSIRVYCRVRPFLPGQKS-------------------------------
VLTTVDHLEDST-LSIATPSK---------------YGKEGQKTFTFNKVFGPSASQ-----------
------------------EAVFADTQPLIRSVLDGYN---------VCIFAYGQ--------------
--------TGSGKTFTMMGPNEL--------TDETLGVNYRALSDLFHLS------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
------K-----------------------------------IRNSTQ----------------DG-
INVPEAT----LVPVSTTSDVIHLMNIGQKNRAVSATAMNDRSSRS-HSCLTVHVQGKDLTSGVT----
------------------LRGSMHLVD----------------------------
LAGSERIDKSEVTG----DRLKEA--------------QHINKSLS------ALGDVIASLSQ------
------KNNHIPYRNSKLTQLLQ-----DALGGQA-
KTLMFIHISPELEDLGETLSTLKFAERVATVDLG------
>AT5G27000-AtKinesin14-II_ATK4/KATD
--------KGNIRVYCRVRPFLPGQESG-------------------------------
GLSAVEDIDEGT-ITIRVPSK---------------YGKAGQKPFMFNKVFGPSATQ-----------
------------------EEVFSDMQPLVRSVLDGYN---------VCIFAYGQ--------------
--------TGSGKTFTMTGPKEL--------TEESLGVNYRALADLFLLSNQRK-----DT--------
-------------------------------------------------TSYEISVQMLEIYN-----
-EQVRDLLAQDG----------------------------------------------------------
-QTKRLE-----------------------------------IRNNSH----------------NG-
INVPEAS----LVPVSSTDDVIQLMDLGHMNRAVSSTAMNDRSSRS-HSCVTVHVQGRDLTSGSI----
------------------LHGSMHLVD----------------------------
LAGSERVDKSEVTG----DRLKEA--------------QHINKSLS------ALGDVISSLSQ------
------KTSHVPYRNSKLTQLLQ-----DSLGGSA-
KTLMFVHISPEPDTLGETISTLKFAERVGSVELG------
>AT2G47500-AtKinesin14-II
```

```
--------KGSIRVYCRVRPFLPGQSS--------------------------------
FSSTIGNMEDDT-IGINTASR----------------HGKS-LKSFTFNKVFGPSATQ-----------
-------------------EEVFSDMQPLIRSVLDGYN---------VCIFAYGQ--------------
--------TGSGKTFTMSGPRDL--------TEKSQGVNYRALGDLFLLAEQRK-----DT--------
-------------------------------------------------FRYDIAVQMIEIYN-----
-EQVRDLLVTDG-------------------------------------------------------
-SNKRLE-------------------------------------IRNSSQ----------------KG-
LSVPDAS----LVPVSSTFDVIDLMKTGHKNRAVGSTALNDRSSRS-HSCLTVHVQGRDLTSGAV----
-------------------LRGCMHLVD----------------------------
LAGSERVDKSEVTG----DRLKEA--------------QHINRSLS------ALGDVIASLAH------
------KNPHVPYRNSKLTQLLQ-----DSLGGQA-
KTLMFVHISPEADAVGETISTLKFAERVATVELG------
>AT3G10310-AtKinesin14-II
--------KGNIRVYCRVRPIFN--SEM------------------------------
DGVIDYIGKDGS-LFVLDPSK----------------PYKDARKTFQFNQVFGPTATQ-----------
-------------------DDVFRETQPLIRSVMDGYN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPPGR--------SATEMGINYLALSDLFLIYIR-----------------
-------------------------------------------------------------------
-------------------------------------------------------------------
-----------------------------------------------TCSSD---------------DDG-
LSLPDAT----MHSVNSTKDVLQLMEAGEVNRAVSSTSMNNRSSRS-HSIFMVHVRGKD-TSGGT----
-------------------LRSCLHLVD----------------------------
LAGSERVDKSEVTG----DRLKEA--------------QYINKSLS------CLGDVISALAQ------
------KNSHIPYRNSKLTLLLQ-----DSLGGQA-
KTLMFAHLSPEEDSFGETISTLKFAQRVSTVELG------
>AT2G22610-AtKinesin14-III
--------KGNIRVFCRCRPLNTEETST--------------------------------
KSATIVDFDGAK---DGELGVI---------------TGNNSKKSFKFDRVYTPKDGQ-----------
-------------------VDVFADASPMVVSVLDGYN---------VCIFAYGQ--------------
--------TGTGKTFTMEG------------TPQNRGVNYRTVEQLFEVARERR-----ET--------
-------------------------------------------------ISYNISVSVLEVYN-----
-EQIRDLLATSPG-------------------------------------------------------
--SKKLE-------------------------------------IKQSSD-----------------
GSHHVPGLV----EANVENINEVWNVLQAGSNARSVGSNNVNEHSSRS-HCMLSIMVKAKNLMNGDC--
---------------------TKSKLWLVD----------------------------
LAGSERLAKTDVQG----ERLKEA--------------QNINRSLS------ALGDVIYALAT------
------KSSHIPYRNSKLTHLLQ-----DSLGGDS-
KTLMFVQISPSEHDVSETLSSLNFATRVRGVELG------
>AT1G72250-AtKinesin14-III
--------KGNIRVFCRCRPLNFEETEA--------------------------------
GVSMGIDVESTK---NGEVIVM---------------SNGFPKKSFKFDSVFGPNASQ-----------
-------------------ADVFEDTAPFATSVIDGYN---------VCIFAYGQ--------------
--------TGTGKTFTMEG------------TQHDRGVNYRTLENLFRIIKARE-----HR--------
-------------------------------------------------YNYEISVSVLEVYN-----
-EQIRDLLVPASQ--------------------------------------------------------
SASAPKRFE-----------------------------------IRQLSE-----------------
GNHHVPGLV----EAPVKSIEEVWDVLKTGSNARAVGKTTANEHSSRS-HCIHCVMVKGENLLNGEC--
---------------------TKSKLWLVD----------------------------
LAGSERVAKTEVQG----ERLKET--------------QNINKSLS------ALGDVIFALAN------
------KSSHIPFRNSKLTHLLQ-----DSLGGDS-
KTLMFVQISPNENDQSETLCSLNFASRVRGIELG------
>AT5G27550-AtKinesin14-III
--------KGNIRVFCRCRPLNQAEIAN--------------------------------
GCASVAEFDTTQ---ENELQIL---------------SSDSSKKHFKFDHVFKPDDGQ-----------
-------------------ETVFAQTKPIVTSVLDGYN---------VCIFAYGQ--------------
--------TGTGKTFTMEG------------TPENRGVNYRTLEELFRCSESKS-----HL--------
-------------------------------------------------MKFELSVSMLEVYN-----
-EKIRDLLVDNSN--------------------------------------------------------
QPPKKLE-------------------------------------VKQSAE-----------------
GTQEVPGLV----EAQVYNTDGVWDLLKKGYAVRSVGSTAANEQSSRS-HCLLRVTVKGENLINGQR--
---------------------TRSHLWLVD----------------------------
LAGSERVGKVEVEG----ERLKES--------------QFINKSLS------ALGDVISALAS------
------KTSHIPYRNSKLTHMLQ-----NSLGGDC-
KTLMFVQISPSSADLGETLCSLNFASRVRGIESG------
```

```
>AT5G27950-AtKinesin14-IV
--------KGSIRVFCRVRPFLLTERRP---------------------------------------
IREPVS-FGPDNVVI--------------RSAGSSKEFEFDKVFHQSATQ-----------------
------------EEVFGEVKPILRSALDGHN---------VCVLAYGQ--------------------
-TGTGKTFTMDG------------TSEQPGLAPRAIKELFNEASMDQ-----TH--------------
-----------------------------------------SVTFRMSMLEIYM------
GNLKDLLSARQS-----------------------------------------------------
LKSYEASAKCN-------------------------------------LNIQVD--------------
SKGSVEIEGLT----EVEVMDFTKARWWYNKGRRVRSTSWTNVNETSSRS-
HCLTRITIFRRGDAVGSKT----------------------EVSKLWMID------------------
----------LGGSERLLKTGAIG----QTMDEG--------------RAINLSLS------
ALGDVIAALRR------------KKGHVPYRNSKLTQILK-----DSLGTRS-
KVLMLVHISPRDEDVGETICSLSFTKRARAVE--------
>AT1G55550-AtKinesin14-IV
--------KGNIRVFCRVKPLGATEK---------------------------------------
LRPPVASDTRNVIIK---------------LSETKRKTYNFDRVFQPDSSQ-----------------
------------DDVFLEIEPVIKSVIDGYN---------ACIFAYGQ--------------------
-TGTGKTYTMEG------------LPNSPGIVPRAIKGLFKQVE-ES-----NH--------------
-----------------------------------------MFTIHFSMLEIYM------
GNLKDLLLSEA------------------------------------------------------
TKPISPIPPS--------------------------------------LSIHTD--------------
PNGEIDIENLV----KLKVDDFNEILRLYKVGCRSRATASTNSNSVSSRS-HCMIRVSVTSL-
GAPERRR----------------------ETNKIWLVD-----------------------------
LGGSERVLKTRATG----RRFDEG-------------KAINLSLS------ALGDVINSLQR------
------KNSHIPYRNSKLTQVLK-----DSLGQDS-
KTLMLVHISPKEDDLCETICSLNFATRAKNIHLG------
>AT5G10470-AtKinesin14-V_KCA1
----------NIKVFCRARPLFEDEGPS---------------------------------
VIEFPGDCTICVNTSDDTLS---------------N---PKKDFEFDRVYGPHVGQ------------
-----------------AALFSDVQPFVQSALDGSN---------VSILSYGQ----------------
------TNAGKTYTMEG------------SNHDRGLYARCFEELFDLANSDS-----TST---------
-------------------------------------------------SRFSFSLSVFEIYN------
EQIRDLLSET-------------------------------------------------------------
----QS-------------------------------------NLPNIN---------------
MDLHESVIELG----QEKVDNPLEFLGVLKSAFLNRGN----YKSKFNVT-
HLIVSIHIYYSNTITGEN----------------------IYSKLSLVD------------------
----------LAGSEGLIMENDSG----DHVTDL--------------LHVMNSIS------
ALGDVLSSLTS------------GKDSIPYDNSILTRVLA-----DSLGGSS-
KTLMIVNICPSVQTLSETISCLNYAARAR-----------
>AT5G65460-AtKinesin14-V_KCA2
----------NVKVFCRARPLFEDEGPS---------------------------------
IIEFPDNCTIRVNTSDDTLS---------------N---PKKEFEFDRVYGPQVGQ------------
-----------------ASLFSDVQPFVQSALDGSN---------VSIFAYGQ----------------
------THAGKTYTMEG------------SNQDRGLYARCFEELMDLANSDS-----TSA---------
-------------------------------------------------SQFSFSVSVFELYN------
EQVRDLLSGC-------------------------------------------------------------
----QS-------------------------------------NLPKIN---------------
MGLRESVIELS----QEKVDNPSEFMRVLNSAFQNRGN----DKSKSTVT-
HLIVSIHICYSNTITREN----------------------VISKLSLVD------------------
----------LAGSEGLTVEDDNG----DHVTDL--------------LHVTNSIS------
ALGDVLSSLTS------------KRDTIPYENSFLTRILA-----DSLGGSS-
KTLMIVNICPSARNLSEIMSCLNYAARAR-----------
>AT5G65930-AtKinesin14-VI_KCBP
--------KGKIRVYCRIRPLNEKESSE---------------------------------
REKQMLTTVDEFTVEHPWK-----------------DDKRKQHIYDRVFDMRASQ------------
-----------------DDIFEDTKYLVQSAVDGYN---------VCIFAYGQ----------------
------TGSGKTFTIYG------------HESNPGLTPRATKELFNILKRDS-----KR----------
-------------------------------------------------FSFSLKAYMVELYQ------
DTLVDLLLPKS------------------------------------------------------------
-ARRLK-------------------------------------LEIKKD--------------
SKGMVFVENVT----TIPISTLEELRMILERGSERRHVSGTNMNEESSRS-
HLILSVVIESIDLQTQSA----------------------ARGKLSFVD------------------
----------LAGSERVKKSGSAG----CQLKEA-------------QSINKSLS------
```

```
ALGDVIGALSS-----------GNQHIPYRNHKLTMLMS-----DSLGGNA-
KTLMFVNVSPAESNLDETYNSLLYASRVRTIVND------
>AT4G14330-AtKinesinOrph-II_PAKRP2
----------PVEVIGRIRDYPDRKEKSPSILQVNTD--------------------------------
------NQTVRVR---------------ADVGYRDFTLDGVSFSEQEGLE-----------------
----------EFYKKFIEERIKGVKVGNK---------CTIMMYGP---------------------
TGAGKSHTMFG-----------CGKEPGIVYRSLRDIL--GDS------DQDG--------------
----------------------------------------VTFVQVTVLEVYN------
EEIYDLLSTNSSNNL-------------------------------------------------------
GIG-WPKGA-----------------------------------STKVRL--------------
EVMGKKAKNAS----FISGTEAGKISKEIVKVEKRRIVKSTLCNERSSRS-
HCIIILDVPTVGGRLMLVD---------------------------------------------------
----------MAGSENIDQAGQTG---FEAKMQT-------------AKINQGN------
IALKRVVESIAN-----------GDSHVPFRDSKLTMLLQ-----
DSFEDDKSKILMILCASPDPKEMHKTLCTLEYGAKAK-----------
>AT3G17360-AtKinesin12-I_POK1
---------HNVQVLIRLRPLGTMERANQ------------------------------G-
YGKCLKQESPQTLVWLGHP--------------------EAR-FTFDHVASETI--------------
-------------SQEKLFRVAGLPMVENCLSGYN---------SCVFAYGQ----------------
------TGSGKTYTMMGEISEA----EGSLGEDCGVTARIFEYLFSRIKMEEEERRDE-----------
-----------------------------------------------NLKFSCKCSFLEIYN------
EQITDLLEPSS-----------------------------------------------------------
---------------------------------------TNLQLRED-----------------
LGKGVYVENLV----EHNVRTVSDVLKLLLQGATNRKIAATRMNSESSRS-HSVFTCTIESLWE--KD-
------------------SLTRSRFARLNLVD-----------------------------LAGSER-
QKSSGAEG---DRLKEA-------------ANINKSLST------LGLVIMSLVDLAH--------
GKHRHVPYRDSRLTFLLQ-----DSLGG-NSKTMIIANVSPSLCSTNETLSTLKFAQRAKLI-------
--
>AT3G19050-AtKinesin12-I_POK2
---------HNVQILIRVRPLNSMERSIN------------------------------G-
YNRCLKQESSQCVAWIGPP--------------------ETR-FQFDHVACETI--------------
-------------DQETLFRVAGLPMVENCLSGYN---------SCIFAYGQ----------------
------TGSGKTYTMLGEVGDL----EFKPSPNRGMMPRIFEFLFARIQAEEESRRDE-----------
-----------------------------------------------RLKYNCKCSFLEIYN------
EQITDLLEPSS-----------------------------------------------------------
---------------------------------------TNLQLRED-----------------
IKSGVYVENLT----ECEVQSVQDILGLITQGSLNRRVGATNMNRESSRS-HSVFTCVIESRWE--KD-
------------------STANMRFARLNLVD-----------------------------LAGSER-
QKTSGAEG---DRLKEA-------------ASINKSLST------LGHVIMVLVDVAN--------
GKPRHIPYRDSRLTFLLQ-----DSLGG-NSKTMIIANASPSVSCAAETLNTLKFAQRAKLI-------
--
>AT3G44050-AtKinesin12-I
---------HNVQVIIRTRPLSSSEISVQ------------------------------G-
NNKCVRQDNGQAITWIGNP--------------------ESR-FTFDLVADENV--------------
-------------SQEQMFKVAGVPMVENVVAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIEGG----TRRHSVNCGMTPRVFEYLFSRIQKEKEVRKEE-----------
-----------------------------------------------KLHFTCRCSFLEIYN------
EQILDLLDPSS-----------------------------------------------------------
---------------------------------------YNLQLRED-----------------
HKKGIHVENLK----EIEVSSARDVIQQLMQGAANRKVAATNMNRASSRS-HSVFTCIIESKWV--SQ-
------------------GVTHHRFARLNLVD-----------------------------LAGSER-
QKSSGAEG---ERLKEA-------------TNINKSLST------LGLVIMNLVSVSN--------
GKSVHVPYRDSKLTFLLQ-----DSLGG-NSKTIIIANISPSSSCSLETLSTLKFAQRAKLI-------
--
>AT4G14150-AtKinesin12-II_PAKRP1
-----------VKVIVRMKPLNK---GEE------------------------------G--
DMIVEKMSKDSLTVSGQ-----------------------TFTFDSIANPES---------------
-------------TQEQMFQLVGAPLVENCLSGFN---------SSVFAYGQ----------------
-----TGSGKTYTMWGPANGLLE--EHLCGDQRGLTPRVFERLFARIKEEQVKHAER-----------
-----------------------------------------------QLNYQCRCSLLEIYN------
EQITDLLDPSQ-----------------------------------------------------------
---------------------------------------KNLMIRED-----------------
VKSGVYVENLT----EEYVKNLTDVSQLLIKGLGNRRTGATSVNTESSRS-HCVFTCVVESRCKNVAD-
------------------GLSSFKTSRINLVD-----------------------------LAGSER-
```

```
QKSTGAAG---ERLKEA-------------GNINRSLSQ------LGNLINILAEISQT-------
GKPRHIPYRDSRLTFLLQ-----ESLGG-NAKLAMVCAVSPSQSCRSETFSTLRFAQRAKAI-------
--
>AT3G23670-AtKinesin12-II_PAKRP1L
-----------VKVIVRMKPPSK---GEE-------------------------------E--
EMIVKKISNDALTINEQ-----------------------TFTFDSIADPES---------------
-------------TQDEIFQLVGAPLVENCLAGFN---------SSVFAYGQ----------------
-----TGSGKTYTMWGPANGLLE--EHLSGDQRGLTPRVFELLFARLSEEQAKHAER------------
-------------------------------------------QLKYQCRCSFLEIYN------
EQITDLLDPSL-----------------------------------------------------------
-------------------------------------KNLMIRED-----------------
VKSGVYVENLT----EEYVKNLKDLSKLLVKGLANRRTGATSVNAESSRS-HCVFTCVVESHCKSVAD-
------------------GLSSFKTSRINLVD-----------------------------LAGSER-
QKLTGAAG---DRLKEA-------------GNINRSLSQ------LGNLINILAEISQT-------
GKQRHIPYRDSRLTFLLQ-----ESLGG-NAKLAMVCAVSPSQSCRSETFSTLRFAQRAKAI-------
--
>AT3G20150-AtKinesin12-II
----------HVKVVVRIKP------TKE-------------------------------Y--
CWKVKKVSKVSYSVRDR-----------------------HFTFDSVLDSNL----------------
-------------NQDDVFQQIGVPLVRDALSGYN---------TSVLSYGQ----------------
-----NGSGKTYTMWGPAGSMLE--DPSPKGEQGLAPRIFQMLFSEIQREKIKSGGK------------
-------------------------------------------EVNYQCRCSFLEIYN------
GQISDLIDQTQ-----------------------------------------------------------
-------------------------------------RNLKIKDD-----------------
AKNGIYVENLT----EEYVDSYEDVAQILMKGLSSRKVGATSTSFQSSRS-
HVILSFIVESWNKGASSR------------------CFNTTRTSRINLVD------------------
----------LAGAGT-NERD-ATK---HCVEEE-------------KFLKKSLSE------
LGHVVNSLAENVHP-------GISDRSLHKTSCLTHLLQ-----ESLGG-
NSKLTILCNIFPSDKDTKRTMSTLRFGERAKAM---------
>AT5G02370-AtKinesin10
----------NVRVVLRVRPFLPREISDESCDG--------------------------
RSCVSVIGGDGGDTSEVAVYLKD-------------PDSCRNESYQLDAFYGREDDNVK---------
-------------------HIFDREVSPLIPGIFHGFN---------ATVLAYGA--------------
--------TGSGKTFTMQGI------------DELPGLMPLTMSTILSMCEKTR---------------
-----------------------------------------------------SRAEISYYEVYM-----
-DRCWDLL-EVKDN---------------------------------------------------------
-------------------------------------EIAVWDD-----------------
KDGQVHLKGLS----SVPVKSMSEFQEAYLCGVQRRKVAHTGLNDVSSRS-HGVLVISVTSQG------
----------------------LVTGKINLID-----------------------------
LAGNEDNRRTGNEGI----RLQES-------------AKINQSLFA------LSNVVYALNN------
------NLPRVPYRETKLTRILQ-----DSLGG-TSRALMVACLN--PGEYQESLRTVSLAARSR-----
-------
>AT5G23910-AtKinesin10
-------------------------STDLAS--------------------------
TKSISVQKPMGDDSETVTISFGA-------------QFAGSKDSYRLDYCYEE-NETTG---------
------------------SILTKEIKPLISTVFEGKD---------ANVIAHGA--------------
--------RNSGKTHLIQGN------------ERELGLAVLTMSEMLSMAEERG---------------
-----------------------------------------------------DAIFVSVYEVSQ-----
-ETVYDLLDQEKR---------------------------------------------------------
-------------------------------------VVSVLEG-----------------
AQGKIQLKGLS----QVPVKSLSEFQNLYFG----FKKSQKLTSDLPTRS-HKGVMIHVTTGNANS---
----------------------GSLGRMNFLD-----------------------------
MAGYEDSRKQ-NSAL----GPLEI-------------ARVNKSIYA------LQNVMYALNA------
------NESHVPYRESKLTRMLK-----DCLKG-SNITLLITCLP--REFSQDSFYMLNLASRI-----
-------
>AT3G54870-AtKinesinARK_ARK1
-----------------------------------------------------------------------
-----------WN-------------------SESYKFDEVFTDTAS--------------------
--------QKRVYEGVAKPVVEGVLSGYN---------GTIMAYGQ---------------------
TGTGKTYTVGKIGKDDAAER--------GIMVRALEDILLNASSASI----------------------
-----------------------------------------SVEISYLQLYM------
ETIQDLLAP-------------------------------------------------------------
-------------------------------EKNNISINEDAK----------------
TGEVSVPGAT----VVNIQDLDHFLQVLQVGETNRHAANTKMNTESSRS-
```

```
HAILTVYVRRAMNEKTEKAKPESLG---------DKAIPRVRKSKLLIVD------------------
----------LAGSERINKSG--TDGH--MIEEA--------------KFINLSLTS------
LGKCINALAEGS------------SHIPTRDSKLTRLLR-----DSFGG-
SARTSLIITIGPSARYHAETTSTIMFGQRAM-----------
>AT1G01950-AtKinesinARK_ARK2/ATKINUB
----------RVRVAVRLRPRNADESVADADFADCVE----------------------------
LQPELKRLKLRKNNWD--------------------TETYEFDEVLTEAAS------------------
-----------QKRVYEVVAKPVVESVLEGYN---------GTVMAYGQ--------------------
--TGTGKTFTLGRLGDEDTAAR--------GIMVRSMEDIIGGTSLDTD--------------------
-----------------------------------------------SISVSYLQLYM------
ETIQDLLDP-----------------------------------------------------------
-----------------------------------TNDNIAIVEDPR----------------
TGDVSLPGAT----HVEIRNQQNFLELLQLGETHRVAANTKLNTESSRS-
HAILMVHVKRSVVENEFPVSNEMESSSH----FVRPSKPLVRRSKLVLVD------------------
----------LAGSERVHKSG--SEGH--MLEEA--------------KSINLSLSA------
LGKCINAIAENS------------PHVPLRDSKLTRLLR-----DSFGG-
TARTSLIVTIGPSPRHRGETTSTILFGQRAM-----------
>AT1G12430-AtKinesinARK_ARK3/ATKINUA
----------RVRVAVRLRPRNGEELIADADFADCVE----------------------------
LQPELKRLKLRKNNWD--------------------TDTFEFDEVLTEYAS------------------
-----------QKRVYEVVAKPVVEGVLDGYN---------GTIMAYGQ--------------------
--TGTGKTYTLGQLGEEDVADR--------GIMVRAMEDILAEVSLETD--------------------
-----------------------------------------------SISVSYLQLYM------
ETVQDLLDP-----------------------------------------------------------
-----------------------------------SNDNIAIVEDPK----------------
NGDVSLPGAT----LVEIRDQQSFLELLQLGEAHRFAANTKLNTESSRS-HAILMVNVRRSMKTRDG--
-LSSESNGN-SHMTKSLKPPVVRKGKLVVVD-----------------------------
LAGSERINKSG--SEGH--TLEEA--------------KSINLSLSA------LGKCINALAENS----
--------SHVPFRDSKLTRLLR-----DSFGG-TARTSLVITIGPSPRHRGETTSTIMFGQRAM----
-------
>AT1G20060-AtKinesinOrph-IV
-----------------------------------------------------------
KKDEEACITLNDSYSVTLTPPQSLQE------------LKRSKTEVYEGFSHVFPADCS----------
-------------------QNDVYDKMVQPLLEDFMKGKS---------GMLAALGP------------
----------SGSGKTHTVFG------------SLKDPGIVPITLRQIFKKNDESCS------------
-----------------------------------------------GSLRSFNLSIFEICSER-
GKGEKAYDLLGGESSELS-------------------------------------------------
-----------------------------------------------------------------
VQQSTIRGLK----EVPIQNLEEAESLIGQAMLKRATATTNSNSQSSRS-QCIINIRA----
SCNGFSN---------------ETKLQSSDAMLTIVD-----------------------------
LAGAER-EKRTGNQA---IDFGLPGTDPYFILFLMPTIPLTMNTRYL------LAVTVGVPEEPKEGI-
-----AETSSKFFGS--LTRYLR-----DYLEG-KKRMALILTVKAGEEDYLDTSYLLRQASPYM----
-------
>Phypa_451243-PpKinesinOrph-IV
-----------------------------------------------------------------
-----------------------------------------------------------------
-----------------------------------------------------------------
-----------------------------------------------------------------
-----------------------------------------------------------------
-----------------------------------------------------------------
-----------------------------------------------------------------
----------------------------MNCESSRS-HCVF--LLTIQQ-----S-------------
-DIED--RSIKTGKIYLVD-----------------------------LAGSEK-VEKTGAEG---
KLLCEA--------------KTINKSLSA------LGNVINALTS-----------
DKPCHVPYRDSKLTRILQ-----DSLGG-NSRTALLCCCSPSTLHASETLSTLRFGTRAKLI-------
--
>Phypa_425592-PpKinesin2
---------ERVQVVVRCRPMLVKENAEG-----------------------------
RNNCVLVDTVGSTIQVKNLK-----------------QPEQEPKLFTFDKTYDATS------------
---------------TQKQLYDDVAHPIVHSVMCGYN---------GTVLAYGQ--------------
--------TASGKTFTMDG---LDDP------PEMRGIIPQAFEGIFTHIQ-D----SQS---------
-----------------------------------------------SDNFLVRASYLEIHN-----
-EEIRDLLATGSQS-----------------------------------------------------
-----------------------------------SSRLELKEN-----------------
```

```
VEGNVYVKNLT----SITVQSVADISHLLTVGKKSRSVGATLMNQDSSRS-HSIF--
TITVEASARSSS-------------AETDGSMHIRVGKLNLVD-----------------------
----LAGSER-LNKTGATG---DRFREL-------------TNINWSLSA------LGNVISALVDD-
----------KSSHVPYRDSKLTRLLQ-----DSLGG-
NTRTVMIANIGPADYNYDESVSTLRYANRAKSI---------
>Phypa_453193-PpKinesin4-Id
----------AVQVALNIRPLIPLERVQG------------------------------
CKECISVVPGEPQVQIG------------------------HHSFTFDYIFGSSD------------
--------------TPSSAIFDKCVKPLVEGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTGYTVGGSTD--------GVIPQVMQTIFKRIETLKHK-------------
-------------------------------------------------ADFQLRVSFIEILK-----
-EEIHDLLDP----NPPSTEY-----------------------------------------------
--------------------------IFGGGLKALAVGKPPIQIRET-----------------
TAGGITLMGVT----EADVKSLEEMAAYLEHGSLSRATGSTNMNSHSSRS-HAIFTITLEQRRKWDPL-
------PDGG--NPSPEDCNEDYLCAKLHLVD----------------------------LAGSER-
AKRTGADG---LRFKEG--------------IHINRGLLA------LGNVISALGDERKRR-------
EGGHVPYRDSKLTRLLQ-----DSLGG-NSRTVMIACVSPADVNAEESINTLKYANRARNIR-------
-
>Phypa_438737-PpKinesin4-Ib
----------AVQVALNIRPLIALERAQG------------------------------
CKDCITVVPGEPQVRIG-------------------------THSFTFDHVFGSSG-------------
---------------LSLSGLYEKCVKSLVDGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTAYTVGGNID--------GVIPHAMQHIFKQIETLKIK-------------
-------------------------------------------------TDFQIRVSYIEILK-----
-EEVHDLLDP----NPPATEA-----------------------------------------------
--------------------------NFGGGSKPITVGKPPIQIRET-----------------
TNGGITLAGVT----ETDVKSLEEMAACLEQGSLCRATASTSMNSQSSRS-HAIFTITLEQRRKWEML-
------PDGN--NALLEDCNEDYLCAKLHLVD----------------------------LAGSER-
AKRTGADG---LRFKEG--------------VQINKGLLA------LGNVISALGDDKKRK-------
EGGHVPYRDSKLTRLLQESGCVDSLGG-NSSTVMIACVSPADSNAEENLNTLKYANRARNIQ-------
-
>Phypa_432365-PpKinesin4-Ic
----------AVQVALNIRPLIALERAQG------------------------------
CKDCITVVPGEPQVQIG-------------------------THSFTFDHVFGSSG-------------
---------------SPLSGLYDKCVKSLVDGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTAYTVGGCID--------GVIPHVMQDIFQHVETLKKK-------------
-------------------------------------------------VDFQIRVSFIEILK-----
-EEVHDLLDP----NPPSTEA-----------------------------------------------
--------------------------NFGGGSKPTTMGKPPIQIRET-----------------
SNGGITLAGVT----EPDVKSLEEMAACLEQGSLCRATGSTNMNSQSSRS-HAIFTIMLEQRRKWEML-
------PDGG--NPLLEDCNEDYLSAKLHLVD----------------------------LAGSER-
AKRTGADG---LRFKEG--------------VHINKGLLA------LGNVISALGDDRKRK-------
EGGHVPYRDSKLTRLLQ-----DSLGG-NSSTVMIACVSPADSNAEETLNTLKYANRARNIQ-------
-
>Phypa_437833-PpKinesin4-Ia
----------AVQVALNVRPLITQERIQG------------------------------
CKDCILVVPGEPQVQIG-------------------------SHSFTFDHVFGSTG-------------
---------------TPLSTIFDRCVAPLVEGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTAYTVGGNSD--------GVIPKVMETIFNKVETLKDS-------------
-------------------------------------------------AEFNLRISFIEILK-----
-EEVHDLLDF----SPPSTELPTSNGTNGL-------------------------------------
--------------------------AFGGGLKTGATVKPPIQIRET-----------------
GNGGITLAGVT----ETEVTTLAEMAICLEQGSLCRATGSTNMNSSSSRS-HAIFTITVEQKRKWDS--
------PTAC--AGVLDDSSDDYLCAKLHLVD----------------------------LAGSER-
AKRTGADG---MRFKEG--------------VHINKGLLA------LGNVISALGDDKKRK-------
EGGHVPYRDSKLTRLLQAS--ADSLGG-NSRTVMIACVSPADSNAEETLNTLKYANRARNIQ-------
-
>Phypa_447296-PpKinesin4-IIa
---------SSVRVAVRARPLIQKEIAEN------------------------------
SKECVFYSKDRKQVVLGK-----------------------GRRFTFDHVFSPVI-------------
---------------SQED-VYNECVKPLVESCCAGYN---------ATVLAYGQ--------------
--------TGSGKTFTMGCGNKSSSLEE------ELGVLPRAIRQLFKIVEERSHE-------------
-------------------------------------------------TEFLVKCAFVEIYN-----
-DEIKDLLHP----DTPPK-------------------------------------------------
```

```
----------------------------------------AISIRED----------------
ANGDIILAGVR----EEEVTNFKSMIRFLEYGSVFRTTGSTLMNQHSSRS-HAIFTIIVEQR-------
------------SILECASRNDVITAKFHLVD-----------------------------LAGSER-
VKRTGAVG---MRFKES-------------VTINCGLLA------LGNVISALGDERKR---------
CQHVPYRESKLTRMLQ-----DSLGG-NSRTCMIACISTADTDLEETLNTLKYANRARNIR--------
>Phypa_446183-PpKinesin4-IIc
----------SVRVAVRARPLVEKELVEN------------------------------
CSECVSYSECGKQVIIGA-----------------------ERRFTFDHVFGAAT------------
--------------SQED-VYTNCVKPLVESCCAGYN---------ATVLAYGQ--------------
--------TGSGKTFTMGCGNNVSLLKE------DLGILPRAIRQLYECVEERSNQ-------------
-------------------------------------------------AEFLIKCAYVEIYN-----
-EEIKDLLHP----DTPSK-----------------------------------------------
----------------------------------------SISIRED----------------
AKGDIVLAGVK----EEVVTNFENMIRLLEHGSMFRTTGSTLMNQHSSRS-HAIFTIIVEQR-------
------------SILDSA-SNEVITAKFHLVD-----------------------------LAGSER-
VKRTGAVG---MRFKES-------------VTINCGLLA------LGNVISALGDERKR---------
CQHVPYRESKLTRMLQ-----DSLGG-NSRTCMIACISTADTNFEETLNTLKYANRARNIR--------
>Phypa_433281-PpKinesin4-IIb
---------SSVRVAVRARPLIEKEIVEK------------------------------
CKECVSYSQDGKQVVLGK-----------------------DRRFTFDHVFGPIV------------
--------------SQED-VYIDCVKPLVESCCAGYN---------ATVLAYGQ--------------
--------TGSGKTFTMGCGNNASLLEE------ELGILPRAIRQLFECVEERSNQ-------------
-------------------------------------------------AEFLIKCAFVEIYN-----
-EEIKDLLHP----DTPSR-----------------------------------------------
----------------------------------------SISIRED----------------
ANGDIVLAGVK----EEVVTSFESMIRFLEHGSVFRTTGSTLMNQHSSRS-HAIFTIIVEQR-------
------------SIVECTNTNDVITAKFHLVD-----------------------------LAGSER-
AKRTGAVG---MRFKES-------------VTINCGLLA------LGNVISALGDERKR---------
CQHVPYRESKLTRMLQ-----DSLGG-NSRTCMIACISTADTNFEETLNTLKYANRARNIR--------
>Phypa_425536-PpKinesin5-c
--------GVAVQVLLRCRPFSEDEKRTK------------------------------
SPQVISCHDQRREVTVFQN----------------IASKQIDRTFTFDKVFGPQS-------------
---------------RQLDLYEQAIVPIVNEVLDGYN---------CTIFAYGQ--------------
--------TGTGKTYTMEGSGRKSKN---GELPPDAGVIPRAIQQIFETLD------RDD---------
-------------------------------------------------QEYSVKVTYLELYN-----
-EELTDLLAPEEYSK--VVIDEKI-----------------------------------------------
-------------------------------------KKPLALMED----------------
GKGGVLVRGLE----EEIVTSANQIYTLLDRGSAKRQTAETLLNKQSSRS-HSIFSITIHMKE------
--------------TTPEGEELMKCGKLNLVD-----------------------------LAGSEN-
ISRSGAKD---NRAREA-------------GEINKSLLT------LGRVITALVEH------------
LGHIPYRDSKLTRLLR-----DSLGG-KTKTCIIATVSPSVHCLEETLSTLDYAHRAKNIKNKPEVNQK
>Phypa_447260-PpKinesin5-b
--------GVAVQVLLRCRPFNEEEKRAK------------------------------
TPQVISCNDSRREVTVCQN----------------IASKQIDRTFTFDKVFGPNS-------------
---------------RQVDLYDQAVVPIVNEVLDGFN---------CTIFAYGQ--------------
--------TGTGKTYTMEGSGRKSKN---GDLPADAGVIPRAVQQIFETLD------RDN---------
-------------------------------------------------QEYSVKVTYLELYN-----
-EELTDLLAPEEYSK--VVIDEKV-----------------------------------------------
-------------------------------------KKPLALMED----------------
GRGGVLVRGLE----EEIVTSANEIYTLLDRGSAKRQTAETLLNKQSSRS-HSIFSIIIHIKE------
--------------TTPEGEELMKCGKLNLVD-----------------------------LAGSEN-
ISRSGAKD---NRAREA-------------GEINKSLLT------LGRVITALVEH------------
LGHVPYRDSKLTRLLR-----DSLGG-RTKTCIIATVSPSVQCLDETLSTLDYAYRAKSIKNKPEVNQK
>Phypa_457162-PpKinesin5-a
-----------VQVLLRCRPLNEEEKRIK------------------------------
NPQVISCNDTRREVTVLQT----------------IASKQIDRTFTFDKVFGPAS-------------
---------------RQVDLYDQAIAPIVNEALDGFN---------CTIFAYGQ--------------
--------TGTGKTYTMEGLGRKSKN---GELPADAGVIPRAIQQIFETLD------KEN---------
-------------------------------------------------QEYSVKVSYLELYN-----
-EELTDLLAPEEYSK--VVIDEKI-----------------------------------------------
-------------------------------------KKPLALMED----------------
GRGGVLVRGLE----EEIVTSANEIYTLLDRGSAKRQTAETLLNKQSSRS-HSIFSITIHIKE------
--------------TTPEGEELMKCGKLNLVD-----------------------------LAGSEN-
```

```
ISRSGAKD---NRAREA-------------GEINKSLLT------LGRVITSLVEH------------
LGHVPYRDSKLTRLLR-----DSLGG-KTKTCIIATVSPSVHCLDETLSTLDYAYRAKNIKNKPEVNQK
>Phypa_423604-PpKinesin5-d
--------GVNVQVLVRCRPLSDDEKKAK------------------------------
SPQVISCNEQRREVTAFQC-----------------SAHKQIDRTFTFDKVFGPQC------------
----------------KQIELYDESIVPIVNEVLDGYN---------CTIFAYGQ--------------
--------TGTGKTFTMEGSGMKSKN---GELPPDAGVIPRAIQQIFETLD------KDE---------
-------------------------------------------------QEYSVKVTYLELYN-----
-EELTDLLAPEEYSK--VVIDEKV-----------------------------------------------
-------------------------------------KKHLQLMED-----------------
GKGGVLVRGLE----EEIVTSASHIYTLLDRGSARRQTADTLLNKQSSRS-HTIFSITIHVKE------
--------------TTPEGEELMKCGKLNLVD-----------------------------LAGSEN-
ISRSGAKD---MRARET-------------GEINKSLLT------LGRVITALVEH------------
LGHIPYRDSKLTRLLR-----DSLGG-KTKTCIIATVSPSVPCLEETLSTLDYAYRAKNIKNKPEVNQK
>Phypa_447411-PpKinesin7-Ia
----------NVSVTVRFRPLSQREIQ---------------------------------
RGDEIAWYADGDT-VRSEL----------------NLSTV-----YAFDRVFGPAT------------
----------------TTRGVYDAAAQHVVSGAMEGVN---------GTVFAYGV--------------
--------TSSGKTHTMHGD------------QKSPGIIPLAVKDVFSIIQE-----TP----------
-------------------------------------------------SREFLLRVSYLEIYN-----
-EVINDLLDPIG-----------------------------------------------------------
-------------------------------------QNLRVRED-----------------
SQSGTYVEGIK----EEVVLSPAHALSLIAAGEEHRHVGSNNFNLLSSRS-HTIFTMTVESSPR-
GDGYT-----------------D-EDVTLSQLNLID-----------------------------
LAGSE--SSKTETTG---LRRKEG-------------SYINKSLLT------LGTVISKLS-------
----DGKASHVPYRDSKLTRLLQ-----SSLSG-HGRISLICTITPATSNNEETHNTLKFAHRAKRI--
-------
>Phypa_437231-PpKinesin7-Ib
----------NVSVTVRFRPLSQREIQ---------------------------------
RGDGIAWYADGDT-VRSEL----------------NLSTV-----YAFDRVFGPAT------------
----------------TTRGVYDAAAQHVVSGAMEGVN---------GTVFAYGV--------------
--------TSSGKTHTMHGD------------QKSPGIIPLAVKDVFSIIQE-----TP----------
-------------------------------------------------SREFLLRVSYLEIYN-----
-EVINDLLDPIG-----------------------------------------------------------
-------------------------------------QNLRVRED-----------------
GQAGTYVEGIK----EEVVLSPAHALSLIAAGEEHRHVGSNNFNLLSSRS-HTIFTMTVESSPR-
GDGYT-----------------D-EDVTLSQLNLID-----------------------------
LAGSE--SSKTETTG---LRRKEG-------------SYINKSLLT------LGTVIAKLS-------
----DGKASHIPYRDSKLTRLLQ-----SSLSG-HGRISLICTITPATSNNEETHNTLKFAHRAKRI--
-------
>Phypa_426030-PpKinesin7-III
----------KIFVSIRVRPLSKAEA---------------------------------
AKGSPWKLGPNSIALCNNSG-----------------APISGQAYKFDKVFGSET--------------
---------------STLEIYETHTKDIIASAVRGFN---------GTVFAYGQ--------------
-------TSSGKTYTMRGN------------SSEPGIIPLAVQEIFKNIQE-----AE-----------
-------------------------------------------------DREFLLRVSYMEIYN-----
EEINDLLAPEN------------------------------------------------------------
-------------------------------------RKLQVHEN-----------------
IERGIFVAGLR----EEIVVSPEQVLDLMTAGENYRHVGETNMNAYSSRS-
HSIFRMVIESRDRSHDDPADP-----------GTVQSCDAVRVSVLNLVD------------------
----------LAGSER-VAKTGAEG---ARLKEG-------------THINKSLMT------
LGTVINKLSEGV--------EKQGGHVPYRDSKLTRILQ-----PALGG-
NAKTAVICNITPAQIHVDETKGTLFFASRANRV---------
>Phypa_454208-PpKinesin7-IIc
----------KIYVTVRVRPLSAKEVA---------------------------------
RSDASDWVCTSEQSIAFKH----ALQERS------PFPAA-----YTLDRVFGPDC------------
----------------LTRRVYEEGAKDVALSALTGLN---------STIFAYGQ--------------
--------TSSGKTYTMRG-----------------VTESAIADIFEYIEH-----NT----------
-------------------------------------------------DREFLLKASALEIYN-----
-EVVKDLLTPEG-----------------------------------------------------------
-------------------------------------VPLRLLDD-----------------
KEKGTVVDKLK----EEVIRDISHLRQLIKICEAQRQVGETSLNDTSSRS-
HQIIRLTVESHPSGVSPG-----------------IPSASLIASLNFVD------------------
----------LAGSER-ASQTHADG---TRLREG-------------AHINRSLLT------
```

```
LSTCIRKLSGGS--------AKKG-HIPFRDSKLTRILQ-----HSLGG-
NARTAIICTMSPAHSHVEQSRNTLAFATRAKEV---------
>Phypa_437822-PpKinesinOrph-IVc
------------------------------------------------------------------
------------------------------------------------------------------
---------MAQYSLTARQAVAKPTR---------------------------------------
--------------------------------CRDLLP--IK-----IV------------------
-------------------------------------DREFLVRVSYMEIYN------
EEINDLLAPDN-------------------------------------------------------
-------------------------------------RKLQIHES-----------------
IERGIFVAGLR----EEIADSVEQVIAVLERGEAQRHLAETDMNVNSSRS-
HTIFRMVIESRDKSHDSTQDS-----------DP-SAQDAVRVSALNLVD-------------------
----------LAGSER-ISKTGAEG---VRLREG--------------AHINKSLTT------
LGMVINKLSEGG--------GKQGAHVPYRDSKLTRILQ-----SALGG-
NARTSIICTINPDEIHIDETRGTLQFASRAKRV---------
>Phypa_458197-PpKinesin7-IIa
----------NIYVTVRVRPLSAKEVA--------------------------------
RSDVSDWVCSNGHTIAYKH----ALPERS------PFPAA-----YTFDRVFGPDC-------------
---------------QTLRVYEEGAKDVALSALTGLN---------ATIFAYGQ--------------
--------TSSGKTFTMRG-----------------VTDSAIADIFDYIQR-----SP----------
-------------------------------------------------DREFVLKVSALEIYN-----
-EVVKDLLATEG------------------------------------------------------------
-------------------------------------APLRLLDD-----------------
KEKGTVVDKLK----EEVVRDINHLRQVIKICEAQRQVGETSLNDVSSRS-
HQIIRLTVESHPFGVAPG-----------------STATSLIASLNFVD-------------------
----------LAGSER-ASQTNADG---ARLKEG--------------AHINRSLLT------
LSTCIRKLSGGS--------KIKG-HIPYRDSKLTRILQ-----HSLGG-
NARTAIICTMSPAHSHVEQSRNTLAFATRAKEV---------
>Phypa_432536-PpKinesin7-IIb
----------KIFVTVRVRPLSAKEAA--------------------------------
RGDVCDWVCTTDNTIAFEH----ALPERS------PFPAA-----YSFDRVFGPGS-------------
---------------RTQRVYEEGAKDVALSVLAGLN---------STIFAYGQ--------------
--------TSSGKTFTMRG-----------------VTESAIADIFEYIQR-----DS----------
-------------------------------------------------EREFVLKVSGLEIYN-----
-EVVKDLLASDN------------------------------------------------------------
-------------------------------------APLRLLDD-----------------
KEKGTVVDKLK----EEVVRDVSHLQQVLKICEAQRQVGETSLNDVSSRS-
HQIIRLTVESHPYGVARG-----------------SPATSLLASLNFVD-------------------
----------LAGSER-ASQTNADG---ARLKEG--------------AHINRSLLT------
LSTCIRKLSGGS--------KTKGGHIPYRDSKLTRILQ-----HSLGG-
NARTAIICTMSPAHSHVEQSRNTLAFATRAKEV---------
>Phypa_453903-PpKinesin8-I
----------TLQVAVRCRPLTAKER-LK-------------------------------
SRDILRVVDDKVVVVLDPDTTKDYLDRV---------QNRSKEKKYTYDVAFSPE--------------
---------------AKNADVYNVTASGIVEGVVRGLN---------ATIFAYGA--------------
--------TGSGKTHTMAG------TPD------DPGLMVLSLQSIFALISK-QEAEY-----------
-------------------------------------------------EFEVTCSYLEVYN-----
-EVIYDLLERSSG------------------------------------------------------------
-------------------------------------HLELRED-----------------
PDQGITVAGLK----RIKVSSAEKILELLTQGNNRRKTESTDANATSSRS-HAVLEIIVKRTQRNQYR-
--------------------AQTLRGKLALVD-----------------------------LAGSER-
ASETNNAG---QKLRDG--------------ANINRSLLA------LANCINALGKQQKK--------
GLAYVPYRNSKLTRLLK-----DGLSG-NSRTVMVATVSCGADQYHHTTNTLKYADRAKEI--------
-
>Phypa_424121-PpKinesin8-Ib
----------TLQVAVRCRPLTAKER-IK-------------------------------
SRDILRVVDDKVVVVLDPDTSKDYLDRV---------QNRSKEKKYTYDVAFSSE--------------
---------------AKNADVYNVTASSIVDGVVRGLN---------ATIFAYGA--------------
--------TGSGKTHTMAG------TPE------DPGLMVLSLQSIFTLISK-QEAEY-----------
-------------------------------------------------DFEVTCSYLEVYN-----
-EVIYDLLERSSG------------------------------------------------------------
-------------------------------------HLELRED-----------------
PDQGITVAGLK----RIKVSSAEKILELLTQGNNRRKTESTDANATSSRS-HAVLEIIVKRTQRNQLR-
--------------------AQTLRGKLALVD-----------------------------LAGSER-
```

```
ASETNNAG---QKLRDG-------------ANINRSLLA------LANCINALGKQQKK--------
GLAYVPYRNSKLTRLLK-----DGLSG-NSRTVMVATVSCGADQYHHTTNTLKYADRAKEI--------
-
>Phypa_458481-PpKinesin8-II
----------RIMVYVRARPLSKKEKEAG------------------------------
SRSCVRIVNKRDVYLTEFALE-TDYLR----------LKRVRGRHFAFDASFPDS--------------
--------------TSQQEVYNTSTAQLVEGVLQGRN---------GSVFCYGA--------------
--------TGAGKTHTMLG------TLQ------SPGVMVLALKDLFAKIKQ-RSKDG-----------
-------------------------------------------------DHVVRLSYLEVYN-----
-ETVRDLLSPGR-------------------------------------------------------
----------------------------------------PLVLRED-----------------
SKQGIVAAGLT----QYQAYSADEVIHLLQRGNLNRTTEPTRVNETSSRS-HAILQVVAEYKLQQETG-
--------------------VTVRVGKLSLID-----------------------------LAGSER-
ALATDQRT---LRSVEG-------------ASINRSLLA------LSSCINALCE------------
GKKHIPFRNSKLTQLLK-----DSLGG-SCRTAMIANISLSDASFGETQNTLHWADRAKEI--------
-
>Phypa_458410-PpKinesin9-a
-----------IKVHVRIR-PTAK-PSPMFHIEDQT-----------------------
NTVLIDLLKQNGGGAPESFVD-------------------QIAFSVNSITQSTD--------------
---------------QSEVYESCGRALVKDFLEGYN---------ATFIAYGQ---------------
-------VGSGKTYTMAGDMKVNAHRG---------FIARAIHQIFEEKEADPGS--------------
-------------------------------------------------GIVLYISYLEIYQ------
E-------------------------------------------------------------------
-------------------------------------RSKDLMIIEES------------------
GYVYIRGLA----KIPVETEAQALMCFSEGEKQRSYACHQINQVSSRS-HTIFTLCMEKRVG-------
-------------RFKTEYDTVVAKLNLVD----------------------------
LAGFERLKKTNTSTGGR--MRVEA-------------CSINKSLCL------LEQAVYAIK-QGQE--
---------YVPFRQSKISIILK-----EALSG-NCRTELILCLWPEEYFLDET---------------
-------
>Phypa_425498-PpKinesin9-b
MPYVDDALLSRIRVYLRLR-PSVK-PSPAINIESET-----------------------
HRVLIDVEKSIGGGPPKAYVN-------------------QIVFNVDNIVQ-----------------
-------------------------------------------------------------------
---------------------VYVHRG---------IIPRAIQQIFEEKEAKPEA--------------
-------------------------------------------------GIVVHMSYMEIYQ------
EGLYDLLQ----------------------------------------------------------
-----------------------------------KRRDDLMIIEDN------------------
QLLNVRGLA----KVRVETETEALKWFQEGEKSRSFGNHFLNSLSSRS-HTILTFYMERRVA-------
-------------RVSTQLALQVAKLNLVD----------------------------
LAGVERLKKTKGDTGSL--MRKEA-------------CINNKTLSF------LEQTIFALR-LKKA--
---------HIPFRHSKVTTLLK-----ESLGN-NHKTVFMVCAWPEEYFLDETIGALRFAQRVK----
-------
>Phypa_428375-PpKinesin9-c
---------STIDIYLRVR-PISSGAKAVLELNQEE-----------------------
GRVTWTIPRHVSLGLANHQRE-------------------HFTFKFTGLFDMESK-------------
----------------QDEVFQKVAHKVVIGSLDGYN---------GTIFAYGQ---------------
-------TGSGKTYTITGGSERYVDRG---------IIPRTISLIFSEIAERSEY--------------
-------------------------------------------------AYTLHFSYMEVYN------
ETGYDLLNPDHETKA----------------------------------------------------
----------------------------------
LEDLPKDFILANEPIIANYQFANAFRVATNPDVHFLQNPAGLGRNRWNSNEEEALNLVFVGDTNRIISS
TPMNMASSRS-HCIFTAHILACK----------------------VGEETVRKSKLHLVD--------
---------------------LAGSERVWKTG--VDG-------------------------------
-------QVIVALQEKFQGKM-------RTHIPYRNSMMTSVLR-----DSIGG-
NCLTVMIATVTIAQDQLPETISTCRFAQRVA-----------
>Phypa_438664-PpKinesin13-a
----------RIRVVVRKRPLNKKEILRK------------------------------
EEDIVTISDMDSSLTVNEPKVKVDLTA------------YTERHEFVFDAVLDQQ-------------
---------------VSNDEVYRSTVEPIVPTIFNRTK---------ATCFAYGQ--------------
--------TGSGKTYTM-----------------QPLPLRACGDMMAIMQQPNYRNQ-----------
-------------------------------------------------GFQLWLSFFEIYG-----
-GKLYDLLNERR-------------------------------------------------------
-----------------------------------KLCMRED-----------------
GRQQVCIVGLK----EFRVSDVELVKEYIDKGNQSRSTGSTGANEESSRS-
```

```
HAILQLVVKKAQEGKEGKE-------------------ISRVVGKISFID------------------
----------LAGSERGADTTDNDR---QTRMEG-------------AEINKSLLA------
LKECIRALDN-----------EQNHIPFRGSKLTEVLR-----DSFVG-
DSRTVMISCISPNAGSCEHTLNTLRYADRVK-----------
>Phypa_456175-PpKinesin13-c
----------RIRVVVRKRPLNKKEISRK------------------------------
EEDIVTISDTDSSLTVNEPKVKVDLTA-----------YTERHEFVFDAVLDQQ-------------
--------------VSNDEVYRSTVEPIVPTIFNRTK---------ATCFAYGQ-------------
--------TGSGKTYTM-----------------QPLPLRACGDIMAIMQQPNYRNQ-----------
-------------------------------------------------GFQLWLSFFEIYG-----
-GKLYDLLNERR-------------------------------------------------------
----------------------------------------KLCMRED-----------------
GRQQVCIVGLK----EFRVSDVELVKEYIDKGNQSRSTGSTGANEESSRS-
HAILQLVVKKAQEGKEGKE-------------------ISRVVGKISFID------------------
----------LAGSERGADTTDNDR---QTRMEG-------------AEINKSLLA------
LKECIRALDN-----------EQNHIPFRGSKLTEVLR-----DSFVG-
DSRTVMISCISPNAGSCEHTLNTLRYADRVK-----------
>Phypa_427794-PpKinesin13-b
----------RIRVVVRKRPLNKKELSRK------------------------------
EEDIITINDVESSLTVNEPKVKVDLTA-----------YIERHEFVFDAVLNQH-------------
--------------VSNDEVYRSTVEPIVPTIFNRTK---------ATCFAYGQ-------------
--------TGSGKTYTM-----------------QPLPLRACQDIMSIMQQPSHRNQ-----------
-------------------------------------------------GLQLWLSFFEIYG-----
-GKLYDLLNERR-------------------------------------------------------
----------------------------------------KLCMRED-----------------
GRQQVCIVGLK----EFRVSDVELVKEYIDKGNASRSTGSTGANEESSRS-HAILQLVVKKAQEGKE--
--------------------VSRVVGKISFID------------------------------
LAGSERGADTTDNDR---QTRMEG-------------AEINKSLLA------LKECIRALDN------
------DQGHIPFRGSKLTEVLR-----DSFVG-DSRTVMISCISPNAGSCEHTLNTLRYADRVK----
-------
>Phypa_438782-PpKinesin14-Ib
--------KGNIRVFCRVRPLMVEEED------------------------------
GNEQATVQFPSSTELQGRAIELAQ--------------PAGGPKHCFQFDKVFGPEVKQ---------
---------------------GGVFEEISQLVQSALDGYK---------VCIFAYGQ------------
----------TGSGKTHTMLGNPE---------IPDEGGVIPRSLEQVFASSQALI-----AQG-----
-----------------------------------------------------WKFCMQASMLEIYN---
---ETIRDLLA-------------------------------------------------------
KGPVNGDAKQM-----------------------------------YVVKHD---------------
QSGNTTVSDLS----LVEVTTWKEVSNLLHRASQSRSTSKTAMNEQSSRS-
HCVFTLRISGVNEGTEQA----------------------VHGVLNLID------------------
----------LAGSERLSRSGATG----DRLKET-------------QAINKSLA------
SLGDVIMAIAN-----------KDPHVPFRNSKLTYLLQ-----PCLGGDS-
KTLMFVNISPDMKSLNESLCSLRFAAKVNACEI-------
>Phypa_439730-PpKinesin14-Ia
--------KGNIRVFCRVRPLMVEEDE-----------------------------
GNESPSVQFPSSTDLEGRAIELVQ--------------PSGGPKHCFQFDKVFGPDVKQ---------
---------------------AGVFEEISQLVQSALDGYK---------VCIFAYGQ------------
----------TGSGKTHTMIGNPE---------IPDEGGVIPRSLEQVFESSQALI-----AQG-----
-----------------------------------------------------WKFCMQASMLEIYN---
---ETIRDLLA-------------------------------------------------------
KGPVNGDVKQM-----------------------------------YVVKHD---------------
PSGNTSVSDLT----LVEVATWKEVSNLLHRASQSRSTSKTLMNEQSSRS-
HCVFTLRISGVNEGTEQA----------------------VHGVLNLID------------------
----------LAGSERLSRSGATG----DRLKET-------------QAINKSLA------
SLGDVIMAIAN-----------KDPHVPFRNSKLTYLLQ-----PCLGGDS-
KTLMFVNISPDMKSLNESLCSLRFAAKVNACEI-------
>Phypa_424496-PpKinesin14-IIIb
--------KGNIRVFCRCRPLSPVEVAA------------------------------
NASSVAEFESAG---NGDIVVR--------------NGTAGKKLFKFDRVFSPQDDQ-----------
------------------ADVFADTAPVVVSVLDGYN---------VCIFAYGQ-------------
--------TGTGKTFTMEG-----------NVANRGVNYRTLEELFNIAAQRK-----GE--------
-------------------------------------------------TNYDISVSVMEVYN-----
-EQIRDLLAPPAA-------------------------------------------------------
QDQSTKKLE-----------------------------------IKQAAE-----------------
```

```
GGHHVPGLV----EAKVTSMEEVWDVLQAGSSSRTVGSTRANDHSSRS-HCMLCVMVKGENLVTGEH--
--------------------TKSKLWLVD----------------------------
LAGSERVAKSDAQG----DRLKEA-------------QNINKSLS------ALGDVIQALSI------
------KSSHIPFRNSKLTHLLQ-----DSLGGDS-
KTLMFVQISPNDADLSETLCSLNFASRVRGVELG------
>Phypa_459874-PpKinesin14-IIIa
--------KGNIRVFCRCRPLSQAELLA------------------------------
NSVSVTEYESAS---SGDIVVR--------------
HGAAGKKLFKFDRVFSPQDDQCKPVALRARSCTLAVKCLHLILISDTLPFTADVFADTAPVVVSVLDGY
N---------VCIFAYGQ----------------------TGTGKTWTMEG------------
STGNRGVNYRTLEELFTIAAQRK-----GE--------------------------------------
-------------------INYDISVSVMEVYN------EQIRDLLVPVAA------------------
-----------------------------------QDQPTKKLE-----------------------
-------------IKQAAE----------------GGHHVPGIV----
EARVTSMAEVWSVLQAGSNSRTVGSTRANDHSSRS-HCMLCVMVRGENTITGEV--------------
--------TKSKLWLVD----------------------------LAGSERVAKSDAQG----
DRLKEA-------------QNINKSLS------ALGDVIQALAM------------
KSSHVPFRNSKLTHLLQ-----DSLGGDS-KTLMFVQISPNEADLSETLCSLNFASRVRGVELG-----
-
>Phypa_439319-PpKinesin14-IIb
--------KGNIRVYCRVRPFLTEEFGR-------------------------------
QTTIDYIGENGE-LMLVNPLKP---------------GAKDSRKSFSFNKCFAPNASQ-----------
-------------------EEVFLDTQPLIRSVLDGFN---------VCIFAYGQ--------------
--------TGSGKTFTMSGPNNM--------TPVDWGVNYRALHDLFHTTQSRH-----DV--------
-----------------------------------------------------FRYEISVQMLEIYN-----
-D-------------------------------------------------------------------
----TLE-------------------------------------IRNNSQ---------------LNG-
LNVPDAS----RMSVRSTEDVLDLMKVGQKNRAVGATALNERSSRS-HSVLTVHVQGTDLESGAI----
-------------------LRGSLHLVD----------------------------
LAGSERVDRSEATG----DRLKEA-------------QHINKSLS------ALGDVIAALAQ------
------KNVHVPYRNSKLTQLLQ-----DSLGGQA-
KTLMFVHISPDVDSFGETVSTLKFAERVSTVEL-------
>Phypa_441550-PpKinesin14-IId
--------KGNIRVYCRVRPFLTKESTR-------------------------------
QTTIDYVGENGE-LILLNPIKL---------------AGKESRRSFVFNRCFNVNASQ-----------
-------------------EEVFLDTQPLIRSALDGFN---------VCIFAYGQ--------------
--------TGSGKTFTMSGPNNL--------TPTTWGVNYRALNDLFFITQSRV-----HV--------
-----------------------------------------------------FRYEIGVQMLEIYN-----
-E-------------------------------------------------------------------
-------------------------------------------------Q---------------LNG-
LNVPDAN----IMPVRSTDDVLELMKLGQKNRAVGSTSLNDRSSRS-HSVLTVHVQGTDLNSGAV----
-------------------FRGSLHLVD----------------------------
LAGSERVDKSEVTG----DRLKEA-------------QHINKSLS------ALGDVISALAQ------
------KNGHVPYRNSKLTQLLQ-----DSIGGQA-
KTLMFVHISPDVESFGETLSTLKFAERVASVELG------
>Phypa_436987-PpKinesin14-IIc
--------KGNIRVYCRVRPFLAEEAGR-------------------------------
LSTLDYIGENGE-LMLVNPLKP---------------GAKDSRKSFTFNKCFPPTASQ-----------
-------------------EEVFLDTQPLIRSVLDGFN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPNNM--------TSIDWGVNYRALHDLFHITQSRQ-----DV--------
-----------------------------------------------------FRYEIGVQMLEIYN-----
-EQVRDLLSVDG-------------------------------------------------------
-AQKKLE-------------------------------------IRNNSQ---------------LNG-
LNVPDAS----RMSVRSTEDVLDLMKVGQKNRAVGATALNERSSRS-HSVLTVHVHGTDLESGAV----
-------------------LRGSLHLVD----------------------------
LAGSERVDRSEATG----DRLKEA-------------QHINKSLS------ALGDVIAALAQ------
------KNGHVPYRNSKLTQLLQ-----DSLGGQA-
KTLMFVHISPDVESFGETVSTLKFAERVSTVELG------
>Phypa_430601-PpKinesin14-IIa
--------KGNIRVYCRVRPFLTEEAGR-------------------------------
LTTLDYIGENGQ-LMLVNPLKP---------------GAKDSRKSFTFNKCFAPNASQ-----------
-------------------EEVFLDTQPLIRSVLDGFN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPNNM--------APVDWGVNYRALHDLFSTTQSRL-----DV--------
-----------------------------------------------------FRYEIGVQMLEIYN-----
```

```
-EQVRDLLAADVV-----------------------------------------------------
STLTLTTLE-------------------------------------IRNNSQ--------------
LNG-LNVPDAS----MMSVRSTEDVLDLMKVGQKNRAVGATALNERSSRS-
HSVLTVHVQGTDLESGAI----------------------LRGSLHLVD------------------
----------LAGSERVDRSEATG----DRLKEA-------------QHINKSLS------
ALGDVIAALAQ-----------KNGHVPYRNSKLTQLLQ-----DSLGGQA-
KTLMFVHISPDVESFGETISTLKFAERVSTVELG------
>Phypa_450599-PpKinesin14-VIb
--------KGKIRVYARWRPLSSKEVKE---------------------------------
RQQNVLIAPDEFTIEHPWK-----------------DDKPKQHQFDHVFDHHATQ------------
-----------------EEVFEDTKYLVQSAIDGYN---------VCIFAYGQ----------------
------TGSGKTFTIYG-----------SDNNPGLTPRATKELFGYLKRDA-----NK----------
-------------------------------------------------FSFALKVYMLEIYQ------
DSLIDLLLPKS-----------------------------------------------------------
AAKPRK-------------------------------------LEIKKD--------------
SKGMVVVENAT----LLPIASHDELQAIVHKGLERRHVSGTHMNAESSRS-
HLILSVIVESTNRQSQVL----------------------VKGKLSFVD------------------
----------LAGSERVKKSGSSG----EQLKEA-------------QSINKSLS------
ALGDVISALAT-----------EEQHIPYRNHKLTMLMS-----DSLGGNA-
KTLMFVNISPAESNLDETHNSLCYATRVRSI---------
>Phypa_428061-PpKinesin14-VIc
--------KGKIRVYARWRPLSEKEVKG---------------------------------
GEQSVLTSCDEFSIEHPWK-----------------DDKIKQHQFDHIFDEFATQ------------
-----------------EQVFEDTKYLVQSAIDGYN---------VCIFAFGQ----------------
------TGSGKTYTIYG-----------TEANPGLTPRITLELFSCIKRDA-----NK----------
-------------------------------------------------FQFSLQVYMLELYQ------
DTLIDLLLSKN-----------------------------------------------------------
GTKPKK-------------------------------------LEIKKD--------------
SKGMVVVENAT----LIPVATREELESVVAKGLEKRHTSGTQMNAESSRS-
HLILSIIVESTNLQSQVL----------------------MKGKLSLVD------------------
----------LAGSERVKKSGSSG----EQLKEA-------------QSINKSLS------
ALGDVISALAT-----------DEQHIPYRNHKLTMLMS-----DSLGGNA-
KALMFVNVSPAGSNVDETHNSLCYAIRVRSIMND------
>Phypa_458819-PpKinesin14-VId
----------KIRVYARWRPLSDKEIRE---------------------------------
GEKLMLTSCDEFTIEHPWK-----------------DDKIKQHQFDHIFDQFATQ------------
-----------------EEVFEDTKYLVQSAIDGYN---------VCIFAFGQ----------------
------TGSGKTYTIYG-----------SNSNPGLTPRVTQELFNCMKRDS-----NK----------
-------------------------------------------------FQFSLQVYMLEIYQ------
DTLVDLLQPKFG-----------------------------------------------------------
FGGKPRK------------------------------------LDIKKD--------------
TKGMVVVENAT----LIPVVTREELDSVIAKGLEKRHTSGTQMNAESSRS-
HLILSIIIESTNLQSQVL----------------------MKGKLSLVD------------------
----------LAGSERVKKSGSSG----EQLKEA-------------QSINKSLS------
ALGDVISALAT-----------DEQHIPYRNHKLTMLMS-----DSLGGNA-
KALMFANISPAGSNLEETHNSLCYATRVRSIIND------
>Phypa_439249-PpKinesin14-VIa
--------KGKIRVYARWRPLSSKEIKE---------------------------------
RQQNVLIAPDEFTIEHPWK-----------------DDKPKQHQFDHVFDHYATQ------------
-----------------EEVFEDTKYLVQSAIDGYN---------VCIFAYGQ----------------
------TGSGKTFTIYG-----------SENNPGLTPRATKELFGYLKRDA-----NK----------
-------------------------------------------------FSFSLKVYMLEIYQ------
DSLIDLLLPKS-----------------------------------------------------------
AAKPRK-------------------------------------LEIKKD--------------
SKGMVVVENAT----LLPIASHDELQAIVHKGLERRHVSGTHMNSESSRS-
HLILSVIVESTNRQSQVL----------------------VKGKLSFVD------------------
----------LAGSERVKKSGSSG----EQLKEA-------------QSINKSLS------
ALGDVISALAT-----------EEQHIPYRNHKLTMLMS-----DSLGGNA-
KTLMFVNISPAESNLDETHNSLCYATRVRSI---------
>Phypa_436446-PpKinesinOrph-IIa
----------PVEVVGRIREHPEGFDKESAIRVLPHS-------------------------------
------RVAVRSE--------------GIGNGFREFSLDGVSLAAVENLQ-----------------
----------TFYGRYVEPRVEDVKAGGR---------CTIMMYGP---------------------
TGAGKSYTMFG-----------AAHEKGVAYHALSQLMTHEASH--DDDDISD--------------
```

```
----------------------------------------KSIEVRATVWEIYN------
EEIYDLLAGVSAPKS-------------------------------------------------------
GFGTLFKMSG--------------------------------SSGGRVKL--------------
EVMGKKVKTCI----SISGTDPQKLLKEISKVEGRRVVKSTNCNDRSSRS-
HCMVTLDVPEVGGKLVLVD---------------------------------------------------
----------MAGSENVEQAGLG----RELKMQT-------------GKINQGN------
GALKRVVEAIAN------------GDSYIPYRDSKLTMLLQ-----
DSFEDDRAKILMILCASPDIRDLHKTICTLEYGAKAK-----------
>Phypa_430757-PpKinesinOrph-IIb
----------PVEVVGRIREHPEGNDKESAIRVLPHS-------------------------------
------RVAVRAE--------------GMGNGCREFSLDGVSLAAMENLQ------------------
----------AFYGRYVESRVEDVKAGGR---------CTIMMYGP----------------------
TGAGKSYTMFG------------AAHEKGVAYHALSQLMTHEASD--GGDDFSD---------------
----------------------------------------KSIEVRATVWEIYN------
EEIYDLLASVSAPKS-------------------------------------------------------
GFGTLFKMSG--------------------------------SSSGRVKL--------------
EVMGKKVKTCM----SISGTDPQKLLKEISKVEGRRVVKSTNCNDRSSRS-
HCMVTLDVPDVGGKLVLVD---------------------------------------------------
----------MAGSENVEQAGLG----RELKMQT-------------GKINQGN------
GALKRVVEAIAN------------GDSYIPYRDSKLTMLLQ-----
DSFEDDRAKILMILCASPDLRDLHKTICTLEYGAKAK-----------
>Phypa_435249-PpKinesin14-IV
--------KGNIRVFCRIRPFLPAEKHA-----------------------------------------
RPGPVTNASENWVKI---------------SGRNSRKEFEFDKVFQPNSVQ------------------
------------DDVFAEIEPIIRSALDGHN---------VCIFAYGQ----------------------
-TGSGKTFTMEG------------SNDDPGVVPRSLRRLFEEASYDT-----NI---------------
----------------------------------------QYSYSLSMLEVYK------
GSLRDLLVARP-------------------------------------------------------
TRHTD-ATKCQLELMGTCS-----------------------------LSIQMG---------------
SKGFIEVENLT----EIPIADVKEASRLYLKGSRRRSTAWTNANDTSSRS-HCLLRINIVCK-
SPHDNKK----------------------RMSKLWLID-----------------------------
LGGSERLLKTNAQG----LTMEEG--------------RAINISLS------ALGDVISALHK------
------RRPHVPYRNSKLTQILR-----DSLGDNS-
KTLMLVHVSPTETDLGETICSLSFATRVRGTHLG------
>Phypa_435597-PpKinesin14-Vb
--------KGNVRVYCRARPQFEDEGPS---------------------------------
STTYPDDFTLRLNSNVTAA-------------------PNKDFELDRIYGPHISQ-------------
-----------------ADIFQDLQPLVQSALDGFN---------VSIFAYGQ----------------
------TGAGKTFTMEG------------PSHDRGLYYRVLEELFDLVNSEA-----TPT---------
-------------------------------------------SSTSFFVTMFELYN------
EQVRDLLKAPD-------------------------------------------------------
----NR-------------------------------------GASTVL---------------
FGEPGRGVELV----DERLDSPSGFARIFKFGKQMRANVDGVKFDRSSRS-
HLVVTIHIHSSDSLTGEE----------------------HYSKLSMVD------------------
----------LAGSERLNKAEANG----DRLTES--------------LHINKSLS------
ALGDVLSALTT------------KKDYIPYDHSKLTELLY-----DSLGGDS-
KAVLIANVNPSNAEVQETIATLNFASRARSAEI-------
>Phypa_437825-PpKinesin14-Va
--------KGNIRVYCRARPQFEDEDSS---------------------------------
FISYPDDFTLRINSNVSTA-------------------PSKDFELDRIYGPHISQ-------------
-----------------GDIFQDLQPLVQSALDGYN---------VSIFAYGQ----------------
------AGSGKSYTMEG------------PSHDRGLYYRAFEELFDLVNAEN-----SPS---------
-------------------------------------------SRTAYYVTMFELHN------
EQVRDLLKTSD-------------------------------------------------------
----SS-------------------------------------GASTVM---------------
MGGLGHGVELV----DERIDSPSGFTRVFKFGSQMRANVDGVKSDRSNRS-
HLVVTIHIYTTDSLTGEE----------------------QYSKLSMVD------------------
----------LASSERFSKAEVNG----DRLTES--------------LHINKSLS------
ALGDVFAALSA------------KKDYIPYGHSKLTQLLA-----DSLGGDS-
KAVLIANVSPSNSDLQETIATLNFVSRARNAEI-------
>Phypa_444072-PpKinesin12-Ia
---------HSVQVLIRARPISSAEIAQQ-----------------------------G-
IARCVKQENAHTISWLGQP--------------------ETR-FTFDHVAGEFV---------------
--------------TQEELFRVAGLPMVENCMAGYN---------SCMFAYGQ----------------
```

```
------TGSGKTHTMLGDIGDF----DQQPNENCGMTPRVFAYLFAKIQKEEEAQKHR-----------
-------------------------------------------KLKYKCRCSFLEIYN------
EQISDLLEPSL-----------------------------------------------------------
-------------------------------------TNLQMRED----------------
LNKGVYVEGLL----EVEVQNVQDVLHLLLLGATNRKVAATNMNKESSRS-HSVFTCIIESQWE--CD-
------------------SMINFRYGRLNLVD----------------------------LAGSER-
QKATGEDG---ERLREA-------------ASINKSLST------LGLVIMVLVDIAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIANISPSSCAASETLSTLKFAQRAKFI-------
--
>Phypa_440218-PpKinesin12-Ib
---------HSVQVLIRARPISSAELSQQ-----------------------------G-
VARCVKQENAHTISWLGQP--------------------ETR-FTFDHVAGEFV---------------
--------------TQEELFRVAGLPMVDNCMAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDMEHF----DQQPNENRGMTPRVFEYLFAKIQKEEESQKHK-----------
-------------------------------------------ELKYKCRCSFLEIYN------
EQISDLLEPAS-----------------------------------------------------------
-------------------------------------TNLPMRED----------------
MNKGVYVEGLL----EVEVQNVQDVLHLLLLGATNRKVAATTMNRESSRS-HCVFTCIIESQWE--SD-
------------------AMINFRFGRLNLVD----------------------------LAGSER-
QKATGADG---ERLREA-------------ASINKSLST------LGLVIMVLVDVAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIANISPSSCASSETLSTLKFAQRAKFI-------
--
>Phypa_431567-PpKinesin12-Il
---------HNVQVVIRCRPPSSKESVKQ-----------------------------C-
FTRCVKQDGPQAITWLGQP--------------------ETR-FTFDHVAGENI---------------
--------------SQEKIFQAVGLPIVENCMAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDICDL----DDRPNEDRGITPRIFEYLFSRIQKEGLARQLE-----------
-------------------------------------------QLRYVCKCSFLEIYN------
EQITDLLEPSS-----------------------------------------------------------
-------------------------------------SNLQIRED----------------
SKKGVYVENLT----ETAVSSVQDVVSLLLKGAANRKVASTNMNRESSRS-HSVFTCTIESRWE--IN-
------------------SLTNMRFGRLNLVD----------------------------LAGSER-
QKSSGAEG---DRLKEA-------------ASINKSLST------LGLVIMILVDVAN--------
GKPRHVPYRDSKLTFLLQ-----DSLGG-NSKTAIIATISPSICCSMETLSTLKFAQRAKFI-------
--
>Phypa_453302-PpKinesin12-Im
---------HNVQVVVRTRPISTKEATKQ-----------------------------D-
VARCLRQESAHAITWLGQP--------------------ETR-FTFDHVAGENI---------------
--------------SQEKLFEVVGLPIVENCMAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDVTDL----GHKPSDNRGMTPRIFEYLFSKIRKEEQHKQLE-----------
-------------------------------------------QLEYVCRCSFLEIYN------
EQITDLLEPSS-----------------------------------------------------------
-------------------------------------TNLHMRED----------------
SKKGVYVENLT----EIVVRSVQDVVVLLLKGAANRKVASTIMNRESSRS-HSVFTCTIESKWV--TN-
------------------SMSNMRFGRLNLVD----------------------------LAGSER-
QKSSGTER---DRLKEA-------------ASINKSLST------LGLVIMILVDIAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTAIIATISPSSCCTMETLSTLKFAQRAKFI-------
--
>Phypa_422285-PpKinesin12-IIb
------------KVIVRMRPLNKKEAAEE-----------------------------A--
TNVVQKLSGDSLSLGDQ-----------------------QFTFDSVAGETE---------------
-------------SQEAVFEMVGRPMVENCLAGFN---------SSIFAYGQ----------------
-----TGSGKTHTMWGILPTSGT--DASVTEERGITPRVFEQLFSRIQQEERNNVEK------------
-------------------------------------------QLRYQCRCSFLEIYN------
EQITDLLEPTQ-----------------------------------------------------------
-------------------------------------KNLLIRED----------------
TKTGVYVEGLT----EEYVSNMDDVISLLVRGSANRRVGSTAMNNESSRS-HSVFTFVIESRSKSVAE-
------------------GVSSVRTSRMNLVD----------------------------LAGSER-
QKQTGAAG---DRLKEA-------------GNINKSLSQ------LGNVINILAEVAQS-------
GKHRHIPYRSSRLTFLLQ-----ESLGG-NAKLAMICAISPASSCRTETLSTLRFAQRAKA--------
--
>Phypa_440124-PpKinesin12-IIc
-----------------------------------------------------------------
---------DQ-----------------------QFTFDAVAGEVS---------------------
```

```
-------SQEAVFNLVGLPMAENCLAGFN---------SSIFAYGQ---------------------
TGSGKTHTMWGVMSDT----QDIPSDDRGLTSRVFEMLFARIQREELNNINK----------------
-------------------------------------QLVYQCRCSFLEIYN------
EQITDLLEPSQ-----------------------------------------------------------
-------------------------------------KNLMIRED----------------
TKTGVYVENLS----EEFVSSVQEVTRVLVKGLANRRVSATSMNSESSRS-HCVFTCVIESRSKGEGE-
------------------GISSVRSSRLNLVD----------------------------LAGSER-
QKQTGSAG---ERLKEA-------------GNINKSLSQ------LGNVINILAEVSQS-------
GKHRHVPYRDSRLTFLLQ-----ESLGG-NARLAMICTVSPASCCRNETLSTLRFAQRAKAI-------
--
>Phypa_428714-PpKinesin12-IIa
------------KVIVRVRPLNKKEEAEE-----------------------------A--
EQVVHRLSATGVSLADQ------------------------LFTYDAVAGEDA----------------
-------------SQEAVFHMVGLPMVENCLAGFN---------SSIFAYGQ-----------------
-----TGSGKTHTMWGVMSES----EDFPSEGRGLTPRVFEKLFARIDEEERINADK------------
-----------------------------------------------QLVYQCRCSFLEIYN------
EQITDLLEPSL-----------------------------------------------------------
-------------------------------------KNLMIRED----------------
TKSGVYVDNLS----EEFVSSVQDVTRILIKGLANRRVGATTMNSESSRS-HSVFTCVIESRSKSEGE-
------------------GISSVRSSKLNLVD----------------------------LAGSER-
QKQTGSAG---ERLKEA-------------GNINKSLSQ------LGNVINILAEVAQS-------
GKHRHIPYRDSRLTFLLQ-----ESLGG-NAKLAMICAVSPASSCKNETLGTLRFAQRAKVI-------
--
>Phypa_441202-PpKinesinOrph-III
----------NIKVYVRTRPLSKDEANRG-------------------------------
DNSCIQVNYDEKSLKVVEQGYG----------------YNVTTRSFKFDGCMGSEVHQ-----------
-----------------RDVLKKVHMKKLLDKVLAGYS---------STVMACGQ--------------
--------TGSGKTFTMCGQEENLLC---DMEGGDNGLIVRSATYLFEAMKACEHNTPNGQK-------
-------------------------------------------------PFTMRASYFEIYT-----
-EQVNDLLRLDN-----------------------------------------------------------
---------------------------------------APRDVKWS-----------------
SRDGYYVDNLL----LVDCDTLSDVISVLNEGSRNRKVGSHELNKDSSRS-
HCIMTLHVDSLCQVGDDAP------------------PITRYGRMLFVD-------------------
----------LAGSERLKKSKSSGE----MLKET--------------GSINRSLFT------
LGKVISALSEGK----------KGDVVPYR-----------------------------------------
-----------------------
>Phypa_455498-PpKinesinARK-a
----------RVRVTVRLRPRNAEELEADLDFADCVE----------------------------
LQPELKRLKLRKNNWE-------------------SETYQFDEILTETAS-----------------
-----------QKRVYEVVAKPVVESVLEGYN---------GTVMAYGQ--------------------
--TGTGKTFTLGKLGDEDTADR--------GIMVRALEDILSNINHADD-------------------
-----------------------------------------------TVTVSYLQLYM------
ESVQDLLAP-------------------------------------------------------------
-------------------------------------ERDNCHIQEDPK----------------
TGDVSVPGAT----QIQLTDHQSFVNLLDVGESNRVAANTKLNTESSRS-HALLLVQVKKAVRTKE---
-PAENG---------KMRAPTIRRSKLLIVD----------------------------
LAGSERVDKSG--SEGH--TLEEA-------------KSINLSLTA------LGKCINALAENS----
--------PHVPIRDSKLTRLLR-----DSFGG-TARTSLIVTIGPSPRHRGETTSTIMFGQRAM----
-------
>Phypa_427907-PpKinesinARK-d
----------RVRVAVRLRPRNAEELEADADFADCVE----------------------------
LQPEFKRLKLRKNNWD-------------------CETYQFDEVLTETAS-----------------
-----------QKRVYEVVAKPVVEGVLEGYN---------GTVMAYGQ--------------------
--TGTGKTFTLGRLGEEDCADR--------GIMVRAMEDILANITPGED-------------------
-----------------------------------------------TVTVSYLQLYM------
ETVQDLLAP-------------------------------------------------------------
-------------------------------------ERDNIAIQEDPK----------------
TGDVSVPGAT----QVLLQDQTSFVRLLDVGEANRFAANTKLNTESSRS-
HALLVVQVKKGYKGRSGDTATNENDNGS-PQTGTGLRAPIIRRSKLLIVD-----------------
----------LAGSERVDKSG--SEGH--TLEEA-------------KSINLSLTA------
LGKCINALAENS-----------PHVPIRDSKLTRLLR-----DSFGG-
TARTSLIVTIGPSPRHRGETTSTILFGQRAM----------
>Phypa_425827-PpKinesinARK-c
```

```
----------RVRVTVRLRPRNAEELESDRDFADCVE----------------------------
LQPELKRLKLRKNNWD-------------------CETYQFDEILTDTAS-----------------
-----------QKRVYEVVAKPVVESVLEGYN---------GTVMAYGQ-------------------
--TGTGKTFTLGKLGDEDTADR--------GIMVRALEDILAVINPVHD-------------------
---------------------------------------------TVTVSYLQLYM------
ESVQDLLSP-----------------------------------------------------------
-----------------------------------EKDNIAIQEDPK---------------
TGDVSVPGAT----QIQVTDHQSFCNLLDVGEANRFAANTKLNTESSRS-HAILLVQVKKAVRNKEV-
VVPPENGNGG-SHSVKGMRAPTIRKSKLLIVD----------------------------
LAGSERVDKSG--SEGH--TLEEA--------------KSINLSLTA------LGKCINALAENS----
--------PHVPIRDSKLTRLLR-----DSFGG-TARTSLIVTIGPSPRHRGETTSTIMFGQRAM----
-------
>Phypa_446331-PpKinesinARK-LIKE
-----------------------------------------------------------------
----------------------------------TEFQFDAVLPPTAL-------------------
--------QVDVYNMAARAVVLDVLNGYN---------GTIMAYGQ---------------------
TGAGKTYTLSDNLTNGGGVTS-----VGGIIPRSAAEIFDRAGLDQDY--------------------
-----------------------------------------EFHVSMSYIQIYM------
EQIQDLLRP-----------------------------------------------------------
-----------------------------------ESCNMQIREG-----------------
MNGVYVSGVE----EVQVKSVEDTMKLLMLGDRHRCLSFTKLNAHSSRS-
HTIVILTVEKKAKYKTSEQKAELADRRR-VSSCFVESER-VLVGKLFLVD------------------
----------LAGSERLKKSG--SEGI--RASEA--------------MSVNLSLTC------
LGKCISARADPAI----------THVPFRDSKLTRLLQ-----ESLGG-
NAKTSLVINIAPCSEYLQESMSSLHFGSRAM----------
>Phypa_453488-PpKinesinARK-b
----------RVRVTVRLRPRNAEELEADLDFADCVE----------------------------
LQPELKRLKLRKNNWE-------------------SETYQFDEILTETAS-----------------
-----------QKRVYEVVAKPVVESVLEGYN---------GTVMAYGQ-------------------
--TGTGKTFTLGKLGDEDTADR--------GIMVRALEDILSSINHVDD-------------------
---------------------------------------------TVTVSYLQLYM------
ESVQDLLAP-----------------------------------------------------------
-----------------------------------ERDNCHIQEDPK---------------
TGDVSVPGAT----QIQLTDHQSFVNLLDVGESNRVAANTKLNTESSRS-HALLLVQVKKAVRSKE---
-PTENGNG-------KMRATTIRRSKLLIVD----------------------------
LAGSERVDKSG--SEGH--TLEEA--------------KSINLSLTA------LGKCINALAENS----
--------PHVPIRDSKLTRLLR-----DSFGG-----------------------------------
-------
>Phypa_455320
---------HNVQVLVRVRPVSASEVSGQ------------------------------G-
FSRCVRQDGPHTITWLGQP--------------------QTR-FSFDHVAGESI---------------
--------------TQEDLFRVAGTPMVENCMRGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIDDL----AIRPSLQRGMTPRVFEYLFARIQQEENLREQE-----------
-----------------------------------------------QLRFVCKCSFLEIYN------
EHITDLLDPTS---------------------------------------------------------
-----------------------------------INLQIRED----------------
VKTGVYVENLK----EVEVKSVHDVVQLLTQGASNRKVAATNMNRESSRS-HSVFACTVESKWQ--AN-
------------------SLTNIRFGRLNLVD----------------------------LAGSER-
QKSSGAEG---DRLKEA--------------ANINKSLST------LGLVIMTLVDIAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTVIATISPSNGNALETMSTLKFAQRAKLI-------
--
>Phypa_442090-PpKinesin12-Ic
---------HNVQVLLRARPISGIEIAQL------------------------------G-
HARCIRQENAYAISWLGQP--------------------ETR-FSFDYVASEFV---------------
--------------TQEELFRVAGPPMVENCMAGNN---------SCVFAYGQ----------------
------TGSGKTHTMLGDVGDF----DRQPNENRGMTPRVFEYLFKKIHEEEVAQRHT-----------
-----------------------------------------------NMKFKCRCSFLEIYN------
EQISDLLEPSS---------------------------------------------------------
-----------------------------------SNLPIRED----------------
STKGIYVDGLV----EVEAQNVRNILHLLLLGAANRKVAATDMNRESSRS-HSVFTCVIESQWE--CD-
------------------ATINSRIGRLNLVD----------------------------LAGSER-
QKSSGVDG---ERFREA--------------ASINKSLST------LGLVIMDLVDIAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIANISPSSCASSETLSTLKFAQRAKFI-------
--
```

```
>Phypa_437642-PpKinesin12-Io
---------HNVQVLVRTRPVSNSESVGA------------------------------G-
FTRCVRQDSAHTITWLGQP-------------------ETR-FTFDHVAGESI--------------
--------------TQEDIFRVAGSPMVDNCLRGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIDDL----DCHPSHQRGVTPRIFEYLFATIQQEVILREQE-----------
---------------------------------------------HLRFVCKCSFLEIYN------
EHITDLLDPSS-------------------------------------------------------
-----------------------------------TNLHIRED-----------------
AKTGVYVENLK----EVEVKSVHDVVQLLIQGASNRRVAATNMNRESSRS-HSVFACSVESKWE--SN-
------------------SLTNIRFGRLNLVD----------------------------LAGSER-
QKSSGAEG---DRLKEA--------------ANINKSLST------LGLVIMILVDVAN--------
GKERHIPYRDSKLTFLLQ-----DSLGG-NSKTTIIATISPSNVNALETLSTLKFAQRAKFI-------
--
>Phypa_426336-PpKinesin12-In
---------HNVQVLVRTRPVSSSEASGH------------------------------C-
FGRCVRQDGPHTITWLGQP-------------------QTR-FSFDHVAGESI--------------
--------------TQEDLFRVAGAPMVENCMRGFN---------SCMFAYGQ----------------
------TGSGKTYTMLGDIHDL----GYRPSPQRGMAPRVFEYLFSRIQQEEKLRELE-----------
---------------------------------------------QLKFSCKCSFLEIHN------
EHITDLLNPTS-------------------------------------------------------
-----------------------------------TNLQIRED-----------------
IKTGVYVENLK----QIEVKTVHDVVQLLIQGASHRKVAATNMNSESSRS-HSVFSCTVESRWQ--SD-
------------------SLSKVRVGRLHLVD----------------------------LAGSER-
QSSSGAEG---DRLKEA--------------ANINKSLST------LGLVIMTLVDIAH--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIATISPANGSALETMSTLKFAQRAKLI-------
--
>Phypa_432190-PpKinesin12-If
---------HNVQVLIRTRPISASEMASQ------------------------------G-
FSKCLRQEGPHQITWLGQP-------------------ETR-FTFDHVAGEII--------------
--------------TQEKLFNVAGLPMVENCMAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIDHI----SDRPSDNRGMTPRVFEYLFSRIKLEEEQRKDE-----------
---------------------------------------------NLKFMTKCSFLEIYN------
EQITDLLEPTS-------------------------------------------------------
-----------------------------------TNLQMRED-----------------
VRKGVYVENLT----EVEVHCVQDVIQLLCQGSANRKVAATNMNRESSRS-HSVFTCIIESRWE--RD-
------------------SMTNIRFGRLNLVD----------------------------LAGSER-
QKTSGAEG---ERLKEA--------------ANINKSLST------LGLVIMILVDVAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIATISPSSCNALETLSTLKFAQRAKLI-------
--
>Phypa_454564-PpKinesin12-Ih
---------HNVQVLIRTRPINKAEMASQ------------------------------G-
FMRCLRQDSPQTITWLGQP-------------------ESR-FTFDHVAGDIV--------------
--------------TQEKMFKVAGLPMVENCMSGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIENI----DLLPSENRGMTPRVFEYLFERIRKEEEMRKDE-----------
---------------------------------------------NLMFMCRCSFLEIYN------
EQITDLLEPTS-------------------------------------------------------
-----------------------------------TNLHMRED-----------------
NRTGVYVENLS----EVEVHNVQDVIRLLIQGASNRRVAATNMNRESSRS-HSVFTCIVESKWE--RD-
------------------SMTNIRFGRLNLID----------------------------LAGSER-
QKSSGAEG---ERLKEA--------------ANINKSLST------LGLVIMILVDVAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIATVSPSGCNSMETLSTLKFAQRAKLI-------
--
>Phypa_432169-PpKinesin12-Ig
---------HNVQVLIRTRPISSSEVISQ------------------------------G-
FSKCLRQESPHTITWLGPP-------------------ETR-FTFDQVAGEIV--------------
--------------SQEKLFNVAGLPMVENCMAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIDHL----GDKPSDNRGMTPRVFEYLFSRIKLEEERRADE-----------
---------------------------------------------NLKFLTKCSFLEIYN------
EQITDLLEPTS-------------------------------------------------------
-----------------------------------TNLQMRED-----------------
VRKGVYVDNLT----EVEVNSVQDVIQLLSQGSANRKVAATNMNRESSRS-HSVFTCIIESKWE--RD-
------------------SMTNIRFGRLNLVD----------------------------LAGSER-
QKTSGAEG---ERLKEA--------------ANINKSLST------LGLVIMILVDVAN--------
```

```
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIATVSPSSCNSLETLSTLKFAQRAKLI-------
--
>Phypa_434464-PpKinesin12-Ie
---------HNVQVLIRLRPINGSEVAQQ------------------------------G-
LARCVKQDSAHTVTWVGQP-------------------ETR-FTFDHVAGESI--------------
--------------TQAELFRVAGVPMVENCLAGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDISDF----GHQPSDNRGMTPRVFESLFTKMKLAEEAQKHE-----------
-----------------------------------------------NLKFKCRCSFLEIYN------
EQITDLLEPSS-----------------------------------------------------------
-------------------------------------SNLQVRED-----------------
ATKGVYVEGLT----EVEVQNEQDVLHLLLLGAANRRVAATNMNNESSRS-HSVFTCIIESQWE--CD-
------------------QMINYRFGRLNLVD-----------------------------LAGSER-
QRASGAEG---ERLKEA--------------ASINKSLST------LGLVIMVLVDTAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIANISPSICASLETLSTLKFAQRAKFI-------
--
>Phypa_432906-PpKinesin12-Ii
---------HNVQVLIRSRPINNAEMASQ------------------------------G-
YTRCLKQENAHCITWLGQP-------------------ESR-FTFDHVAGDSV--------------
--------------TQDKLFRVVGLPMVENCMSGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDLERL----DRSPSDNRGITPRVFEYLFERIRQEEESRKHE-----------
-----------------------------------------------KLMFMCRCSFLEIYN------
EQITDLLEPTS-----------------------------------------------------------
-------------------------------------TNLHMRED-----------------
TRTGVYVENLS----EVEVQNVQDVIDLLIQGASNRRVAATNMNRESSRS-HSVFTCIVESKWE--CD-
------------------SLTNIRFGRLNLVD-----------------------------LAGSER-
QKSSGAEG---DRLKEA--------------ASINKSLST------LGLVIMTLVDIAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTIIIATVSPASCNAVETLSTLKFAQRAKLI-------
--
>Phypa_445541-PpKinesin12-Ij
---------HNVQVIIRTRPISASESALK------------------------------G-
FSTCVRQENSRSITWIGPP-------------------EAR-FTFDHVAGEHI--------------
--------------SQEKLFKVSGVPMVENCMAGYN---------SCMFCYGQ----------------
------TGSGKTHTMLGDIEDL----ENQPSESRGITPRVFEYLFSRIKREEEARTNE-----------
-----------------------------------------------HMKYVCKCSFLEIYN------
EQIIDLLEPTS-----------------------------------------------------------
-------------------------------------TNLQLREG-----------------
GKKGVYVENLL----EIEVGSDQDVVQLLLLGSANRKVAATTMNRESSRS-HSVLICTIESRWE--LN-
------------------SMTNSRFGRLNLVD-----------------------------LAGSER-
QKYSGAEG---DRLKEA--------------SNINKSLST------LGLVIMILVDVAG--------
GKQRHVPYRDSRLTYLLQ-----DSLGG-NSKTIMIANISPSSCCALETLSTLKFAQRAKFI-------
--
>Phypa_437562-PpKinesin12-Id
---------HNVQVLIRTRPINDSEASQQ------------------------------G-
HSRCVRQESAHSITWLGQP-------------------ESR-FTFDHVAGESI--------------
--------------SQEELFRVAGLPMVENCMAGYN---------
SCMFAYGQVASPLGYSLRRSLSDKQNLNSCTGSGKTHTMLGDISDL----
DSQPSDNRGMTPRVFESLFAKIREAEELQKHE-----------------------------------
--------------------NLKFTCRCSFLEIYN------EQIGDLLEPSS----------------
-----------------------------------------------------------------
------------TNLQMRED-----------------ANKGVYVEGLV----
EVEVQSVQDVLHLLLLGAANRRVAATNMNKESSRS-HSVFSCIIESQWE--SD----------------
---HMINFRFGRLNLID-----------------------------LAGSER-QRASGAEG---
ERLKEA--------------ACINKSLST------LGLVIMVLVDTAN--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIANISPSSCASLETLSSLKFAQRAKFI-------
--
>Phypa_452429-PpKinesin7-IV
----------KICVAIRLRPPTKQNV---------------------------------
VRGHHWKVSDNSISLLSAAG-----------------SVVSGHTFAFDTIFGADA--------------
--------------KNINIYEQHAKDVVLSAVAGFN---------GTVFAYGQ----------------
-------TSSGKTYTMRGS------------ESEPGITRLAVNEIFRHIQK-----AS-----------
-----------------------------------------------DREYLIRVSYMEIYN------
EEINDLLTPDS-----------------------------------------------------------
-------------------------------------RKLQVHEN-----------------
LEKGVFVAGLR----EEIVDSVEQVLGLLEAGEVQRHFGETNMNINSSRS-HSIFRMVIESRSKCHG--
```

```
-GQ-----------GS-DDVDAVRVSELNLVD----------------------------LAGSER-
IAKTGAGG---VRLKEG-------------GHINKSLMC------LGNVINKLCEGG--------
AKQGAHIPYRDSKLTRILQ-----TALGG-NARTAIICTMTPDEEQIDESRGTLQFASRAKRV------
---
>Phypa_422406-PpKinesin12-Ik
---------HNVQVILRTRPISSSESALK-----------------------------G-
FGRCVRQENSQSITWIGQP--------------------ESR-FTFDHVAGEHI--------------
-------------TQEKLFRVAGIPMVENCMAGYN---------SCMFCYGQ----------------
------TGSGKTYTMLGDIVDL----EHRPSDNRGITPRVFEYLFSRIQKEEESRIHE-----------
-----------------------------------------------HLKYVCKCSFLEIFN------
EQITDLLEPTS-----------------------------------------------------------
-------------------------------------TNLQMREG-----------------
GKKGVYVENLS----EVEVESVQDVVHLLLLGAANRKVAATNMNRESSRS-HSVFTCTIESKWE--FN-
------------------SMTSIRFGRLNLVD----------------------------LAGSER-
QKSSGSDG---DRLKEA-------------AHINKSLST------LGLVIMILVDVAN--------
GKQRHVPYRDSKLTYLLQ-----DSLGG-NSKTIMIANISPSSCCALETLSTLKFAQRAKFI-------
--
>Phypa_431083-PpKinesinOrph-IVa
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
-----------------------------------MRE----------------------ITLMGVT--
--KAGVNSLEEMATYLEHGYLSNTTGSTNMNSHSSRS-HAIFTITLEQRRKWDPI-------PDSG--
SPLSEDCSEDYLCAKLHLVD----------------------------LAGSER-AKRTGAGG---
LRFKE--------------------GLLA------LGNVISALGDEKKRK-------
EGGHVPYRDSKLTRLLQ-----DSLGG-NSRTVMIACVSPADVNVEESINNLKYANRARNIR-------
-
>Phypa_453299-PpKinesinOrph-Ib
----------KVKVVVRIRPFTADEERPLPPVIETVDDRT--------------VIVRDNE--------
--HGQRQESLKFT---------------AHYVQDDSLAIEQTNSDKNDSQN------------------
----------AMFEVLGVPCIEHALDGLN---------TTILAYGH----------------------
TGSGKTYTMLG--DS--------SYQGRGIMPRLSSELMTRIAEKR-----EQG---------------
------------------------------------------EDIKAEVCYFEIYN------
ERIRDLLVADKSPEEK---
QSEPEISVKGRTSMTRTGSLGDLKQARGSKQGLGLKHVPSSGSLSDFKRGSPTQLVQQSPPKEVLGVQR
GSKRMPNWKSFQAAQTDFAKEQQYLKVREH---------------PVNGPYVEGLM----
WKNVETWMDIKTFLKYGSALRTTHTTDAN-AHSSRSHALFTIRITKVIQPTSFSY--------------
--------LWHYYCVIFFSPRLG----------------------HGSERPSDLLTVET---
ATRGEES--------------RAINLSL------TMLNEVILSLS-------------
KGTHPSYRSSVLTWLLR-----DSLGGNN-KTFMVANISATEHDLRETLSTLRYA--------------
-
>Phypa_457477-PpKinesinOrph-Ia
------------------------------------------------------------------------
-----------------------------------------------EESQR-----------------
----------CMFEILGRPCVENSLDGFN---------TTIMTYGQ----------------------
MGSGKTYTIVG--DG--------TTCGRGLVPRIVNELMRRVQDDQ-----SSG---------------
-----------------------------------------VHLTIQVSYLEIYQ------
EKVRDLLVDKKAEHT--------------------------AEESGVNCSLRCTELP------
LIDGGGSSFFLH------------------------------GQVKDQLRVREH---------------
PETGTYVESPR----WKVVTAHEEMDKLLKLGAANRMLGSTISHARLSSRGHTLFTIKITKTNEKSQ-
HT----------------------
SVSHINMVDLAGENSYHISFLSTVAVICTKTSQIEFCIVSCSEKMALGQKPS---AERTYES-------
-------KYINRSL------AQLNDMFTNLPNGRS---------NGKFVSYRSSALTMLLR-----
ESLSENS-KTYLVANISPAEQDFQESIHTLRCAAKAKRI---------
>MvKinesinARKa
----------RVRVAVRLRPRNTDEILADADFGDYVE----------------------------
LMPELKRLRLRKNNWD-------------------SETFQFDEVLTETAS-----------------
-----------QKRVYEVVAKPVVESVLEGYN---------GTVMAYGQ--------------------
--TGTGKTYTLGKLGEEDTAGR--------GIMVRAMEDILADTTSEQD--------------------
-----------------------------------------------SVTVSYLQLYM------
ETVQDLLAP-------------------------------------------------------------
```

```
------------------------------------EKDNISIVEDPK---------------
TGDVSLPGAT----LVEIKDQKSFVELLEIGEANRFAANTKLNTESSRS-
HAILLVNVKKMAKLKPERELSSLNENGSNIQLSKNHRIPTIRKSKLLIVD------------------
----------LAGSERVDKSG--SEGH--TLEEA-------------KYINLSLTA------
LGKCINALAENS------------PHVPIRDSKLTRLLR-----DSFGG-
SARTSLIITIGPSPQHRGETTSTILFGQRAM-----------
>MvKinesinARKb
----------RVRVAVRMRPRNSEEMLADADFADCVE----------------------------
LQPELKRLKLRKNNWD--------------------CETYEFDEILTEFSS------------------
-----------QKRVYEVVAKPVVESVLEGYN---------GTVMAYGQ--------------------
--TGTGKTFTLGRLGEEDASDR--------GIMVRAMEDILADVSPESD--------------------
-----------------------------------------------MVTVSYLQLYM------
ETVQDLLAP-----------------------------------------------------------
------------------------------------ERDNIPIVEDPK---------------
TGDVSLPGAT----LVEIRDQSSFLDLLQLGEANRFAANTKLNTESSRS-
HAILMVNVKKHLKGKTGDMIVMDENGMT-MQMAKGFRAPTVRKSKLVVVD------------------
----------LAGSERLDKSG--SEGH--TLEEA-------------KSINLSLTA------
LGKCINALAEGS------------SHVPIRDSKLTRLLR-----DSFGG-
TARTSLVITIGPSPRHRGETASTILFGQRAM-----------
>MvKinesinARKc
----------RVRVAVRLRPRNAEEQVSDADFADCVE-----------------------------
LEPELHRLKLRKNNWD--------------------ADTYQFDEVLTEHAS------------------
-----------QKRVYEVVGKPVVESVLQGYN---------GTIMAYGQ--------------------
--TGTGKTYTLGQLGDEDDSAR--------GIMVRAFEDILSGVSLRED--------------------
-----------------------------------------------TVTVSYLQLYM------
EMIQDLLSP-----------------------------------------------------------
------------------------------------EKENIPIVEDPK---------------
MKDVQLPGAS----VVEIKDYNSFVELLKVGEANRAVANTRLNAESSRS-
HAMLVVNVRKLVRDSSEDLNNSENGETL--FGSKDFSVPTARRSKLVVVD------------------
----------LAGSERIDKSG--VIGS--SLDEA-------------KSINLSLTA------
LGKCINAIAEGN------------SHVPVRDSKLTRLLR-----DSFGG-
TARTSLVITIGPSPRHRAETASTIMFGQRAM-----------
>MvKinesinARK-LIKE
----------RVRVTLRARPSTKQEKQEEDNSLVYIN-----------------------------
PDKRTVIVRRNGSSVD--------------------YTEYKFDAVLSDSAT------------------
-----------QADVYRVAAQPVVLDVLHGYN---------GTIMAYGQ--------------------
--TGAGKTYTLSDISINETSTSR-----IDGIIPRSAANIFEYTSNDKNH------------------
-----------------------------------------------EYRISMSYIQIYL------
ETIQDLLNP-----------------------------------------------------------
------------------------------------ESSNLQIREGE----------------
TGGIFIAGVH----EVEIKSLEDLVRLLMIGDSNRTNAFTNMNAHSSRS-
HAIVIITVEKKSIGNLCGRTSIVDSGRR-TPRPIGSQEQQILVGKLFLVD------------------
----------LAGSERLKRSG--SEGL--RATEA-------------MSVNMSLTA------
LAKCISARADPSN-----------THVPFRDSKLTRLLQ-----ESLGG-NSKTSLIINIAPCSLY-
HETLSSLKFGMRAM-----------
>MvKinesin12-Ia
---------HNVQVVIRIRPLSSTELASQ------------------------------G-
HSKCVRQESANTITWVGHP--------------------ESR-FTFDIVASEAI--------------
-------------TQEKIFKVAGQPMVDNCLSGYN---------SCMFAYGQ----------------
------TGSGKTFTMLGDLEGA----DHRPSKNRGMTPRVFEYLFAKIEQEEESRRDE-----------
-----------------------------------------------RLRFICKCSFLEIYN------
EQILDLLDPTT---------------------------------------------------------
-------------------------------------TNLQMRED----------------
AKRGVYVENLS----EVEVTNVHDVIRLLLQGAANRKVAATNMNRESSRS-HSVFTCTIESKWT--SQ-
------------------SISSGRFGRLNLVD-----------------------------LAGSER-
QKTSGAEG---ERLKEA-------------ANINKSLST------LGLVIMSLVDIAN--------
GKQRHVPYRDSKLTFLLH-----DSLGG-NSKTTIIANISPSNCNALETLSTLKFAQRAKFI-------
--
>MvKinesin12-Ib
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
------------------------------------MLFLILD-EEKKQSGK------------------
-----------------------------------------NVQFSVKCSSLQIYN------
```

```
EQVKDLLSPHS-----------------------------------------------------------
-------------------------------------TNLTIHED-----------------
AKMGMIVDGVQ----EVEVTTPEATYEVLKRGSINRHVGATAMNSESSRS-HGVFILNLESQRQ--NM-
------------------GVLSKRVSKFYLVD-----------------------------LAGSER-
QKQTEAVG---LRLKES-------------GSINRSLSA------LGNVIKALLETSE--------
GKLRHIPYRDSKLTYLLK-----DALGG-NSRCTLIANVSPSITNSEETLCTLKFAQRAK---------
--
>MvKinesin12-Ic
---------HNVQVLIRCRPLNSSELSAH-----------------------------G-
YSRCIRQETSHTLTWIGHP-------------------ECR-FTFDHVAGEKI---------------
--------------TQEKLFEIAGIPMIDNCMSGYN---------SCMFAYGQ----------------
------TGSGKTHTMLGDIGDL----EQRPSENRGMTPRIFEYLFRRITLEEENRKNE-----------
-----------------------------------------------RLKFTCKCSFLEIYN------
EQITDLLEPSS-----------------------------------------------------------
-------------------------------------SNLQMRED-----------------
LKKGVYVESLS----EVEVNSVQDVIFLLLQGAQNRRVAATNMNKESSRS-HSVFTCIIESQWE--SD-
------------------NMTNIRFGRLNLVD-----------------------------LAGSER-
QKSSGAEG---ERLKEA-------------ANINKSLST------LGLVIMILVDVAQ--------
GKHRHVPYRDSKLTFLLQ-----DSLGG-NSKTTIIANVSPSVGCASETLSTLKFAQRAKFI-------
--
>MvKinesin12-Ie
---------HNVQVLIRIRPLNAAERSAN-------------------------------G-
PLTCVRQESPHSLSWLGQP--------------------ETM-FTFDHVCCSST---------------
--------------TQEEIFEVAGMPMVDNCINGYN---------SSIFAYGQ----------------
------TGSGKTHTMLGDIEEI----ENMPNPNRGVTPRIFEYLFSKISMEEEKHRKD-----------
-----------------------------------------------HLGITCRCSFLEIYN------
EQITDLLDPSS-----------------------------------------------------------
-------------------------------------TNLQIHED-----------------
PKKGIFVENLK----EVEVKNTGDVMELLFQGTANRRVAQTRMNQESSRS-HCVFTCVIKSKWT--SN-
------------------AGTRVRYGRLNLVD-----------------------------LAGSER-
QKSSGAEG---ERLREA-------------VNINKSLST------LGLVIMTLVDVAH--------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTAIIATVSPSIICASETLSTLKFAQRTKFI-------
--
>MvKinesin12-Id
---------HNVQVLIRLRPLNTHESAAS-------------------------------G-
MYMCLKQTSPKSLTWTGHP--------------------ETR-FTFDHVASPSI---------------
--------------TQESLFQVVGLPMVDNCIQGYN---------SSIFAYGQ----------------
------TGSGKTYTMLGDIFEK----ENELNPNRGIIPRIFEYLFSKAEMEEKAREQE-----------
-----------------------------------------------HFTITCRCSFLEIYN------
EQITDLLDPAS-----------------------------------------------------------
-------------------------------------TNLQIHED-----------------
PRKGVFVENLT----EIKVKKATDVLGLLLQGTANRRVAQTRMNKESSRS-HCVFTCTIESSWI--KE-
------------------SFTNSRFGRLNLVD-----------------------------LAGSER-
QKASGAEG---ERLREA-------------VNINRSLSA------LGLVIMNLVDVAQ---------
GKQRHVPYRDSKLTFLLQ-----DSLGG-NSKTAIIATISPSISCANETLSTLKFAQRAKFI-------
--
>MvKinesin12-II
-----------VKVIVRMRPRNSKEELEE-------------------------------A--
VEIAEKVSCDSLRVDEH-----------------------QFTFDQVAGTDA---------------
-------------SQQAIFKTIGLPFVENCLQGFN---------SSIFAYGQ----------------
-----TGSGKTYTMWGSLENLEARNHHVYSEDRGLAPRIFEYLFHRTNEEEERNKDK-----------
-----------------------------------------------QLMHLCRCSFLEIYN------
EQITDLLEPVP-----------------------------------------------------------
-------------------------------------KSLQIRED-----------------
TKSGIYVENLT----EEYVSNADDVLRLMLKGFANRRVGSTSMNAESSRS-HTVFTCIIESRCKNPGD-
------------------GGSSVRTSRMNLVD-----------------------------LAGSER-
QKSTKAAG---QRLKEA-------------GNINRSLSQ------LGNVINILAEISQT-------
GKPRHIPYRDSRLTFLLQ-----ESLGG-NAKLAMICAISPMSSCKNETLSTLRFAQRAKSI-------
--
>MvKinesin13b
----------RIKVVVRKRPLNKKELARK-------------------------------
EEDIVAIDGNGCSLTVHEPKLKVDLTA------------YVEKHDFTFDAVLNDQ--------------
--------------VSNDEVYRETVEPIIPTIFQRTK---------ATCFAYGQ--------------
--------TGSGKTYTM-----------------QPLPLRACQDILNLMQHPAYRNQ-----------
```

```
--------------------------------------------------GFQLWLSFFEIYG-----
-GKLYDLLNERR-------------------------------------------------------
-----------------------------------------KLCMRED-----------------
GRQQVVIVGLK----EFSVSHVEVVKDYIEKGNSSRSTGSTGANEESSRS-
HAILQLAIKKHSNKKDEK--------------------GGKPVGKFSFID------------------
----------LAGSERGADTTDNDR---QTRIEG-------------AEINKSLLA------
LKECIRALDS------------EQGHIPFRGSKLTEVLR-----DSFVG-
DSRTVMISCISPSTGSCEHTLNTLRYADRVK-----------
>MvKinesin13c
----------KIKVVVRKRPINKKEITRG------------------------------
EEDIITVADSANSLIVHEPKLKVDLTA------------YVEKHEFVFDAILDEN--------------
---------------VTNHEVYLETVEPIIPGLFQQTK---------ATCFAYGQ--------------
--------TGSGKTFTM-----------------RPLPLKASEYILYLMQQPGYIEE-----------
--------------------------------------------------GFQLWLSFFEIYG-----
-GKLFDLLNGKS-------------------------------------------------------
-----------------------------------------KLCMRED-----------------
GRQQVCIVGLK----EFQISSLESLKGYIDLGSSARSTGSTGANEESSRS-
HAILQLCIKRPQESTDKK--------------------RSRIVGKMSFID------------------
----------LAGSERGADTTDNDR---QTRLEG-------------AEINKSLLA------
LKECIRALDS------------DQNHIPFRGSKLTEVLR-----DSFVG-
NSKTVMISCISPSSGSCEHTLNTLRYADRVK-----------
>MvKinesin13a
----------RIKVVVRKRPLNKKEIARK------------------------------
EEDIVSIDCNGSALSVHEPKLKVDLTA------------YVEKHDFLFDAVLDDH--------------
---------------VSNDEVYRKTVQPIIPTIFQRTK---------ATCFAYGQ--------------
--------TGSGKTFTM-----------------QPLPLRACQDILDLMKHPVYRNE-----------
--------------------------------------------------GLQLWLSFFEIYG-----
-GKLYDLLTDRR-------------------------------------------------------
-----------------------------------------KLCMRED-----------------
GRQQVCIVGLR----EFPVSNVEVVKEYIDKGNATRSTGSTGANEESSRS-
HAILQLVIKKHSNKKDEK--------------------AGKLVGKFSFID------------------
----------LAGSERGADTTDNDR---QTRIEG-------------AEINKSLLA------
LKECIRALDH------------EQGHIPFRGSKLTEVLR-----DSFVG-
DSRTVMISCISPNAGSCEHTLNTLRYADRVK-----------
>MvKinesin14-Ia
--------KGNIRVFCRVRPMLPDDESSPC--------------------------
GGDDVPVIQYPTSTELLGHGIELIQ--------------AQG-QKHTFSFDKVFGPEAGQ--------
---------------------EDIFVEISQLIQSALDGYK---------VCIFAYGQ-----------
-----------TGSGKTYTMLGNPD---------DIDQRGVIPRSLEQIFKASQELG-----AQG----
-----------------------------------------------------WSFQMQASMLEIYN--
----ETIRDLLA-------------------------------------------------------
PTSKCETTGKQ-----------------------------------YTVKHD---------------
PNGNTSVSDLT----LVEVTKWKEVSSLLQRASQSRSVSKTAMNEQSSRS-
HCVFTLRISGLNESIDQQ----------------------VNGVLNLID------------------
----------LAGSERLSRSCVSG----DRLKET-------------QAINKSLA------
SLGDVILAIAN------------KEQHIPYRNSKLTYLLQ-----PCLGGDS-
KTLMFVNISPDVKSTSESLCSLRFAAKVNACEIG------
>MvKinesin14-Ib
--------KGNIRVFCRVRPLLVEDEDGE-----------------------------
QVQSVVQYPQFGDLVGRGIELTQ--------------AEG-QKYAFSFDKVFGPDFTQ----------
-------------------ANVFEEISQLVQSALDGYK---------VCIFAYGQ-------------
---------TGSGKTYTMLGRPD---------QDEHKGLIPRSLEQIFRSSQSLE-----SQG------
-----------------------------------------------------WKFKMQASMLEIYN----
--ETIRDLLINPKL-D-A-------------------------------------------------
QKADVG-AVAKV-----------------------------------HNIKHD--------------
-NMGNTFVSDLT----LVEVTSWKEVSALLRQAAQSRTVGRTNMNEQSSRS-
HCVFTLRISGVNESTEQQ----------------------VHGILNLVD------------------
----------LAGSERLSKSGATG----DRLKET-------------QAINKSLA------
CLGDVILAIAN------------KEQHVPYRNSKLTYLLQ-----PCLGGDS-
KTLMFVNIAPDCKSLHESLCSLRFAAKVNACEIG------
>MvKinesin14-Ic
--------KGNIRVFCRVRPLLG-DEDPE-----------------------------HVP-
AVQYPQYGDLVGRGVELVQ---------------TEG-QKYTFSFDKVFGPDMKQ--------------
----------------ENVFEEISQLVQSALDGYK---------VCIFAYGQ-----------------
```

```
-----TGSGKTYTMLGQPD---------NESHKGLIPRSLEQIFKSSQILQ-----GQG----------
-------------------------------------------WTFKMQASMLEIYN------
ETIRDLLSTSKS-EPP-----------------------------------------------------
QKPDSTPTAAKQ-----------------------------------YAIKHD--------------
-TLG-THVSDLT----LVEVTSWKEVSTLLHQAAQSRTVGRTAMNEQSSRS-
HCVFTLRIVGTNENTDQQ----------------------VNGVLNLVD------------------
----------LAGSERLSKSQVTG----DRLKET-------------QAINKSLS------
SLGDVILSIAN-----------KEQHVPYRNSKLTYLLQ-----PCLGGDS-
KTLMFVNIAPDSKSLHESLCSLRFAAKVNSCEIG------
>MvKinesin14-IIa
--------KGNIRVYCRVRPFLPGQRN--------------------------------
VSTVDYIGDRGT-IAIQNPTK---------------QGKDQRKSFTFNKVFGPSASQ-----------
-------------------EEVFLDTQPLIRSVLDGYN---------VCIFAYGQ--------------
--------TGSGKTHTMMGPNNP--------CPDDWGVNFRALNDLFYISHERQ-----DV--------
-------------------------------------------------VRYEVAVQMMEIYN-----
-EQVRDLLCSDG-----------------------------------------------------------
-TNKKLE-----------------------------------IRNNSQ--------------QNG-
LNVPDAS----LHPVNSTEAVLDLMALGHKNRAVGSTALNERSSRS-HSVLTVHVKGTDLTSGDA----
-------------------LRGCLHLVD----------------------------
LAGSERVDKSEAIG----ERLKEA--------------QHINRSLS------ALGDVIAALAQ------
------KSSHVPYRNSKLTQLLQ-----DALGGQA-
KTLMFVHVSPDQDSYGETISTLKFAERVAKVEL-------
>MvKinesin14-IId
--------KGNIRVYCRVRPFLSGQNTK--------------------------------
YDSVEFIGENGD-LLICNPLKP---------------QAKDARRMFNFNKVYRPSATQ-----------
-------------------EEIFSDTQPLIRSVLDGFN---------VCIFAYGQ--------------
--------TGSGKTYTMSGPSSM--------SKHDWGVNYRALDDLFQISQSRK-----DV--------
-------------------------------------------------ISYEVGVQMIEIYN-----
-EQVRDLLFIDT-----------------------------------------------------------
-SNRRLE-----------------------------------IRNNSQ--------------LNG-
LNVPDAC----MLRVNSTADVLKLMKIGQKNRAVGATALNERSSRS-HSVLTVHVRGTELATGNV----
-------------------LHGCLHLVD----------------------------
LAGSERVDRSEARG----DRLKEA--------------QHINKSLS------ALGDVISALAQ------
------KNSHVPYRNSKLTQLLQ-----DSLGGQA-
KTLMFVHIGPDADSYGETISTLKFAERVASVEL-------
>MvKinesin14-IIb
--------KGNIRVYCRVRPFLPGQTNT--------------------------------
QTTVDFVGEKGS-IIIANPNK---------------QGKDQRKSFNFNKVFGPNVAQ-----------
-------------------EDVFLDTQPLIRSVLDGFN---------VCIFAYGQ--------------
--------TGSGKTYTMTGPNNP--------TPKDWGVNFRALNDLFQISLDRK-----DF--------
-------------------------------------------------VKYEVGVQMMEIYN-----
-EQVRDLLSSDG-----------------------------------------------------------
-SNKKLE-----------------------------------IRNNSQ--------------QNG-
LNVPDAT----MLPVACTNDVLEFMNLGHKNRAVSSTALNERSSRS-HSVLTVHVKGTDMSNGSV----
-------------------YRGCLHLVD----------------------------
LAGSERVDRSEVTG----DRLKEA--------------QHINKSLS------ALGDVIAALAQ------
------KNSHVPYRNSKLTQLLQ-----DSLGGQA-
KTLMFVHISPDEDSYAETISTLKFAERVATVEL-------
>MvKinesin14-IIc
--------KGNIRVYCRVRPFLGGQETK--------------------------------
QSVVEFIGENGD-LLLANPK----------------QVKDGHKMFTFNKTFGPSASQ-----------
-------------------EEVFMDTQPLIRSVLDGYN---------VCIFAYGQ--------------
--------TGSGKTFTMTGPNSL--------TEKDWGVNYRALNDLFHISQSRE-----DV--------
-------------------------------------------------VKYEVAVQMVEIYN-----
-EQVRDLLMSDC-----------------------------------------------------------
-PNKKLE-----------------------------------IRNYSQ--------------LNG-
LNLPDAT----MLPVSSTSDVLELIKIGQKNRAVGATALNERSSRS-HSVLTVHVQGTDLASGSI----
-------------------LRACLHLVD----------------------------
LAGSERVDRSEVTG----DRLKEA--------------QHINKSLS------ALGDVVSALAQ------
------KNSHVPYRNSKLTQLLQ-----NSLAGQA-
KTLMFVHISPDTESYGETISTLKFAERVASVELG------
>MvKinesin14-IIIa
--------KGNIRVFCRCRPLSSSDINV--------------------------------
GASSVVDFDSSR---DNELAVR---------------CNGGRKLFKFDRVFTPSNNQ-----------
```

```
------------------TDVFADTAPVVVSVLDGYN---------VCIFAYGQ--------------
--------TGTGKTHTMEG-----------TENDRGVNYRTLEELFRLASERK-----GQ--------
-------------------------------------------------FEYTISVSVLEVYN-----
-EQIRDLLASPPP-----------------------------------------------------
SGQSAKKLE-----------------------------------IKQVAD-----------------
GVHHVPGLT----EAKVESMEEVWEVLQTGKSARAVGSTNCNEHSSRS-HCMLCVMVKGESVITGEC--
--------------------YRSKLWLVD----------------------------
LAGSERIAKSDVQG----DRLKEA-------------QNINKSLS------ALGDVIYALST------
------KSSHVPYRNSKLTHLLQ-----DSLGGES-
KTLMFVQISPSENDAGETLCSLNFASRVRGIEL-------
>MvKinesin14-IIIb
----VVELKGNLRVYCRCRPLSSQEVAR-------------------------------
GEQPVVDYDIYN---INELYVN--------------ASEGQKKVFKFDRIFSPEHDQ-----------
------------------AAVFKEVAPLVVSVLDGFN---------VCIFAYGQ--------------
--------TGTGKTFTMQG-----------SESNRGVNYRTLEKLFNLAAERE-----GH--------
-------------------------------------------------FEYNMFVSVLEVYN-----
-EEIRDLLAPSGL-----------------------------------------------------Q-
--NGKKLE-----------------------------------VKQISE-----------------
GVHHVPGVV----EAPVTCVEQAWKVLEAGGRGRAVGSTNANKHSSRS-HGLVCIVVKGENVITGET--
--------------------VNSKLWLID----------------------------
LAGSERVGKTDAQG----DRLKEA-------------QNINKSLS------ALGDVIHALAK------
------RSSHVPYRNSKLTHLLQ-----DSLGGQS-
KTLMLVQISPSGQDVGETLCSLNFASRARGIEFG------
>MvKinesin14-IV
--------KGNVRVFCRIRP--PARKEQ-------------------------------------
QAAVVPSLNKIHVAV---------------SGK--RKIFELDKVFLPASTQ-----------------
------------DDVFAEVSPLVRSALDGHN---------VCVFAYGQ--------------------
-TGAGKTFTMEG-----------NRELPGIVPRTLQTLFHQASSDR-----SR---------------
-----------------------------------------SYIFTFSMLELYM------
GSLRDLLVSAP-------------------------------------------------------
QRITDPAPKC-----------------------------------LSIQMD---------------
VKGWVEIENLS----EFVIKDAKQARKLYRTGSRARATACTNSNEVSSRS-HCLIRVTMACT-
DHSGENP----------------------ILSKLWLVD----------------------------
LGGSERLLKTNAKG----QILEEG-------------KAINLSLT------ALGDVISALQK------
------KQPHIPYRNSKLTQILR-----DSLGEDA-
KTVMLVHVSPSKEDAGETVCSLTFATRARGIHLG------
>MvKinesinOrphIV
----------
KIRVFLRIRPAKVVRSGKVTKKAPKQGDMVNRSIQNWKKPSAAEAKSANEAPEVCLFPQTSDSVSLIPP
TSVSG------------PRRSKAEEFNGFTHIFTPDAT----------------------------
QEEVFDKIVHPRLVDLLKGQS---------SLVVAMGP----------------------
TSAGKTHTMLG------------TGEDVGLLPRSLRFLLKDNQDINS----------------------
-----------------------------------------ASIR-
IAISMFEIYSDQQARSERLIDLLQ-DGVELS-----------------------------------
-------------------------------------------------------------------
------------LLQFKIKGLH----EAFVSSIEEADSVLLSGLKRRTTASTAANDRSSRS-
HCIINISANVPKDCEGQDD----------------VIYLKK--ATLTIAD------------------
----------LAGFER-EKKTGNQG---VRLNES-------------SFINNTSMI------
FGQCLRALLEHQKNP------KKLLQKHFQYSMLTRYLK-----PYMEA-
RGNMTLIVNVSPCEEDYLDTAFVLRQAA-------------
>MvKinesin14-V
--------KGNIRVYCRVRPQFEHEGPS--------------------------------
VTEFPDDFLIRVNTASLTAN--------------PIGLPKKEFELDRVYGPHVGQ-------------
-----------------GDFFQDVQPFVQSALDGYN---------VCVFAYGQ---------------
------SGSGKTYTMEG-----------PSNDWGVFFRAFEELFDLSNNDM-----TST---------
-------------------------------------------------SKFSFAMSIFELNN-------
EIRDLLLGS---------------------------------------------------------
---TR-----------------------------------SMGTVQ--------------
MGYSGKPVELT----QERVENPIEFSRIYKSALQGRS-----KDASANPA-
HLVLTIHIRYTNSFSGES----------------------YYSKLSMVD------------------
----------MVASESLSREDAIG----DRLTEL-------------LHINKSFS------
ALGDVLSALTA-----------KKEYVPYGNSKLTQILA-----DSLGGDA-
KTLLIVNLSPCQNDVQETVSSLQFAARARNVEL-------
>MvKinesin14-VIb
```

```
--------KGKIRVYARWRPLSVKEKTE---------------------------------
GQKSVLVSPDEFTLEHPWK-----------------DDKPKQHQFDHVFDDTATQ------------
----------------DDVFESTKFLVQSAVDGYN---------VCIFAYGQ---------------
------TGSGKTFTIYG-----------SDNNPGLTPRATEELFRVIHRDR-----NK----------
-----------------------------------------------FCFTLKAYMVELYQ------
DTLVDLLLPKN-------------------------------------------------------
AKK-QK-------------------------------------LEIKKD--------------
SKGMVVVENAT----LLTIVSREELQAVVTKGIEQRHTSGTQMNAESSRS-
HLILSVIVESTNLQTQIQ----------------------VKGKLSFVD------------------
----------LAGSERVKKSGSAG----EQLKEA-------------QSINKSLS------
ALGDVISALST------------ETQHI-----------------------------------------
------------------------
>MvKinesin14-VIa
--------KGKIRVFARWRPLSEKEKGE---------------------------------
NQRAVLTRPDEFTLEHPWK-----------------DDKPKQFQFDRVFDETSTQ------------
-----------------EDIFEDTKYLVQSAVDGYN---------VCIFAYGQ---------------
------TGSGKTFTIYG-----------SEANPGLTPRATRELFSILKRDS-----NK----------
-----------------------------------------------FSFSLKAYMIELYQ------
DTLVDLLLPKN-------------------------------------------------------
AKR-PK-------------------------------------LEVKKD--------------
SKGMVLVENCT----LVPVSTLEDLEAIVMKGLDRRHISGTQMNSESSRS-
HLVLSIIIESTNLQTQVQ----------------------VKGKLSFVD------------------
----------LAGSERVKKSGSSG----EQLKEA-------------QSINKSLS------
ALGDVISALAT-----------EEQHIPYRNHKLTMLMS-----DSLGGNA-
KTLMFVNISPAESNIDETYNSLGYASRVRAITN-------
>MvKinesinOrphIII
----------NINVFVRVRPLTKDENTRG-----------------------------
DKPCVQVLKDTQFIKVVDYGYG----------------YNALTRKFQFDGCLSHEVKQ-----------
-----------------KQVMEKCRVSRLLDSVLEGYS---------SAVIACGQ--------------
--------TGSGKTFTMCGRNNEQER---NTE--DDGLIAQCVSYIFSAMKKFDD--AGEKM-------
-----------------------------------------------SFCLKASYYEVYN-----
-EQVNDLLRLDC-------------------------------------------------------
-----------------------------------------APREVKWS-----------------
LKDGYYVDNLL----LVECESVDDVFSVLSEGAKNRKVGSHDLNKDSSRS-
HCIMTLYVDTVTDIGDGSP-------------------PIVRYGKMLFVD------------------
----------LAGSERLKKSRSSGE----MLRET-------------GNINRSLFT------
LGKVISALAEGK----------KGDLVPYRESMLTKLLM-----ETLGG-
NSLALMIACVSPAASAVEETLSTLYYAT-------------
>MvKinesin10
-----------VRVVARIRPSLPYEIHNHVP-----------------------------
TACFSFTRDSPEETTLHVKDLSSRRVLGSQLGSDGSLSDTCREGTYKLDSCYNG-DDEIS---------
-------------------VLFDNEVKHFLPKLFEGQN---------ATVFAYGA--------------
--------TGSGKTYTMQGS-----------DGHPGLMTLAMKNILEMAAQKPN---------------
-----------------------------------------------YRVEVAYYQIYN-----
-ERILDLLDESNAD-----------------------------------------------------
-----------------------------------------QIKVLED-----------------
TDGKTYLRGLS----QVHVETMEKFNELLVDGCLRRRVGQTGLNIVSSRS-HAALVVTIVNHDN-A---
----------------------FCHGKLNLID----------------------------
LAGNEDNKKTGNEGN----RLVES-------------ARINQSLFV------LSNVITALNS------
------NNPRVPYRDSKLTRILQ-----DSLGG-TSHTIMIACLN--RRSYQEANQTLTIAARS-----
-------
>MvKinesin2
---------ERVQVVVRCRPISERELTAG-----------------------------
HKCCISIDTERRTVEVHNVGG-------------RFAADNIPKSFTFDRVYNEKS-------------
----------------TQRQIYKDVAYSIVHSVMCGYN---------GTVLAYGQ--------------
--------TASGKTYTMEG---CDHS------PDLWGIIPNAFEHIFQHIR-Q----SQS---------
-----------------------------------------------SDSFLVRISYLEIYN-----
-EEIRDLLSP--AT-----------------------------------------------------
-------------------------------------SKKLELKES----------------
VETGVYVKNLT----SLTVNSFADIRQLLMLGKKNRAIGATAMNQDSSRS-HSIF--TITVES---
STC--------------DPTGGKTHVRVGKLNLVD----------------------------
LAGSER-LSKTGATG---ERFKEM-------------TKINWSLSA------LGNVISALVDG-----
------KSSHIPYRDSKLTRLLQ-----DSLGG-NTRTVMVANIGPADYNYEESISTLRYANRAKNI--
-------
```

```
>MvKinesin4-Ia
----------AVKVAVHVRPLIASERLQG-----------------------------
CKDCVSVVPGEPQVQVG-----------------------NFGFTFDHVYGNTA------------
--------------LPSSRIFDECVAPLVEGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTTYTVGDDKN--------GIIPQVMDLIYDRMRSSKKN-------------
-------------------------------------------------INYQVLVSFIEILN-----
-EEVNDLLDS----NSPTGTRQDS-----------------------------------------------
-------------------------YVGFCTRVSTPPKASIQIRET-----------------
TNGEITLSGVS----EVEVNNLEEMAALLEQGSLCRATASTNMNSQSSRS-HAILTITLRQQRKLELN-
------ADNE--TSLLDHIPDDNLCAKLHLVD----------------------------LAGSER-
VKRTGADG---IRFKEG--------------VHINKGLLA------LGNVISALGDEKKRK-------
EGGHVPYRDSKLTRLLQ-----DSLGG-NSRTVMIACISPADSNAEETLNTLKYANRARNIQ-------
-
>MvKinesin4-Ic
----------SVKVAVNIRPLIGHELVQG-----------------------------
CKDCVSVVPGEPQVQLG-----------------------NHSFTFDHVYGSTG------------
--------------APSSCIFNDCVHPLVDGLFHGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTGYTVGGSTE--------GVIPKVMQMIFDRVAELKSK-------------
-------------------------------------------------ADFHIRVSFIEILK-----
-EEVHDLLDP----NPPIALKSEP-----------------------------------------------
-------------------------GNGAGSKAGPVGKPPIQIREN-----------------
TNGEITLAGVT----EVDVRSQMEMGNCLEQGSLCRATASTNMNSRSSRS-HAIFTITVEQKR------
--------IS--EPNSTDGGDDFLCAKLHLVD----------------------------LAGSER-
AKRTGADG---VRFKEG-------------VHINKGLLA------LGNVISALGDDKKRK-------
EGGHVPYRDSKLTRLLQ-----DSLGG-NSRTVMIACISPADSNAEETLNTLKYANRARNIQ-------
-
>MvKinesin4-Ib
----------SVVVALRIRPLTSSEHKQG-----------------------------
CKDCITVIPNEPQIQLG-----------------------SNFFTFDHVYGSTG------------
--------------SRTTDIFDECVAPLIDGLFQGYN---------ATVLAYGQ--------------
--------TGSGKTYTMGTAFTVGGGAE--------GITPRVMETLFKKIEKSKDG-------------
-------------------------------------------------VESQLRVSFIEILK-----
-EEVHDLLDP----NPAAMARADLN----------------------------------------------
--------------------------FLTVNCKNPSTGKPVIQIRET-----------------
TNGEIMVAGVR----EVEVANHQEMALCLEQGALSRATGSTNMNATSSRS-HAIFTVTLEQRKKLNMN-
------SRDN--NTAVDEI-DDFLCAKLHLVD----------------------------LAGSER-
AKRTGTDG---LRFKEG--------------VHINKGLLA------LGNVISALCDERKRK-------
EGGHIPYRDSKLTRILQ-----DSLGG-NSRTVMIACVSPADVNAEETLNTLKYANRARNIQ-------
-
>MvKinesin4-II
------------------PLLEKEIQDH-----------------------------
CQECVNYNSERAEITLGK-----------------------EKRFTFDHVFGPDS------------
--------------SQEE-IYLDCVKPLVDSCIAGYN---------ATVVAYGQ--------------
--------TGSGKTHTMGSANSNVIQES------EMGILPRVIRQLYKSIEEHKN-------------
-------------------------------------------------AEFLVKCSFVEIYN-----
-EEIKDLLHP----ETPSK-----------------------------------------------
---------------------------------------SIFIRED-----------------
ANGDIILAGVR----EEVVTSFENMMKFLELGSTSRTTGSTLMNQHSSRS-HAIYTIIVQQR-------
------------TIEDCSSDVETITAKFHLVD----------------------------LAGSER-
AKRTGAVG---ARFKES--------------ITINSGLLA------LGNVISALGDERKR---------
GQHVPYRQSKLTRLLQ-----DSLGG-NSRTCMIACISTADVNNEETLNTLKYANRARN----------
>MvKinesin5a
--------TVNVQVLLRCRPLCDDEIRNN-----------------------------
DPYVVHCDEERSEVIVSS--------------------KLFDRTFTFDRVYGPET------------
----------------EQKDLYDESIANIVNDVLEGFN---------CTIFAYGQ--------------
--------TGTGKTYTMEGLLRNSKD--AEELPAGAGIIPRALQQIYNALE------SQR---------
-------------------------------------------------MDYSMKATYLEIYN-----
-EEITDLLAPDEN----LFSPSPR-----------------------------------------------
-------------------------------------KPSLVLMED-----------------
GKGGVMVRGLE----EELVFSEKQIFGLLERGSSKRRTAETLLNKQSNRS-HSIFSIILHMRD------
--------------ANLVSEELIKCGRLNLVD----------------------------LAGSEN-
ILRSGARE---ERAREA--------------GEINKSLLT------LGRVISSLAEH------------
SGHVPYRDSKLTRLLR-----ESLGG-KTKTCIIATIAPSLRCLEETLSTLDYAYRAKNIKNRPEVNQK
>MvKinesin5b
```

```
--------GVNVQVVVRCRPFNDDELRLN------------------------------
GPHVVMCNEARREVTVTT--------------------KLLDRMFTFDKVFGQQA------------
---------------QQKDLYDQAIAPIVNEVLEGYN---------CTVFAYGQ-------------
--------TGTGKTYTMEGPARKSKN--AGELPTEAGVIPRAVQQIFHTLE------SQH---------
-------------------------------------------------AEYNMKVTFLELYN-----
-EEITDLLAPDEISR--LVDDKPR-----------------------------------------------
-------------------------------------KP-LALMED-----------------
GKGGVLVRGLE----EEVVYSANDIFNLLERGSAKRRTAETLLNKQSSRS-HSIFSIIIHIKE------
--------------SNIGGEELIKCGRLNLVD----------------------------LAGSES-
ISRSGARE---ARAREA--------------GEINKSLLT------LGRVITSLVEH------------
LGHVPYRDSKLTRLLR-----ESLGG-KAKTCIIATIGPSFQCLEESLSTLDYAYRAKSIKNKPEMNQ-
>MvKinesin5c
--------GVNVQVLLRCRPFNDEELRTS------------------------------
APQVVSCNEHRREVSVNMN----------------IAAKQIDRTFTFDKVFGPNA------------
---------------RQQDLYEQAIVPIVNEVLEGFN---------CTIFAYGQ-------------
--------TGTGKTFTMEGSGRKPKD---GGMPTDAGVIPRAVQQIVDTLE------AQN---------
-------------------------------------------------AEYSMKVTFLELYN-----
-EEITDLLAPDDVVRPLPVDDRQP-----------------------------------------------
-------------------------------------KKPLALMED----------------
GKGGVLVRGLE----EEIVYSANEIYTLLERGTAKRRTAETLLNKQSSRS-HSIFSITIHIKE------
--------------ATPEGEELLKCGKLNLVD----------------------------LAGSEN-
ISRSGARD---GRAREA--------------GEINKSLLT------LGRVITALVEH------------
LGHIPYRDSKLTRLLR-----DSLGG-KTKTCIIATVSPSVHCLEETLSTLDYAHRAKNIKNKPELNQK
>MvKinesin5d
---------VNVQVLLRCRPFSEDELLAN------------------------------
APQVITCNEVKREVSVNQN----------------VQGKQVDRTFVFDKVFGPQS------------
---------------KQKDLYDQAIIPIVNEVLEGFN---------CTIFAYGQ-------------
--------TGTGKTYTMEGSGRRTGN---DELPPDAGVISRAIKQIFDTLE------LQN---------
-------------------------------------------------AEYSVKVTFLELYN-----
-EETTDLLAPEDFSK---IPDEK-----------------------------------------------
-------------------------------------KRPLSLMED----------------
GKGGVIVRGLE----EVVVNNATEIFNLLDRGSAKRRTAETLLNKQSSRS-HSIFSITIHIKE------
--------------STPDGEELIKCGKLNLVD----------------------------LAGSEN-
VCRSGARE---GRAREA--------------GEINKSLLT------LGRVITSLVEH------------
LGHVPYRDSKLTRLLR-----DSLGG-RTKTCIIATVSPSVHCIEETLSTLDYAQRAKNIKNKPEVNQK
>MvKinesin7-Ia
----------SVTVTVRFRPLNNREIQ---------------------------------
RGDDIVWFPDGDNIVRSQL---------------HPTAA-----YAFDRVFGPST------------
---------------ITSNVYDAAARHVVHGAMDGIN---------GTVFAYGV-------------
--------TSSGKTHTMHGD-----------QKSPGIIPLAIKDVFSIIQE-----TP----------
-------------------------------------------------SREYLLRVSYLEIYN-----
-EVINDLLDPAG-----------------------------------------------------------
-------------------------------------QNLRVRED-----------------VQ-
GTYVEGIK----EELVLSPAHCLSLIATGEEHRHVGSNNFNLVSSRS-HTIFTLTIESSTR-GNFCF--
---------------E-DDVTLSQLNLID----------------------------LAGSE--
SSKTETTG---LRRKEG------------AYINKSLLT------LGTVISKLT-----------
DGKAIHIPYRDSKLTRLLQ-----PSLSG-HGRISLICTITPASSSNEETHNTVKFAHRAKHI------
---
>MvKinesin7-Id
----------SVSVTVRFRPLSLREIQ---------------------------------
KGDEVAWYADGDTIVRSEY----------------NPSTA-----YAFDKVFGPAT------------
---------------TTRSVYDVAAKHVVQGALDGIN---------GTVFAYGV-------------
--------TSSGKTHTMHGD-----------HKSPGIIPLAVKDVFRIIQE-----TP----------
-------------------------------------------------DREFLLRLSYLEIYN-----
-EVINDLLNPSG-----------------------------------------------------------
-------------------------------------QNLRIRED----------------SQ-
GPYVEGLQ----EEVVLSPAHALSLIAAGEEHRHVASNNFNLLSSRS-HTVFTLTIESSSRESDRYD--
---------------EYEELRISQLNLID----------------------------LAGSE--
SSKTETTG---LRMKEG-------------SYINKSLLT------LGTVIGKLS-----------
EGRAVHVPYRDSKLTRLLQ-----PSLGG-NGKVSLICTLTPASSNLEETHNTLKFAQRAKRVT-----
---
>MvKinesin7-Ib
----------NVTVTVRFRPLSSREIQ---------------------------------
KGDEIAWYADGETTVRSEY----------------NAATS-----YAFDRVFGPAT------------
```

```
----------------TTRNVYDVAARHVVNGAMRGVN---------GTVFAYGV--------------
--------TSSGKTHTMHGD------------QKSPGIIPLAVKEVFSTIQE-----TP----------
-------------------------------------------------GREFLLRVSYLEIYN-----
-EVINDLLDPAG-----------------------------------------------------------
---------------------------------------QNLRVRED----------------AQ-
GTYVEGIK----EEVVLSPAHCLSLIATGEEHRHVGSNNFNLLSSRS-HTIFTLTIESSPR-GDNYS--
---------------D-EEVTLSQLNLID----------------------------LAGSE--
SSKTETTG---LRRKEG--------------AYINKSLLT------LGTVISKLT-----------
DGKATHVPYRDSKLTRLLQ-----SSLSG-HGRVSLICTVTPSSSSTEETHNTIKFAHRAKHV------
---
>MvKinesin7-Ic
----------SVAVTIRFRPLSTRESQ--------------------------------
RGDEIAWYPDGDKIVRSEF----------------NHQAA-----YAFDRVFGPAT-------------
----------------TTIAVYDAAAKHVVRGAMDGVN---------GTVFAYGV--------------
--------TSSGKTHTMHGD------------QKSPGIIPLAVKDVFNFIQD-----TP----------
-------------------------------------------------GREYLLRVSYLEIYN-----
-EVINDLLNPTQ-----------------------------------------------------------
---------------------------------------TNLRIRED----------------AQ-
GTYVEGVK----EEVVLSASHALSLIASGEEHRHVASNNFNLVSSRS-HTIFTMTIESSAH-SPDAD--
---------------D-EDVTLSLLNLID----------------------------LAGSE--
SSKTETTG---LRRKEG--------------SFINKSLLT------LGTVIGKLS-----------
EGKPTHVPFRDSKLTRLLQ-----SSLSG-HGRVSLICTITPASAYYEETHNTLKFAHRAKRV------
---
>MvKinesin7-IIa
------SNEEKINVSVRIRPLNSKEIS--------------------------------
NNDYPVWDCTDLKTITCLY----TRPDRN-------YPQS-----YVFDRVFRQEN-------------
----------------GTREVYEKGAKEVTLSALSGKN---------ATIFAYGQ--------------
--------TCSGKTYTMNG-----------------ITEYAADDIFNYIKQ-----HD----------
-------------------------------------------------DHVFLIKFSALEIYN-----
-EVATDLLNPGS-----------------------------------------------------------
---------------------------------------GPLRIMDD-----------------
PERGTVIEKLV----EEIVKDKDHLLSLLSICEDHRQVGETVMNDISSRS-
HQIIRLTIERSSRVVCDE------------------RPGKSLVAALNFVD-------------------
----------LAGSER-ASLTHSEG---TTLKEG--------------CYINRSLLS------
LSTVIRKLSDPN--------RPRSTHIPYRESKLTRILS-----NALGG-
NARTAIICTMSLSNRHFEQTRNTLFFASCAKEV---------
>MvKinesin7-IIb
----------KIYVTVRVRPLNEKELA--------------------------------
RNEFQVWDCPDESTVAYTF----TMPERS------SFPQK-----YDFDQVFGLQA-------------
----------------TTQDVYDRGAKDVALSALKGKN---------ATIFAYGQ--------------
--------TSSGKTYTMHG-----------------IMESAADDIFGHIQQ-----NA----------
-------------------------------------------------ERIYLLKFSALEIYN-----
-EVTSDLLNPES-----------------------------------------------------------
---------------------------------------GPLRLLDD-----------------
PERGTIIEKLE----EKIVRDKEHLQNLVAFCESHRQVGETSLNDYSSRS-
HQIVRLTIESSPRIVADD------------------KPSKSLLAVLNFVD-------------------
----------LAGSER-ASLTNSEG---TRLKEG--------------CYINRSLLT------
LSSVIRKLSEGS--------RARPGHVPYRDSKLTRILQ-----NSLGG-
NARTAIICTMSCSNRHVEQTRNTLFFASCAKEV---------
>MvKinesin7-IIc
----------KILVTVRVRPLSSREIA--------------------------------
QNDTSAWECVDDRSIIHRP-PASMLPERS------PHHHP---KSYVFDHVFGPDS-------------
----------------VTLQVYERGAKDVALSALSGIN---------ATIFAYGQ--------------
--------TSSGKTYTMRG-----------------ITENAVRDMYAFMEK-----HS----------
-------------------------------------------------ERDFVIKISAIEIYN-----
-EVVRDLLNTDAS-----------------------------------------------------------
---------------------------------------SGPLRLLDD-----------------
PERGTRIEKLV----EDVVRDSQHLQKILSVIEAQRQVGETQLNETSSRS-
HQIIRLTVESFPKDVGDG------------------TLVKSLIASLNLVD-------------------
----------LAGSER-VSQTQAEG---TRLKEG--------------CHINRSLLT------
LTTIIRKLS-QG--------RVKTGHLPYRDSKLTRILQ-----NSLGG-
NARTAIICTMSPAQAHVEQSRNTLFFATQAKEV---------
>MvKinesin7-IId
```

```
----------KIQVAVRIRPLSSREIS--------------------------------
QRETSAWDCVDSRTIVQRPAPTPPTPERS------PHHHSQQSKSYTFDHVFGPES------------
---------------ATEQVYEEGAKDIALSALCGVN---------ATIFAYGQ-------------
--------TSSGKTFTMRG-----------------VTQNTIRDIFNYIEK-----HS----------
-------------------------------------------------ERNFVIKISAIEIYN-----
-EVVKDLLNTDTN-------------------------------------------------------
-------------------------------------IGPLRLMDD-----------------
PEKGTIVERLV----EDIVRDKNHFQQILSVIEAQRQVGETSLNETSSRS-HQIIRLTIESFPR-
VADG-----------------GLVKSLVANLNLVD----------------------------
LAGSER-ASQTHSEG---TRLKEG-------------CHINRSLLT------LTNIIRKLSGPS----
----REKAAHLPYRDSKLTRILQ-----NSLGG-NARTAIICTMSPAQTHMEQSRNTLFFATQAKEV--
-------
>MvKinesin7-IIe
----------KILVTVRVRPLSSREIA--------------------------------
QRDPSAWDCIDFKSVVFKV----SHPDRS------PQTKS-----YKFDRVFGPDS------------
---------------VTNAVYEEGAKDVALSALKGLN---------ATIFAYGQ-------------
--------TSSGKTFTMRG-----------------IVENAIRDIYSFIEQ-----QR----------
-------------------------------------------------DRNFILKVSALEIYN-----
-EIVKDLLVADG---------------------------------------------------------
-------------------------------------GPLRLLDD-----------------
PEKGTVVEKLT----EDTVKDIDHLKRILSNVEAHRQVGETMLNEASSRS-
HQIIRLTVENFPQKLTEEP----------------AVVNSLVATLNFVD------------------
----------LAGSER-ASQTLAEG---NRLKEG-------------SHINRSLLT------
LTTIIRKLSSQV--------KGKSVHLPYRDSKLTRILQ-----NSLGG-
NARTAIICTMSPAYSHVEQSRNTLFFATQAKEV---------
>MvKinesin7-III
----------KISVSVRVKPLDKAEV----------------------------------
AKGIPWKIHNNAIALCPASG-----------------SQLPNHVFVFDNVFSMES-------------
--------------TTEEIYNTTTKKIVSSAMDGFN---------GTVFAYGQ---------------
-------TSSGKTFTMRGY-----------DKDPGIIPLAIYDIFRNLEK-----AD-----------
-------------------------------------------------DREYLLHMSYMEIYN------
EDINDLLAPEQ----------------------------------------------------------
-------------------------------------TKLQVHES-----------------
LERGVFVSGLR----EEIVTSPEQALQLMEFGESRRHFGETNMNLHSSRS-
HTIFRLVIESRDKNQDDSID---------------LSCDSVRVSVLNLVD------------------
----------LAGSER-VAKTGAEG---TRLKEG-------------THINKSLMV------
LGTVINKLSEGI--------QGQGGHIPYRDSKLTRILQ-----PALGG-
NARTAVICTITPAVIHVDESKGTLQFASRAMRV---------
>MvKinesin7-IV
----------KINVAVRVRPAKNNDS----------------------------------
DNVNCWKVVENTVSLCSPYG-----------------TPVSGHTFAFDYVFGQDA-------------
--------------KTSSIYELHTKGVIESALQGFN---------GTVFAYGQ---------------
-------TSSGKTFTMRGS-----------KDDLGLIALSVHEVFKHIEQ-----IP-----------
-------------------------------------------------DREFLIRVSYMEIYN------
EEINDLLAPEN----------------------------------------------------------
-------------------------------------RKLQVHEN-----------------
LEKGIFVAGLR----EEIVSGPEHVFELLEYGEAQRHVGQTNMNLYSSRS-HTIFRMVIESKDKDAH--
--DE-----------G--VCPDAVRVSVLNLVD----------------------------LAGSER-
AAKTGAGG---VRLKEG-------------SHINKSLMT------LGNVIKKLSDGV--------
ARQGGHIPYRDSKLTRILQ-----PALGG-NAKTAIICTIAPEEVHIDETRGTLQFASRAKRV------
---
>MvKinesin8-I
----------TLQVAVRCRPLTPAER-SR------------------------------
YREVIRIENDKVVVVSDSDGS-----------------RKQEKRYTFDYALGPE-------------
---------------YKNQDIYNRVVAPMIQGVCQGLN---------ATVFAYGA-------------
--------TGSGKTYTMAG------RPD------DPGLMVLSLQEIFRCVSK-EEKDH-----------
-------------------------------------------------KYEVTCSYLEVYN-----
-EVIYDLLDKSSG---------------------------------------------------------
-------------------------------------HLELRED-----------------
PEQGIVVAGLR----RVQVNSTAKILELLHVGNSRRKTEKTDANAISSRS-HAVLEIMVKKFYRNHHG-
-------------------SQVLQGKMALVD----------------------------LAGSER-
ASETNNVG---RKLRDG-------------ANINKSLLA------LANCINALGKQQKK--------
GLAYVPYRNSKLTRILK-----DGLSG-NSRTCMIATVSFADHQYHHTMNTLKYANRAKEI--------
-
```

```
>MvKinesin8-IIb
----------RIRVYVRIRPISKKEKEVG------------------------------
ARCCVKAANGKEIYLTEMALE-SDYLR----------LKRLKGRYFIFDGAFPEN-------------
--------------TNQEEVYRTSTEQLVEAVLEGKN---------SSVFCYGA-------------
--------TGAGKTFTMLG------TMK------DPGVMVLALKDLFAKLKQ-RSSEG-----------
-------------------------------------------------EHSVRLSYLEVYN-----
-ETVRDLLSPGR-------------------------------------------------------
-----------------------------------ALVLRED-----------------
LKQGIVAAGLT----QYKACSADEVMALLQQGNQNRTTEPTRLNETSSRS-HAILQIFVEYSARVGVS-
-------------------LVSRTGKLSLID----------------------------LAGSER-
AIATDQRT---LRSLEG--------------ANINRSLLA------LSSCISALVE------------
GKKHIPFRNSKLTQLLK-----DSLGG-SCQTAMIANISPSNMSFGETQNTLYWADRAKEI--------
-
>MvKinesin8-IIa
----------RIMVYVRMRPLSKAEKESG------------------------------
ARSCVRVVNKKDVYLTEFASE-TDYLR----------LKRLKGRHFVFDAAFNDS-------------
--------------SNQQEVYNTSAAELVEGVLQGRN---------GSVFCYGA-------------
--------TGAGKTHTMLG------TTQ------NPGVMVLALKDLFNKLRQ-RCREG-----------
-------------------------------------------------DYVVRLSYLEVYN-----
-ESVRDLLSPGR-------------------------------------------------------
-----------------------------------PLVLRED-----------------
SKQGIIAAGLT----QYTAYSADEVMSLLHQGNQNRTTEPTRVNETSSRS-HAILQVVVEYKVRNDSH-
-------------------YVCRTGKLSLID----------------------------LAGSER-
ALATDQRT---IRSLEG--------------ANINRSLLA------LSSCINALVE------------
GKRHIPFRNSKLTQLLK-----DSLGG-ACQTSMIANISPSTLSFGETQNTLHWADRAKQI--------
-
>MvKinesin9B
-----------IEIYLRMR-PIT-EAPATYEIDEDE------------------------
GRVQFHMPRQVAAGMANHQKE--------------------RYDFFFTHIFDTTAM-------------
---------------QDTIFERVARKVVLGSLDGFN---------GTIFAYGQ---------------
-------TGSGKTYTITGGAERYVDRG---------IIPRTISLIYSELGKRSDY--------------
-------------------------------------------------SYTIHFSYLEIYN------
ETGIDLLNPDHETTP----------------------------------------------------
-----------------------------------LEELPKVSLLEDD-----------------
ENVIHMRNLS----SHLATNEEEALNLLFVGDTNRMISSTPMNMASSRS-HCIFTAYIEARK-------
---------------AGEDIVRKSKLHLVD----------------------------
LAGSERVSKTG--VDGQ--ILKEA--------------KYINLSLHF------
LEQVIVALQEKSQGKA-------RHHIPYRNSMMTSVLR-----DALGG-
NCQTVMLATASIAQDQLEETISTCRFAQRVA-----------
>MvKinesin9A
----------RIASHLRLK-PSAR-PSPALHVDAPN------------------------
SSLRVDLKHS-AGGPPRSHGD--------------------QIVFHLDSVIQSTS-------------
---------------QESTFNRCALKIVEDVLCGYN---------GTVLAYGQ---------------
-------SGSGKSYTMSGDPKNSMHKG---------IVPRAIQRIFAEKAARPES--------------
-------------------------------------------------EMAIHVSYLEIYN------
EVIYDLLSDG----------------------------------------------------------
-----------------------------------SQASRVVTLLEEN-----------------
SLLEMKGLS----QFHCSTEEEALKYFLRGERHRTIAPNMHHKMSNRS-HCMFTIYVERHT--------
-------------KVRDYPDRTAAKLNLVD----------------------------
LAGCERLKNAN--SVKQ--WEKEI--------------SNINKSLTF------LEQAVADLR-RGQG--
---------HVSFRQSRLTMLLK-----DALGG-NSRTVLIVCALQEHDSLDETISALRFAQRVK-----
-------
>MvKinesinOrphI
----------------RMRPFFEKEKVCPYRQVLCLLGNR--------------
ILIKQHPSTLKTKRTCMSKKKFDELELE---------------VDYVQDDSYVQVKKEDKKEESQR---
------------------------HLFSTIGVQAVDRLLNGQS---------VTILSYGQ---------
--------------AGTGKTYTIYG--DG--------TESGRGLIPRITCDFLHKIQAK------GRD-
-------------------------------------------------------
DECVVEVSFLEIYQ------
EKLRDLLAVNRARETPFELLMRRSPDLSTEYPEVSSSSDQDSDSDSDSGPSLGFRDIPDKVHVHRYQQG
TKSSIRHP--------------------------VRPTSNYLKLREH--------------
PVKGTYVEGLI----WKKVCTWSDMDRLLKQGAARRSTVATDSN-
RQSSRSHTLFTMKVTWLSTPRHGGR---------------------TVSYLNLVDLAG----------
-------------------NEKL---QNVS---IEKQKDM-------------KYINKSL------
```

```
SQLNSVILNLS--RS-----------TFVPYRNSALTWLLK-----ESLQGDN-
ITFLVANVSPAEKDFNETLATLNYAFKAKRI---------
>MvKinesinOrphII
----------PVEVISRIREHPDGPDK-PSVLQVLQN--------------------------------
------GHTVRVR---------------TDQGYRDFGLDAVSLASNEDLQ-----------------
----------SFYKKYVESRIETVKLGGR---------CTIMMYGP---------------------
TGAGKSHTMFG-----------SAKEPGIAYRALQNILNVGNANLENAGDESG--------------
----------------------------------------NKIKVTVKVLEIYN------
EDIFDLLATTSS-----------------------------------------------------
GPGNWLK-------------------------------------SRVRL--------------
EVMGKKAKNAI----SITGTDPDKISKEIAKIEKKRIIKSTACNERSSRS-
HCLVMVDVPSLGGRLVLVD-----------------------------------------------
----------MAGSENIEQAGVT----LDLKMQT-------------GKINQGN------
IALKRVVEAIAN-----------GDSYIPFRDSKLTMLLQ-----
DSFEDDEAKILMILCASPDPKDIHKTIGTLEYGSKAKCIVR-------
>HsKinesin1-KIF5B
--------ECNIKVMCRFRPLNESEVN--------------------------------
RGDKYIAKFQGEDTVVIA------------------SKP-----YAFDRVFQSST------------
---------------SQEQVYNDCAKKIVKDVLEGYN---------GTIFAYGQ-------------
--------TSSGKTHTMEG---KLHD------PEGMGIIPRIVQDIFNYIY-S----MDE---------
-------------------------------------------------NLEFHIKVSYFEIYL-----
-DKIRDLLDV--S-----------------------------------------------------
----------------------------------------KTNLSVHED-----------------
KNRVPYVKGCT----ERFVCSPDEVMDTIDEGKSNRHVAVTNMNEHSSRS-HSIF--LINVKQ-----
E--------------NTQT--EQKLSGKLYLVD---------------------------LAGSEK-
VSKTGAEG---AVLDEA-------------KNINKSLSA------LGNVISALAEG------------
STYVPYRDSKMTRILQ-----DSLGG-NCRTTIVICCSPSSYNESETKSTLLFGQRAKTI---------
>HsKinesin2-KIF3A
---------DNVKVVVRCRPLNEREKSMC-------------------------------
YKQAVSVDEMRGTITVHKTD-----------------SSNEPPKTFTFDTVFGPES------------
---------------KQLDVYNLTARPIIDSVLEGYN---------GTIFAYGQ--------------
--------TGTGKTFTMEG---VRAI------PELRGIIPNSFAHIFGHIA-K----AEG---------
-------------------------------------------------DTRFLVRVSYLEIYN-----
-EEVRDLLGK--DQ----------------------------------------------------
----------------------------------------TQRLEVKER-----------------
PDVGVYIKDLS----AYVVNNADDMDRIMTLGHKNRSVGATNMNEHSSRS-HAIF--TITIEC----
SE--------------KGIDGNMHVRMGKLHLVD----------------------------
LAGSER-QAKTGATG---QRLKEA-------------TKINLSLST------LGNVISALVDG-----
------KSTHVPYRNSKLTRLLQ-----DSLGG-NSKTMMCANIGPADYNYDETISTLRYANRAKNI--
-------
>HsKinesin2-KIF17
---------EAVKVVVRCRPMNQRERELR-------------------------------
CQPVVTVDCARAQCCIQNPG-----------------AADEPPKQFTFDGAYHVDH------------
---------------VTEQIYNEIAYPLVEGVTEGYN---------GTIFAYGQ--------------
--------TGSGKSFTMQG---LPDP------PSQRGIIPRAFEHVFESVQ-C----AEN---------
-------------------------------------------------TKFLVRASYLEIYN-----
-EDVRDLLGA--DT----------------------------------------------------
----------------------------------------KQKLELKEH-----------------
PEKGVYVKGLS----MHTVHSVAQCEHIMETGWKNRSVGYTLMNKDSSRS-HSIF--TISIEM----
SA--------------VDERGKDHLRAGKLNLVD----------------------------
LAGSER-QSKTGATG---ERLKEA-------------TKINLSLSA------LGNVISALVDG-----
------RCKHVPYRDSKLTRLLQ-----DSLGG-NTKTLMVACLSPADNNYDETLSTLRYANRAKNI--
-------
>HsKinesin3-KIF1A
---------ASVKVAVRVRPFNSREMS-------------------------------
RDSKCIIQMSGSTTTIVNPK-----------------QPKETPKSFSFDYSYWSHTSPEDINY------
---------------ASQKQVYRDIGEEMLQHAFEGYN---------VCIFAYGQ--------------
--------TGAGKSYTMMG----KQE------KDQQGIIPQLCEDLFSRINDT----TND---------
-------------------------------------------------NMSYSVEVSYMEIYC-----
-ERVRDLLNP--KN----------------------------------------------------
----------------------------------------KGNLRVREH-----------------
PLLGPYVEDLS----KLAVTSYNDIQDLMDSGNKARTVAATNMNETSSRS-HAVFNIIFTQKR-----
H--------------DAETNITTEKVSKISLVD---------------------------LAGSER-
ADSTGAKG---TRLKEG-------------ANINKSLTT------
```

```
LGKVISALAEMDSGPNKNKKKKKTDFIPYRDSVLTWLLR-----ENLGG-
NSRTAMVAALSPADINYDETLSTLRYADRAKQIRCNAVIN--
>HsKinesin4-KIF4A
---------IPVRVALRCRPLVPKEISEG------------------------------
CQMCLSFVPGEPQVVVGT-----------------------DKSFTYDFVFDPS-------------
---------------TEQEEVFNTAVAPLIKGVFKGYN---------ATVLAYGQ-------------
--------TGSGKTYSMGGAYTAEQENEP-----TVGVIPRVIQLLFKEIDK-KSD-------------
-------------------------------------------------FEFTLKVSYLEIYN-----
-EEILDLLCP----SRE-------------------------------------------------------
---------------------------------------KAQINIRED-----------------
PKEGIKIVGLT----EKTVLVALDTVSCLEQGNNSRTVASTAMNSQSSRS-HAIFTISLEQRKK-----
------SDKN--SS---------FRSKLHLVD-----------------------------LAGSER-
QKKTKAEG---DRLKEG--------------ININRGLLC------LGNVISALGDDKK----------
GGFVPYRDSKLTRLLQ-----DSLGG-NSHTLMIACVSPADSNLEETLNTLRYADRARKIK--------
>HsKinesin4-KIF21A
---------SSVRVAVRIRPQLAKEKIEG------------------------------
CHICTSVTPGEPQVFLGK-----------------------DKAFTFDYVFDIDS-------------
---------------QQEQ-IYIQCIEKLIEGCFEGYN---------ATVFAYGQ-------------
--------TGAGKTYTMGTGFDVNIVEE------ELGIISRAVKHLFKSIEEKKHIAIKNGLP------
-------------------------------------------------APDFKVNAQFLELYN-----
-EEVLDLFDTTRDIDAKSKK-----------------------------------------------------
---------------------------------------SNIRIHED-----------------
STGGIYTVGVT----TRTVNTESEMMQCLKLGALSRTTASTQMNVQSSRS-
HAIFTIHVCQTRVCPQIDADNATDNKII--SESAQMNEFETLTAKFHFVD------------------
----------LAGSER-LKRTGATG---ERAKEG-------------ISINCGLLA------
LGNVISALGDKSKR---------ATHVPYRDSKLTRLLQ-----DSLGG-
NSQTIMIACVSPSDRDFMETLNTLKYANRARNIK--------
>HsKinesin4-KIF27
---------IPVKVAVRIRPLLCKEALHN------------------------------
HQVCVRVIPNSQQVIIGR-----------------------DRVFTFDFVFGKN-------------
---------------STQDEVYNTCIKPLVLSLIEGYN---------ATVFAYGQ-------------
--------TGSGKTYTIGGGHIASVVEG------QKGIIPRAIQEIFQSISEHPS-------------
-------------------------------------------------IDFNVKVSYIEVYK-----
-EDLRDLLEL----ETSMK-----------------------------------------------------
---------------------------------------DLHIRED-----------------
EKGNTVIVGAK----ECHVESAGEVMSLLEMGNAARHTGTTQMNEHSSRS-HAIFTISICQVHKN----
-------MEA--AEDGSWYSPRHIVSKFHFVD-----------------------------LAGSER-
VTKTGNTG---ERFKES--------------IQINSGLLA------LGNVISALGDPRRK---------
SSHIPYRDAKITRLLK-----DSLGG-SAKTVMITCVSPSSSNFDESLNSLKYANRARNIR--------
>HsKinesin5-KIF11
--------GKNIQVVVRCRPFNLAERKAS------------------------------
AHSIVECDPVRKEVSVRTGG----------------LADKSSRKTYTFDMVFGAST-------------
----------------KQIDVYRSVVCPILDEVIMGYN---------CTIFAYGQ-------------
--------TGTGKTFTMEGERSPNEE-YTWEEDPLAGIIPRTLHQIFEKLT------DNG---------
-------------------------------------------------TEFSVKVSLLEIYN-----
-EELFDLLNPSSDVS-----------------------------------------------------
---------------------------------------ERLQMFDDPR-----------------
NKRGVIIKGLE----EITVHNKDEVYQILEKGAAKRTTAATLMNAYSSRS-HSVFSVTIHMKE------
--------------TTIDGEELVKIGKLNLVD-----------------------------LAGSEN-
IGRSGAVD---KRAREA--------------GNINQSLLT------LGRVITALVER------------
TPHVPYRESKLTRILQ-----DSLGG-RTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQK
>HsKinesin6-KIF23
---------DPVGVYCRVRPLGFPDQECC---------------------------------
IEVINNTTVQLHTPEGYRLNR-------------NGDYKETQYSFKQVFGTHT----------------
-------------TQKELFDVVANPLVNDLIHGKN---------GLLFTYGV-----------------
-----TGSGKTHTMTGS------------
PGEGGLLPRCLDMIFNSIGSFQAKRYVFKSNDRNSMDIQCEVDALLERQKREAMPPKTSSSKRQVDPEF
ADMITVQEFCKAEEVDEDSVYGVFVSYIEIYN------NYIYDLLEEVPFDPIKPK-------------
-----------------------------------------------------------------------
------PPQSKLLRED----------------KNHNMYVAGCT----
EVEVKSTEEAFEVFWRGQKKRRIANTHLNRESSRS-HSVFNIKLVQAPLDADGDN-------------
VLQEKEQITISQLSLVD----------------------------LAGSERTNRTRAEGN----
RLREA--------------GNINQSLMT------LRTCMDVLRENQMYG-------
```

```
TNKMVPYRDSKLTHLFK-----NYFDG-EGKVRMIVCVNPKAEDYEENLQVMRFAEVTQ----------
-
>HsKinesin7-KIF10
----------AVAVCVRVRPLNSREESLG-----------------------------
ETAQVYWKTDNNVIYQVDGS----------------------KSFNFDRVFHGNE------------
---------------TTKNVYEEIAAPIIDSAIQGYN---------GTIFAYGQ-------------
--------TASGKTYTMMGS-----------EDHLGVIPRAIHDIFQKIKK-----FP----------
-----------------------------------------------DREFLLRVSYMEIYN-----
-ETITDLLCGTQK-------------------------------------------------------
-------------------------------------MKPLIIRED-----------------
VNRNVYVADLT----EEVVYTSEMALKITK-GEKSRHYGETKMNQRSSRS-HTIFRMILESREKGEPS-
-----------------NCEGSVKVSHLNLVD----------------------------LAGSER-
AAQTGAAG---VRLKEG-------------CNINRSLFI------LGQVIKKLSDG----------
QVGGFINYRDSKLTRILQ-----NSLGG-NAKTRIICTITP--VSFDETLTALQFASTAKYM-------
--
>HsKinesin8-KIF19
----------QLMVALRVRPISVAELEEG-----------------------------
ATLIAHKVDEQMVVLMDPMEDPDDILR----------AHRSREKSYLFDVAFDFT-------------
--------------ATQEMVYQATTKSLIEGVISGYN---------ATVFAYGP-------------
--------TGCGKTYTMLG------TDQ------EPGIYVQTLNDLFRAIEE-TSNDM-----------
-------------------------------------------------EYEVSMSYLEIYN-----
-EMIRDLLNPSLG-------------------------------------------------------
-----------------------------------------YLELRED-----------------
SKGVIQVAGIT----EVSTINAKEIMQLLMKGNRQRTQEPTAANQTSSRS-
HAVLQVTVRQRSRVKNIL-------------------QEVRQGRLFMID------------------
----------LAGSER-ASQTQNRG---QRMKEG-------------AHINRSLLA------
LGNCINALSDK--G--------SNKYINYRDSKLTRLLK-----DSLGG-
NSRTVMIAHISPASSAFEESRNTLTYAGRAKNI---------
>HsKinesin9-KIF9
----------KVHAFVRVK-PTDDFAHEMIRYGDDK------------------------
RSIDIHLKKDIRRGVVNNQQT-------------------DWSFKLDGVLHDAS-------------
---------------QDLVYETVAKDVVSQALDGYN---------GTIMCYGQ--------------
-------TGAGKTYTMMGATENYKHRG---------ILPRALQQVFRMIEERPTH-------------
-------------------------------------------------AITVRVSYLEIYN------
ESLFDLLSTLPYV-------------------------------------------------------
-----------------------------------GPSVTPMTIVENP-----------------
QGVFIKGLS----VHLTSQEEDAFSLLFEGETNRIIASHTMNKNSSRS-HCIFTIYLEAHS--------
-------------RTLSEEKYITSKINLVD----------------------------
LAGSERLGKSG--SEGQ--VLKEA-------------TYINKSLSF------LEQAIIALGDQKRD--
---------HIPFRQCKLTHALK-----DSLGG-NCNMVLVTNIYGEAAQLEETLSSLRFASRMK----
-------
>HsKinesin9-KIF6
----------TIQIFARVKPPVRKHQQGIYSIDEDEKLI----------------------
PSLEIILPRDLADGFVNNKRE-------------------SYKFKFQRIFDQDAN-------------
---------------QETVFENIAKPVAGSVLAGYN---------GTIFAYGQ--------------
------TGSGKTFTITGGAERYSDRG---------IIPRTLSYIFEQLQKDSSK-------------
-------------------------------------------------IYTTHISYLEIYN------
ECGYDLLDPRHEASS-----------------------------------------------------
-----------------------------------LEDLPKVTILEDP----------------
DQNIHLKNLT----LHQATTEEEALNLLFLGDTNRMIAETPMNQASTRS-HCIFTIHLSSKE-------
---------------PGSATVRHAKLHLVD----------------------------
LAGSERVAKTG--VGGH--LLTEA-------------KYINLSLHY------LEQVIIALSEKH----
-------RSHIPYRNSMMTSVLR-----DSLGG-NCMTTMIATLSLEKRNLDESISTCRFAQRVA----
-------
>HsKinesin10-KIF22
----------RVRVAVRLRPFV------DGTAG--------------------------
ASDPPCVRG-MDSCSLEIANWRN---------------HQETLKYQFDAFYGE-RSTQQ---------
------------------DIYAGSVQPILRHLLEGQN---------ASVLAYGP-------------
--------TGAGKTHTMLGS-----------PEQPGVIPRALMDLLQLTREEGAEGRPWA--------
-------------------------------------------------LSVTMSYLEIYQ-----
-EKVLDLLDPASG--------------------------------------------------------
-----------------------------------DLVIRED----------------
CRGNILIPGLS----QKPISSFADFERHFLPASRNRTVGATRLNQRSSRS-
HAVLLVKVDQRERLAPFR---------------------QREGKLYLID------------------
```

```
----------LAGSEDNRRTGNKGL----RLKES-------------GAINTSLFV------
LGKVVDALNQ-----------GLPRVPYRDSKLTRLLQ-----DSLGG-
SAHSILIANIAPERRFYLDTVSALNFAARSK-----------
>HsKinesin11-KIF26A
----------KVKVMLRIWPAQGAQRSAEAMSFLKVDP--------------------
RKKQVILYDPAAGPPGSAGPRRA--------------ATAAVPKMFAFDAVFPQDSEQAE--------
-------------------VCSG-TVADVLQSVVSGAD---------GCIFSFGH-------------
---------MSLGKSYTMIGK-DS--------SPQSLGIVPCAISWLFRLIEER----RERTG------
-------------------------------------------------TRFSVRVSAVEVCGRD--
--QSLRDLLAEVAP-------------------------------------------------------
---GSLQDTQS--------------------------------PGVYLRED---------------
PVCGAQLQNQS----
ELRAPTAEKAAFYLDAALAARSTSRAGCGEDARRSSHMLFTLHVYQYRMEKCGRG--------------
--------GM-----------------------------------SGGRSRLHLIDLGS---
CEAAAGR--------------AGEAAGGPLCLSLSALGSVILALVN------------
GAKHVPYRDHRLTMLLR-----ESLATAGCRTTMIAHVSDAPAQHAETLSTVQLAARIH----------
-
>HsKinesin12-KIF15
---------DAIKVFVRIRPPAERSGSAD-----------------------------
GEQNLCLSVLSSTSLRLHSNP--------------------EPKTFTFDHVADVDT-------------
----------------TQESVFATVAKSIVESCMSGYN---------GTIFAYGQ--------------
--------TGSGKTFTMMGPSES-----DNFSHNLRGVIPRSFEYLFSLIDREKE-KAGA---------
-------------------------------------------------GKSFLCKCSFIEIYN-----
-EQIYDLLDSAS-------------------------------------------------------
-----------------------------------------AGLYLREH-----------------
IKKGVFVVGAV----EQVVTSAAEAYQVLSGGWRNRRVASTSMNRESSRS-HAVFTITIESMEK--SN-
------------------EIVNIRTSLLNLVD-----------------------------LAGSER-
QKDTHAEG---MRLKEA--------------GNINRSLSC------LGQVITALVDVGN--------
GKQRHVCYRDSKLTFLLR-----DSLGG-NAKTAIIANVHPGSRCFGETLSTLNFAQRAKLI-------
--
>HsKinesin13-KIF2C
---------HRICVCVRKRPLNKQELAKK-----------------------------
EIDVISIPSK-CLLLVHEPKLKVDLTK------------YLENQAFCFDFAFDET--------------
---------------ASNEVVYRFTARPLVQTIFEGGK---------ATCFAYGQ--------------
--------TGSGKTHTMGGDLSGKAQNA------SKGIYAMASRDVFLLKNQPCYRKL------------
-------------------------------------------------GLEVYVTFFEIYN-----
-GKLFDLLNKKA-------------------------------------------------------
-----------------------------------------KLRVLED-----------------
GKQQVQVVGLQ----EHLVNSADDVIKMIDMGSACRTSGQTFANSNSSRS-HACFQIILRAK-------
--------------------GRMHGKFSLVD-----------------------------
LAGNERGADTSSADR---QTRMEG--------------AEINKSLLA------LKECIRALGQ------
------NKAHTPFRESKLTQVLR-----DSFIGENSRTCMIATISPGISSCEYTLNTLRYADRVK----
-------
>HsKinesin13-KIF24
---------EKIRVCVRKRPLGMREVRRG-----------------------------
EINIITVEDK-ETLLVHEKKEAVDLTQ------------YILQHVFYFDEVFGEA--------------
---------------CTNQDVYMKTTHPLIQHIFNGGN---------ATCFAYGQ--------------
--------TGAGKTYTMIG------THE------NPGLYALAAKDIFRQLEVSQPRK------------
-------------------------------------------------HLFVWISFYEIYC-----
-GQLYDLLNRRK-------------------------------------------------------
-----------------------------------------RLFARED-----------------
SKHMVQIVGLQ----ELQVDSVELLLEVILKGSKERSTGATGVNADSSRS-HAVIQIQIKDS-------
--------------------AKRTFGRISFID-----------------------------
LAGSERAADARDSDR---QTKMEG--------------AEINQSLLA------LKECIRALDQ------
------EHTHTPFRQSKLTQVLK-----DSFIG-NAKTCMIANISPSHVATEHTLNTLRYADRVK----
-------
>HsKinesin14A-KIFC1
--------KGNIRVFCRVRPVLPGEPTPPPGLLLFPSGP-----------------
GGPSDPPTRLSLSRSDERRGTLSGA--------------PAPPTRHDFSFDRVFPPGSGQ--------
---------------------DEVFEEIAMLVQSALDGYP---------VCIFAYGQ-----------
-----------TGSGKTFTMEGGPGG--------DPQLEGLIPRALRHLFSVAQELS-----GQG----
-------------------------------------------------WTYSFVASYVEIYN--
----ETVRDLLATG-----------------------------------------------------
TRKGQGGECEI-----------------------------------RRAGP---------------
```

```
GSEELTVTNAR----YVPVSCEKEVDALLHLARQNRAVARTAQNERSSRS-
HSVFQLQISGEHSSRGLQ----------------------CGAPLSLVD------------------
----------LAGSERLDPGLALGPGERERLRET-------------QAINSSLS------
TLGLVIMALSN-----------KESHVPYRNSKLTYLLQ-----NSLGGSA-
KMLMFVNISPLEENVSESLNSLRFASKVNQCVIG------
>HsKinesin14B-KIFC3
--------KGNIRVIARVRPVTKEDGEG------------------------------
PEATNAVTFDADDDSIIHLLH-----------------KGKPVSFELDKVFSPQASQ----------
------------------QDVFQEVQALVTSCIDGFN---------VCIFAYGQ--------------
--------TGAGKTYTMEG-----------TAENPGINQRALQLLFSEVQEKA-----SD--------
-------------------------------------------------WEYTITVSAAEIYN-----
-EVLRDLLGKEP-----------------------------------------------------------
--QEKLE-------------------------------------IRLCPD--------------
GSGQLYVPGLT----EFQVQSVDDINKVFEFGHTNRTTEFTNLNEHSSRS-
HALLIVTVRGVDCSTGLR----------------------TTGKLNLVD------------------
----------LAGSERVGKSGAEG----SRLREA-------------QHINKSLS------
ALGDVIAALRS-----------RQGHVPFRNSKLTYLLQ-----DSLSGDS-
KTLMVVQVSPVEKNTSETLYSLKFAERVRSVELG------
```

Appendix I-2

MSA of kinesin-1, ARK-LIKE, 'orphan'-I, and 'orphan'-IV sequences in *Arabidopsis*, *Marsilea*, *Selaginella*, *Physcomitrella*, and *Chlamydomonas* used to determine if kinesin-1 architectures exist in plants.

```
>SELMODRAFT_92509
------------------------------------------------------------------
----------------------------FKFDAVLPP-SA----------
TQADVYNVSAQAVIQDVLDGYNGTIMAYGQTGAGKTYTLSDMVFDDVGSFHSTGIIPRSAADIYIRAER
DKD--HEYR-ISMSYIQIYME------MIQDLL-----------------------------------
----------RP--------EN-----------------------------------------
SNLSIRETEAGG-IFVAGIEEVQVKSIEDVMKLLMIGDRNRRFAFTRLNAH-SSRSHTIAMLTVEK---
KAP--------------------GISEKVL----VGKLFLVDLAGSERLKKSGSE-GLRA----
SEAMSV-----NMSLTALGKCISARADPSVL------------
HVPFRDSKLTRLLQESLGGNAKTSLIVNIAPCSEYLQETLSSLQFGARVSX--
>Phypa_455498-PpKinesinARK-a
-RVRVTVRLRP-----------------------------------
RNAEELEADLDFADCVELQPEL----------KRLKLRKN--NWESETYQFDEILTE-TA---------
-SQKRVYEVVAKPVVESVLEGYNGTVMAYGQTGTGKTFTLGKL--GDEDTAD-
RGIMVRALEDILSNINH-----ADDT-VTVSYLQLYME------SVQDLL------------------
--------------------------AP--------ER-----------------------------
------------DNCHIQEDPKTGDVSVPGATQIQLTDHQSFVNLLDVGESNRVAANTKLNTE-
SSRSHALLLVQVKK---AVR------------TKEP-AENGKMRAPTIR---
RSKLLIVDLAGSERVDKSGSE-GHTL----EEAKSI-----NLSLTALGKCINALAENSP---------
----HVPIRDSKLTRLLRDSFGGTARTSLIVTIGPSPRHRGETTSTIMFGQRAM---
>Phypa_453488-PpKinesinARK-b
-RVRVTVRLRP-----------------------------------
RNAEELEADLDFADCVELQPEL----------KRLKLRKN--NWESETYQFDEILTE-TA---------
-SQKRVYEVVAKPVVESVLEGYNGTVMAYGQTGTGKTFTLGKL--GDEDTAD-
RGIMVRALEDILSSINH-----VDDT-VTVSYLQLYME------SVQDLL------------------
--------------------------AP--------ER-----------------------------
------------DNCHIQEDPKTGDVSVPGATQIQLTDHQSFVNLLDVGESNRVAANTKLNTE-
SSRSHALLLVQVKK---AVR----------SKEPTE-NGNGKMRATTIR---
RSKLLIVDLAGSERVDKSGSE-GHTL----EEAKSI-----NLSLTALGKCINALAENSP---------
----HVPIRDSKLTRLLRDSFGG---------------------------------
>Phypa_425827-PpKinesinARK-c
-RVRVTVRLRP-----------------------------------
RNAEELESDRDFADCVELQPEL----------KRLKLRKN--NWDCETYQFDEILTD-TA---------
-SQKRVYEVVAKPVVESVLEGYNGTVMAYGQTGTGKTFTLGKL--GDEDTAD-
RGIMVRALEDILAVINP-----VHDT-VTVSYLQLYME------SVQDLL------------------
--------------------------SP--------EK-----------------------------
------------DNIAIQEDPKTGDVSVPGATQIQVTDHQSFCNLLDVGEANRFAANTKLNTE-
SSRSHAILLVQVKK---AVR-NKEVVVPPENGNGGS-HSVKGMRAPTIR---
KSKLLIVDLAGSERVDKSGSE-GHTL----EEAKSI-----NLSLTALGKCINALAENSP---------
----HVPIRDSKLTRLLRDSFGGTARTSLIVTIGPSPRHRGETTSTIMFGQRAM---
>Phypa_427907-PpKinesinARK-d
-RVRVAVRLRP-----------------------------------
RNAEELEADADFADCVELQPEF----------KRLKLRKN--NWDCETYQFDEVLTE-TA---------
-SQKRVYEVVAKPVVEGVLEGYNGTVMAYGQTGTGKTFTLGRL--GEEDCAD-
RGIMVRAMEDILANITP-----GEDT-VTVSYLQLYME------TVQDLL------------------
--------------------------AP--------ER-----------------------------
------------DNIAIQEDPKTGDVSVPGATQVLLQDQTSFVRLLDVGEANRFAANTKLNTE-
SSRSHALLVVQVKK---GYKGRSGDTATNENDNGSP-QTGTGLRAPIIR---
RSKLLIVDLAGSERVDKSGSE-GHTL----EEAKSI-----NLSLTALGKCINALAENSP---------
----HVPIRDSKLTRLLRDSFGGTARTSLIVTIGPSPRHRGETTSTILFGQRAM---
```

```
>Phypa_446331-PpKinesinARK-LIKE
------------------------------------------------------------------------
-------------------------TEFQFDAVLPP-TA----------
LQVDVYNMAARAVVLDVLNGYNGTIMAYGQTGAGKTYTLSDNLTNGGGVTSVGGIIPRSAAEIFDRAGL
DQD--YEFH-VSMSYIQIYME------QIQDLL-----------------------------------
----------RP--------ES----------------------------------------
CNMQIRE-GMNG-VYVSGVEEVQVKSVEDTMKLLMLGDRHRCLSFTKLNAH-SSRSHTIVILTVEK---
KAK-YKTSEQKAELADRRR-VSSCFVESERVL---VGKLFLVDLAGSERLKKSGSE-GIRA----
SEAMSV-----NLSLTCLGKCISARADPAIT------------
HVPFRDSKLTRLLQESLGGNAKTSLVINIAPCSEYLQESMSSLHFGSRAM---
>Phypa_457477-PpKinesinOrph-Ia
------------------------------------------------------------------------
-------------------------------------EE----------
SQRCMFEILGRPCVENSLDGFNTTIMTYGQMGSGKTYTIV----GDGTTCG-
RGLVPRIVNELMRRVQDDQSSGVHLT-IQVSYLEIYQE------KVRDLL------------------
VDKKAEHTAEESGVNCSLRCTELPLIDGGGSSFFLHGQVK----------------------------
------------DQLRVREHPETG-
TYVESPRWKVVTAHEEMDKLLKLGAANRMLGSTISHARLSSRGHTLFTIKITKTNEKSQHTSVSHINMV
DLAGENSYHISFLSTVAVICTKTSQIEFCIVSCSEKMALGQKPSAERT----YESKYI-----
NRSLAQLNDMFTNLPNGRSNGK----------
FVSYRSSALTMLLRESLSENSKTYLVANISPAEQDFQESIHTLRCAAKAKRI-
>Phypa_453299-PpKinesinOrph-Ib
-KVKVVVRIRP-----------------------------------
FTADEERPLPPVIETVDDRTVIVRDNEHGQRQESLKFTAH--YVQDDSLAIEQTNSDKND---------
-SQNAMFEVLGVPCIEHALDGLNTTILAYGHTGSGKTYTML----GDSSYQG-
RGIMPRLSSELMTRIAEKREQGEDIK-AEVCYFEIYNE------
RIRDLLVADKSPEEKQSEPEISVKGRTSMTRTGSLGDLKQARGSKQGLGLKHVPSSGSLSDFKRGSPTQ
LVQQSPPKEVLGVQRGSKRMPNWKSFQAAQTDFAKEQQYLKVREHPVNG-
PYVEGLMWKNVETWMDIKTFLKYGSALRTTHTTDANAH-SSRSHALFTIRITK---VIQPTSFSYLWH-
-------YYCVIFFSPRLG---HGSERPSDLLTVET--------ATRG----EESRAI-----
NLSLTMLNEVILSLSKGT--------------
HPSYRSSVLTWLLRDSLGGNNKTFMVANISATEHDLRETLSTLRYA-------
>Phypa_431083-PpKinesinOrph-IVa
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
-----------------------------------------------------MRE-
ITLMGVTKAGVNSLEEMATYLEHGYLSNTTGSTNMNSH-SSRSHAIFTITLEQ---RRK----------
-WDPIP-DSGSPLSEDCSEDYLCAKLHLVDLAGSERAKRTGAG-GLRF----KEG-----------
LLALGNVISALGDEKKRKEGG--------
HVPYRDSKLTRLLQDSLGGNSRTVMIACVSPADVNVEESINNLKYANRARNIR
>Phypa_451243-PpKinesinOrph-IVb
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
-----------------------------------------------MNCE--------------------
---------------------------SSRSHCVFLLTIQQ------------------------
SDIEDRSIK---TGKIYLVDLAGSEKVEKTGAE-GKLL----CEAKTI-----
NKSLSALGNVINALTSDKPC------------
HVPYRDSKLTRILQDSLGGNSRTALLCCCSPSTLHASETLSTLRFGTRAKLI-
>Phypa_437822-PpKinesinOrph-IVc
------------------------------------------------------------------------
------------------------------------------------------
MAQYSLTARQAVAKPTRC------------------------------RDLLPIKIVD---------
---REFL-VRVSYMEIYNE------EINDLL-------------------------------------
--------AP--------DN----------------------------------------
RKLQIHESIERG-IFVAGLREEIADSVEQVIAVLERGEAQRHLAETDMNVN-SSRSHTIFRMVIES---
RDK-----------SHDST-QDSDPSAQDAVR---VSALNLVDLAGSERISKTGAE-GVRL----
REGAHI-----NKSLTTLGMVINKLSEGGGKQGA---------
HVPYRDSKLTRILQSALGGNARTSIICTINPDEIHIDETRGTLQFASRAKRV-
>AT3G63480-AtKinesin1
```

```
SNVTVCARFRP-------------------------------------
RSSKEMRDPSRDGVCARPIDA-----------ETFVFQDD--KEDEFTFSLDRVFYE-DS---------
-TQAAVYEFLALPIMRDAVNGINGTIITYGQTGAGKTYSMEGPGIQDCDEHN-
KGLLPRVVHGMFEQISSSNDI-ARYT-VKLSMVEIYME------KVRDLL------------------
--------------------------DL--------SK-----------------------------
------------ANIQIKENKTQG-ILLSGVTEVPVSDSVEALQHLCTGLANRAVGETQMNMS-
SSRSHCAYLFTIQQ-------------------------DSVKDKRVK---
TGKLILVDLAGSEKADKTGAE-GRVL----EEAKTI-----NKSLSALGNVINALTSGPSSKGN-----
----HIPYRDSKLTRILQDALGGNSRMALLCCCSPSTLNASETLSTLRFGMRAKHI-
>AT3G54870-AtKinesinARK_ARK1
------------------------------------------------------------------
----------------------WNSESYKFDEVFTD-TA----------
SQKRVYEGVAKPVVEGVLSGYNGTIMAYGQTGTGKTYTVGKI--GKDDAAE-
RGIMVRALEDILLNASS-----ASIS-VEISYLQLYME------TIQDLL------------------
--------------------------AP--------EK-----------------------------
------------NNISINEDAKTGEVSVPGATVVNIQDLDHFLQVLQVGETNRHAANTKMNTE-
SSRSHAILTVYVRR---AMNEKTE--------KAKP-ESLGDKAIPRVR---
KSKLLIVDLAGSERINKSGTD-GHMI----EEAKFI-----NLSLTSLGKCINALAEGSS---------
----HIPTRDSKLTRLLRDSFGGSARTSLIITIGPSARYHAETTSTIMFGQRAM---
>AT1G01950-AtKinesinARK_ARK2/ATKINUB
-RVRVAVRLRP-------------------------------------
RNADESVADADFADCVELQPEL----------KRLKLRKN--NWDTETYEFDEVLTE-AA---------
-SQKRVYEVVAKPVVESVLEGYNGTVMAYGQTGTGKTFTLGRL--GDEDTAA-
RGIMVRSMEDIIGGTSL-----DTDS-ISVSYLQLYME------TIQDLL------------------
--------------------------DP--------TN-----------------------------
------------DNIAIVEDPRTGDVSLPGATHVEIRNQQNFLELLQLGETHRVAANTKLNTE-
SSRSHAILMVHVKR---SVV---ENEFPVSNEMESS-SHFVRPSKPLVR---
RSKLVLVDLAGSERVHKSGSE-GHML----EEAKSI-----NLSLSALGKCINAIAENSP---------
----HVPLRDSKLTRLLRDSFGGTARTSLIVTIGPSPRHRGETTSTILFGQRAM---
>AT1G12430-AtKinesinARK_ARK3/ATKINUA
-RVRVAVRLRP-------------------------------------
RNGEELIADADFADCVELQPEL----------KRLKLRKN--NWDTDTFEFDEVLTE-YA---------
-SQKRVYEVVAKPVVEGVLDGYNGTIMAYGQTGTGKTYTLGQL--GEEDVAD-
RGIMVRAMEDILAEVSL-----ETDS-ISVSYLQLYME------TVQDLL------------------
--------------------------DP--------SN-----------------------------
------------DNIAIVEDPKNGDVSLPGATLVEIRDQQSFLELLQLGEAHRFAANTKLNTE-
SSRSHAILMVNVRR---SMK---TRDGLSSESNGNS-HMTKSLKPPVVR---
KGKLVVVDLAGSERINKSGSE-GHTL----EEAKSI-----NLSLSALGKCINALAENSS---------
----HVPFRDSKLTRLLRDSFGGTARTSLVITIGPSPRHRGETTSTIMFGQRAM---
>AT1G20060-AtKinesinOrph-IV
------------------------------------------------
KKDEEACITLNDSYSVTLTPP----------QSLQELKR--SKTEVYEGFSHVFPA-DC---------
-SQNDVYDKMVQPLLEDFMKGKSGMLAALGPSGSGKTHTVF----GSLKDP---
GIVPITLRQIFKKNDESCS--GSLRSFNLSIFEICSE-RGKGEKAYDLL---------------------
--------------------------GG--------ES-----------------------------
-----------SELSVQQ------STIRGLKEVPIQNLEEAESLIGQAMLKRATATTNSNSQ-
SSRSQCIINIRAS-------------------------
CNGFSNETKLQSSDAMLTIVDLAGAEREKRTGNQ-
AIDFGLPGTDPYFILFLMPTIPLTMNTRYLLAVTVGVPEEPKEGIAETS--SKFF--
GSLTRYLRDYLEGKKRMALILTVKAGEEDYLDTSYLLRQASPYM---
>KT986231-MvKinesinARKa
-RVRVAVRLRP-------------------------------------
RNTDEILADADFGDYVELMPEL----------KRLRLRKN--NWDSETFQFDEVLTE-TA---------
-SQKRVYEVVAKPVVESVLEGYNGTVMAYGQTGTGKTYTLGKL--GEEDTAG-
RGIMVRAMEDILADTTS-----EQDS-VTVSYLQLYME------TVQDLL------------------
--------------------------AP--------EK-----------------------------
------------DNISIVEDPKTGDVSLPGATLVEIKDQKSFVELLEIGEANRFAANTKLNTE-
SSRSHAILLVNVKK---MAKLKPERELSSLNENGSNIQLSKNHRIPTIR---
KSKLLIVDLAGSERVDKSGSE-GHTL----EEAKYI-----NLSLTALGKCINALAENSP---------
----HVPIRDSKLTRLLRDSFGGSARTSLIITIGPSPQHRGETTSTILFGQRAM---
>KT986232-MvKinesinARKb
-RVRVAVRMRP-------------------------------------
RNSEEMLADADFADCVELQPEL----------KRLKLRKN--NWDCETYEFDEILTE-FS---------
-SQKRVYEVVAKPVVESVLEGYNGTVMAYGQTGTGKTFTLGRL--GEEDASD-
```

```
RGIMVRAMEDILADVSP-----ESDM-VTVSYLQLYME------TVQDLL------------------
-------------------------AP--------ER--------------------------------
------------DNIPIVEDPKTGDVSLPGATLVEIRDQSSFLDLLQLGEANRFAANTKLNTE-
SSRSHAILMVNVKK---HLK-GKTGDMIVMDENGMTMQMAKGFRAPTVR---
KSKLVVVDLAGSERLDKSGSE-GHTL----EEAKSI-----NLSLTALGKCINALAEGSS---------
----HVPIRDSKLTRLLRDSFGGTARTSLVITIGPSPRHRGETASTILFGQRAM---
>KT986233-MvKinesinARKc
-RVRVAVRLRP-----------------------------------
RNAEEQVSDADFADCVELEPEL----------HRLKLRKN--NWDADTYQFDEVLTE-HA---------
-SQKRVYEVVGKPVVESVLQGYNGTIMAYGQTGTGKTYTLGQL--GDEDDSA-
RGIMVRAFEDILSGVSL-----REDT-VTVSYLQLYME------MIQDLL------------------
--------------------------SP--------EK------------------------------
------------ENIPIVEDPKMKDVQLPGASVVEIKDYNSFVELLKVGEANRAVANTRLNAE-
SSRSHAMLVVNVRK---LVR--DSSEDLNNSENGETLFGSKDFSVPTAR---
RSKLVVVDLAGSERIDKSGVI-GSSL----DEAKSI-----NLSLTALGKCINAIAEGNS---------
----HVPVRDSKLTRLLRDSFGGTARTSLVITIGPSPRHRAETASTIMFGQRAM---
>KT986234-MvKinesinARK-LIKE
-RVRVTLRARP-----------------------------------------
STKQEKQEEDNSLVYINPDK----------RTVIVRRNGSSVDYTEYKFDAVLSD-SA----------
TQADVYRVAAQPVVLDVLHGYNGTIMAYGQTGAGKTYTLSDISINETSTSRIDGIIPRSAANIFEYTSN
DKN--HEYR-ISMSYIQIYLE------TIQDLL-------------------------------------
----------NP--------ES-------------------------------------------
SNLQIREGETGG-IFIAGVHEVEIKSLEDLVRLLMIGDSNRTNAFTNMNAH-SSRSHAIVIITVEK---
KSIGNLCGRTSIVDSGRRT-PRPIGSQEQQIL---VGKLFLVDLAGSERLKRSGSE-GLRA----
TEAMSV-----NMSLTALAKCISARADPSNT-----------
HVPFRDSKLTRLLQESLGGNSKTSLIINIAPCSLYH-ETLSSLKFGMRAM---
>KT986283-MvKinesinOrphI
-------RMRP-----------------------------------
FFEKEKVCPYRQVLCLLGNRILIKQHPSTLKTKRTCMSKK--
KFDELELEVDYVQDDSYVQVKKEDKKEESQRHLFSTIGVQAVDRLLNGQSVTILSYGQAGTGKTYTIY-
---GDGTESG-RGLIPRITCDFLHKIQAKGR--DDECVVEVSFLEIYQE------KLRDLL-----
AVNRARETPFELLMRRSPDLSTEYPEVSSSSDQDSDSDSDSGPSLGFRDIPDKVHVHRYQQGTKSSIRH
PVRPTS-------------------NYLKLREHPVKG-
TYVEGLIWKKVCTWSDMDRLLKQGAARRSTVATDSNRQ-SSRSHTLFTMKVTW----------------
------------LSTPRHGGRTVSYLNLVDLAGNEKLQNVS---IEKQ----KDMKYI-----
NKSLSQLNSVILNLSRST-------------
FVPYRNSALTWLLKESLQGDNITFLVANVSPAEKDFNETLATLNYAFKAKRI-
>KT986286-MvKinesinOrphIV
-
KIRVFLRIRPAKVVRSGKVTKKAPKQGDMVNRSIQNWKKPSAAEAKSANEAPEVCLFPQTSDSVSLIPP
-----------TSVSGPRR--SKAEEFNGFTHIFTP-DA----------
TQEEVFDKIVHPRLVDLLKGQSSLVVAMGPTSAGKTHTMLGT--GED-----
VGLLPRSLRFLLKDNQDINS--ASIR-IAISMFEIYSDQQARSERLIDLL--------------------
--------------------------Q---------DG-----------------------------
------------VELSLLQ------FKIKGLHEAFVSSIEEADSVLLSGLKRRTTASTAANDR-
SSRSHCIINISANV---PKD-----------------CEGQDDVIYLK---
KATLTIADLAGFEREKKTGNQ-GVRL----NESSFI-----NNTSMIFGQCLRALLEHQKNPKKLL---
----QKHFQYSMLTRYLKPYMEARGNMTLIVNVSPCEEDYLDTAFVLRQAA------
>Cre13.g588600.t1.1-CrKinesin1a
-RIRPPLREEE-----------------------------------
TPGALAWTEGDKGQLTIFRPDA-------------------PVPQSDFVFTKVLGP-SC---------
-SQADVYTSAVSDVVADVLRGYNGTILAYGQTGAGKTYTLGNT------
APDAIGMIPRAAAELFAAAARDPL--HSYH-VTMSYIQIYME------MIQDLL---------------
-------------------------------NP--------TA--------------------------
---------------DNLPIREDGSG--VFVAGACEVPVASLEECLHYLELGEQNRVFAFTHLNAH-
SSRSHAVVMLTAVK---GRK-----YLTNEERAAAEAPDQDGVVTQKVT---
VGKLYMVDLAGSERLKKSKSV-GLRA----TEARSI-----NLSLTMLGMCISARAQDSP---------
----HVPFRDSKLTRLLQESLGGNAKTSLILCLSDVRQHADESLQSLQFGARA----
>Cre17.g735200.t1.2-CrKinesin1b
-------RFRP-----------------------------------RSSKEAE-----
ARCVEFLGNSDS--------GAIGFTGP--DGVKHNFAFDKVYGE-TS----------
SQEEVFADLTH-VVDAALAGYNGTILAYGQTGSGKTHTLI----
GRVADPAQRGVVPRAVAHLADGIGAAGEREGADFQVVLSVVEIYCE------RVRDLL-----------
-----------------------------------AEGGGGGGGGGS---------------------
```

```
--------------------DNLQVKQDALRG-
VYIEGATELCVTDEAQLVGCMGRGLAQRSVAATSMNAE-SSRSHCIVTVRVER---TRP----------
--------------DGAVQ---TGKLVMVDLAGSERADRTGAA-GTTL----VEGSLI-----
NKSLSCLSNVIYALTDDKGGGGAGGAGGGAGRHVPYRDSKLTRVLQDSLGGTARTVLIICCSPCAENSA
ETLSSLRFGARAKGV-
```

Appendix I-3

MSA of kinesin-2 motor domain sequences used to a build phylogenetic tree.

```
>Mv_c21605_g1_i1_(Mv_Kinesin-2)
-ERVQVVVRCRPISERELTAGHKCCISIDTERRTVEVHNVGGRFAADNI------
PKSFTFDRVYNEKSTQRQIYKDVAYSIVHSVMCGYNGTVLAYGQTASGKTYTMEGCDHSPDLWGIIPNA
FEHIFQHIR--QSQS-SDSFLVRISYLEIYNEEIRDLLSPA--TSKKLE---LKESVETGVYVK-
NLTSLTVNSFADIRQLLMLGKKNRAIGATAMNQDSSRSHSIFTITVESS----TCDP----------
TGGKTHVR-VGKLNLVDLAGSERLSKT-----------------------
GATGERFKEMTKINWSLSALGNVISALVDGKSSHIPYRDSKLTRLLQDSLGGNTRTVMVANIGPADYNY
EESISTLRYANRAKNI-------
>XP_396164_(Am_Kinesin-2A)
-----------PXNGKELD-GHCKTXYVWIHKXXITVENPNA--AQGEP------
PKVFSFDAVFDTDSTQVDIYNETARPIVDKVLQGYNGTIFAYGQTGTGKTYTMSGAKTSPQLRGIIPNT
FAHIFGHIA--KAHD-NQKFLVRATYLEIYNEEVRDLLGKD--QNTRLE---VKERPDIGVFVK-
DLSGYVVNNADDLDRIMSLGNKNRVVGATAMNVSSSRSHAIFTITVESS----QLGE-----------
DGEQHVK-MGKLHLVDLAGSERQSKT-----------------------
KASGVRLREATKINLSLSTLGNVISALVDGQSSHVPYRNSKLTRLLQDSLGGNSKTLMCANVSPADINY
DETISTLRYANRAKNI-------
>XP_393174_(Am_Kinesin-2B)
--------RCRPMDEKELARGYMRVVDVFPSRGVVEIRHPRDDPSSDNV-------
KVFTFDAVYDWNSSQQDLYEETVRPLVSSVLDGFNGTIFAYGQTGTGKTYTMEGLKTDHERRGVIPRSF
EHIFNHIG--RSE--NMQYLVRASYLEIYQEEIRDLLQPD--QSLRFE---LKEKPDIGVFVK-
DLSTSVCKSAAEIQQLMNTGNQNRTIGATNMNEHSSRSHAIFLITIEMG----SIGD-----------
TGG--IR-VGRLNLVDLAGSERQSKT-----------------------
GSSGERLKEASKINLSLSALGNVISALVDGKTTHVPYRDSKLTRLLQDSLGGNSKTIMVANIGPASYNY
EETLTTLRYANRAKNI-------
>XP_395281_(Am_Kinesin-2C)
--SVKVAVRCRPMNARELQQGCRNVVTIDPPSKCCTLECPAT--GTGNG-------
KVYQFDAAFNPEATTESVYDNVGSVIVEAVLDGYNGTVFAYGQTGCGKSHTM---------
RGFIERTLDHIFEATS--TASA-EMRYLALLSYLEIYNERLRDLLQDG--MSNMLT---
LKEDPNRGTYVAGGLKEVTVKDAAECARLVEQGDRRRAAAATKMNAASSRSHAVLTLSLETL----
AINE--------EDSKAENTVK-RGRLHLVDLAGSERQART-----------------------
GATGDRLKEAASINLSLSALGNVISALAAGHGRHVPYRDSKLTRLLRDSLGGNARTLMIACISPSDVDA
EETLSTLRYAARARCIKNKPVIN
>Ce_KLP-11_(Ce_Kinesin-2B)
-ETVKVIVRCRPLSSQEIANNYSKIVHMRPQRGQIELKNPKE---QDEP------
SKDFTFDAIYDENSTQSDLYEETFRDLVDSVLNGYNATIFAYGQTGTGKTHTMEGKSSDPEQRGVIYKC
IDHIFEHMA--ASH--NQEYLVRASYLEIYQEELRDLLEAE--SNKKLE---IKERPDGGVYVK-
DLTSKLTRTVGEIHEVMIRGNGHRSVGRTNMNEHSSRSHAIFIITVECS----RIGE-----------
DGESHIT-VGRLNLVDLAGSERQSKT-----------------------
GATGERFKEATKINLSLSALGNVISALVDAKSAHIPYRDSKLTRLLQDSLGGNSKT------------
-ETLGTLRYANRAKNI-------
>Ce_KLP-20_(Ce_Kinesin-2A)
-EKVKVVVRCRPISTTEKLQGHKIAVTCNDEEKAVNIKSLSQ----EDP------
PRTFYFDAVFSPNTDQMTVYNVAARPIVENVLKGYNGTIFAYGQTGTGKTFTMAGDLEPVEMRGIIPNS
FAHIFDHIA--KCQH-DTTFLVRVSYLEIYNEEIRDLLSKD--HNGNLE---IKERPDVGVYVR-
NLSNPTVENASKMQALMEFGSKNRKVGATAMNLESSRSHAMFTVTIESC-------------------
RNGLVT-QGKLQLVDLAGSERQSKT-----------------------
GAQGERLKEAAKINLSLSTLGNVISSLVDGKSTHIPYRNSKLTRLLQDSLGGNSKTVMIANVGPATYNY
DETLSTLRYANRAKNI-------
>Ce_OSM-3_(Ce_Kinesin-2C)
-ESVRVAVRCRPFNQREKDLNTTLCVGMTPNVGQVNLNAPDG-----AA-------
KDFTFDGAYFMDSTGEQIYNDIVFPLVENVIEGYNGTVFAYGQTGSGKTFSMQGIETIPAQRGVIPRAF
DHIFTATA--TTE--NVKFLVHCSYLEIYNEEVRDLLGAD--NKQKLE---IKEQPDRGVYVA-
GLSMHVCHDVPACKELMTRGFNNRHVGATLMNKDSSRSHSIFTVYVEGM------------------
TETGSIR-MGKLNLVDLAGSERQSKT-----------------------
```

```
GATGDRLKEATKINLSLSALGNVISALVDGKSKHIPYRDSKLTRLLQDSLGGNTKTIMIACVSPSSDNY
DETLSTLRYANRAKNI-------
>Cr_FLA10_(Cr_Kinesin-2)
-ESVKVVVRCRPLNGKEKADGRSRIVDMDVDAGQVKVRNPKA--DASEP------
PKAFTFDQVYDWNCQQRDVFDITARPLIDSCIEGYNGTIFAYGQTGTGKSHTMEGKDEPPELRGLIPNT
FRYVFEIIA--RDSG-TKEFLVRSSYLEIYNEEVRDLLGKD--HSKKME---LKESPDRGVYVK-
DLSQFVCKNYEEMNKVLLAGKDNRQVGATLMNQDSSRSHSIFTITIECI----
EKLESAAAQKPGAKKDDSNHVR-VGKLNLVDLAGSERQDKT-----------------------
GATGDRLKEGIKINLSLTALGNVISALVDGKSGHIPYRDSKLTRLLQDSLGGNTKTVMVANIGPADWNY
DETMSTLRYANRAKNI-------
>Cr_FLA8_(Cr_Kinesin-2)
-ECVKVAVRCRPLNGKEKGDNRATIVEVDNKTGQVTLNNPKG----DEP------
PKTFTFDNAFDWNVTQRDVYDVVARPIVNSVMDGYNGTIFAYGQTGTGKTHTMEGFPT-
PELQGIIPNCFDHVFETVN--SST--GKQWMVRASYLEIYNEEVRDLLSKD--PKNKLE---
LKEHKDSGVYVK-GLNAFVVKGVPELKNVLEVGKKNRSVGATLMNQDSSRSHSIFTITIETI----
EQTQ----------AQPEGHIR-VGKLNLVDLAGSERQSKT-----------------------
GATGDRLKEATKINLSLSALGNVISALVDGKSGHVPYRDSKLTRLLQDSLGGNTKTIMCANMGPADWNY
DETLSTLRYANRAKNI-------
>Dm_KIF3C_(Dm_Kinesin-2C)
-ENIKVVVRCRPMNQTEKERNCQNIVEINEF--AVSVTNPSA--RISQQ-------
KKFIFDSVYNMKTDTEVIYDEMCYSLVESTIEGYNGTIFAYGQTGCGKTHTMQGDENFSNSTGIIPKCF
DHIFERIS--MTT--NVRYLALVTYLEIYNERIRDLLNKN--ENTNVINHFLKELPGIGVSVP-
TLTTQPVVNANQCYDWLHFGNKNRVTAATLMNKNSSRSHTIFTITLEQSPFLNSIGS-----------
DAFGGIC-RGKLSLVDLAGSERQRKT-----------------------
GAQGDRLKEASQINLSLSALGNVISSLVDGKAKHVPFRDSKLTRLLQDSLGGNTKTLMISCISPTDIHY
DETISTLRYASRAKNI-------
>Dm_KLP64D_(Dm_Kinesin-2A)
-ENVRVVVRTRPMDKNELSAGALSAISVDKINRAITVMKPNA--TANEP------
PKTYYFDNVFDGGSNQMDLYVDTARPIVDKVLEGYNGTILAYGQTGTGKTYTMSGNPDSPQTKGIIPNA
FAHIFGHIA--KAKE-NQKFLVRVSYMEIYNEEVRDLLGKD--VGKSLE---VKERPDIGVFVK-
DLSGYVVHNADDLENIMRLGNKNRAVGATKMNQESSRSHAIFSITVERS----ELGE-----------
GDVQHVR-MGKLQLVDLAGSERQSKT-----------------------
QASGQRLKEATKINLSLSVLGNVISALVDGKSTHIPYRNSKLTRLLQDSLGGNSKTVMCATISPADSNY
METISTLRYASRAKNI-------
>Dm_KLP68D_(Dm_Kinesin-2B)
-ECVQVVVRCRPMSNRERSERSPEVVNVYPNRGVVELQNVVD--GNKEQ------
RKVFTYDAAYDASATQTTLYHEVVFPLVSSVLEGFNGCIFAYGQTGTGKTFTMEGVRGNDELMGIIPRT
FEQIWLHIN--RTE--NFQFLVDVSYLEIYMEELRDLLKP---NSKHLE---VRER-GSGVYVP-
NLHAINCKSVEDMIKVMQVGNKNRTVGFTNMNEHSSRSHAIFMIKIEMC------DT-----------
ETNXTIK-VGKLNLIDLAGSERQSKT-----------------------
GASAERLKEASKINLALSSLGNVISALAES-
SPHVPYRDSKLTRLLQDSLGGNSKTIMIANIGPSNYNYNETLTTLRYASRAKSI-------
>Gg_KIF3B_(Gg_Kinesin-2B)
-ESVRVVVRCRPMNSKEKSASYEKVVNVDVKLGQVSVKNPRG--TSHEL------
PKTFTFDAVYDWNSKQVELYDETFRPLVDSVLQGFNGTIFAYGQTGTGKTYTMEGVRGDPEKRGVIPNS
FDHIFTHIS--RSQ--NQQYLVRASYLEIYQEEIRDLLSKD--QSKRLE---LKERPDTGVYVK-
DLSSFVTKSVKEIEHVMNVGNQNRSVGATNMNEHSSRSHAIFVITIECS----ELGL-----------
DGENHIR-VGKLNLVDLAGSERQAKT-----------------------
GAQGERLKEATKINLSLSALGNVISALVDGKSTHIPYRDSKLTRLLQDSLGGNAKTVMVANIGPASYNV
EETLTTLRYANRAKNI-------
>XP_001708236_(Gl_Kinesin-2D)
-
DNVMVMVRVRPFNKREEQEGATEIIEMDKTLCTVTLHKPVEKGAGSATSECLPSKKVFTYDAVYPSNST
QVEVFDESVREMIDGCLEGYNATVFAYGQTGSGKTHTMMGQKDNP---GMIPLAFQRIFDFIA--QAK-
-DDQFLVRASFVEIYNEDLKDLLT----GATHLQ---LKEDPVKGVFIK-
DLSEHPVSDERHIDKLIQKGNESRAVAATLMNATSSRSHSIFQVVLERM-----TVI-----------
DGRECIR-VGKLNLVDLAGSERQEKT-----------------------
GATGDRLKEAAKINLSLTTLGCVISKLVEG-
SKHIPYRDSKLTRLLQDSLGGNSKTLMVVAVSPASTNYDETMSTLRYADRAKQI-------
>XP_001706504_(Gl_Kinesin-2D)
-DNIKVIVRCRPLNARETRENALNIIRMDEASAQVIVDPPEQEKSATQAKKV---
PRTFTFDAVYDQTSCNYGIFQASFKPLIDAVLEGFNSTIFAYGQTGAGKTWTMGGNKEEP---
GAIPNSFKHLFDAIN--SSSS-NQNFLVIGSYLELYNEEIRDLIK----NNTKLP---
LKEDKTRGIYVD-GLSMHRVTTAAELSALMDKGFANRHVAATQMNDTSSRSHSIFMVRIECS----
```

```
EVIE------------NKEVIR-VGKLNLVDLAGSERQSKT-----------------------
GATGETLVEGAKINLSLSALGLVISKLVEG-
ATHIPYRDSKLTRLLQDSLGGNSKTLMCANISPASTNYDETMSTLRYADRAKQI-------
>Hs_KIF17_(Hs_Kinesin-2C)
-EAVKVVVRCRPMNQRERELRCQPVVTVDCARAQCCIQNPGA---ADEP------
PKQFTFDGAYHVDHVTEQIYNEIAYPLVEGVTEGYNGTIFAYGQTGSGKSFTMQGLPDPPSQRGIIPRA
FEHVFESVQ--CAE--NTKFLVRASYLEIYNEDVRDLLGAD--TKQKLE---LKEHPEKGVYVK-
GLSMHTVHSVAQCEHIMETGWKNRSVGYTLMNKDSSRSHSIFTISIEMS----AVDE-----------
RGKDHLR-AGKLNLVDLAGSERQSKT-----------------------
GATGERLKEATKINLSLSALGNVISALVDGRCKHVPYRDSKLTRLLQDSLGGNTKTLMVACLSPADNNY
DETLSTLRYANRAKNI-------
>Hs_KIF3A_(Hs_Kinesin-2A)
-DNVKVVVRCRPLNEREKSMCYKQAVSVDEMRGTITVHKTDS---SNEP------
PKTFTFDTVFGPESKQLDVYNLTARPIIDSVLEGYNGTIFAYGQTGTGKTFTMEGVRAIPELRGIIPNS
FAHIFGHIA--KAEG-DTRFLVRVSYLEIYNEEVRDLLGKD--QTQRLE---VKERPDVGVYIK-
DLSAYVVNNADDMDRIMTLGHKNRSVGATNMNEHSSRSHAIFTITIECS----EKGI-----------
DGNMHVR-MGKLHLVDLAGSERQAKT-----------------------
GATGQRLKEATKINLSLSTLGNVISALVDGKSTHVPYRNSKLTRLLQDSLGGNSKTMMCANIGPADYNY
DETISTLRYANRAKNI-------
>Hs_KIF3B_(Hs_Kinesin-2B)
-ESVRVVVRCRPMNGKEKAASYDKVVDVDVKLGQVSVKNPKG--TAHEM------
PKTFTFDAVYDWNAKQFELYDETFRPLVDSVLQGFNGTIFAYGQTGTGKTYTMEGIRGDPEKRGVIPNS
FDHIFTHIS--RSQ--NQQYLVRASYLEIYQEEIRDLLSKD--QTKRLE---LKERPDTGVYVK-
DLSSFVTKSVKEIEHVMNVGNQNRSVGATNMNEHSSRSHAIFVITIECS----EVGL-----------
DGENHIR-VGKLNLVDLAGSERQAKT-----------------------
GAQGERLKEATKINLSLSALGNVISALVDGKSTHIPYRDSKLTRLLQDSLGGNAKTVMVANVGPASYNV
EETLTTLRYANRAKNI-------
>Hs_KIF3C_(Hs_Kinesin-2B)
-EALKVVARCRPLSRKEEAAGHEQILTMDVKLGQVTLRNPRA--APGEL------
PKTFTFDAVYDASSKQADLYDETVRPLIDSVLQGFNGTVFAYGQTGTGKTYTMQGTWVEPELRGVIPNA
FEHIFTHIS--RSQ--NQQYLVRASYLEIYQEEIRDLLSKE--PGKRLE---LKENPETGVYIK-
DLSSFVTKNVKEIEHVMNLGNQTRAVGSTHMNEVSSRSHAIFIITVECS----ERGS-----------
DGQDHIR-
VGKLNLVDLAGSERQNKAGPNTAGGAATPSSGGGGGGGSGGGAGGERPKEASKINLSLSALGNVIAAL
AGNRSTHIPYRDSKLTRLLQDSLGGNAKTIMVATLGPASHSYDESLSTLRFANRAKNI-------
>XP_001682337_(Lm_Kinesin-2D)
--NIKVLVRCRPFSEKENAMGHKSCVDLDMVQNTVTVKSIIG-----EP-------
DRWTFDAVINNSFSQEDIFTQFIMPLTESVLGGFNATVFAYGQSGSGKTHTMTGVMGNSTLEGVIPRCV
KHIFDSVQKMRDEAPSTTVSMYVSFMELYNGKVRDLLAK---QQVSLD---IRENKDHTFFVK-
GAVVAQVKFPEDVIRHLEEGTDRRRVASTELNADSSRSHSVFSLILECT----ETLE-----------
DGSTRAV-SSKLNLVDLAGSERQGKT-----------------------
GASGDTLKEGCNINLSLSALGTVIDTIVKG-
GTHVPFRSSPLTMLLKDSLGGNSKTVMFANINPSERNVSETVSTLRFADRAK---------
>XP_001685383_(Lm_Kinesin-2D)
--NIRVVIRCRDILPYEAERGDKALVRLDLATNQVVVQHPIG-----DA-------
DVFAFDAVYNNSFTQRDIFLQEVQPLADAVLQGYNATVFAYGQSGSGKTHTMTGKLSQRNMWGMMPQVV
DYLFSEIK--KLTSSTKTFKVKVSYVELYNGKSRDLLSS---KQVNLE---IKQNTSKNFYVK-
GAEMPEVTSFEDAIKWFNAGTERRQTASTDLNDTSSRSHSLFTVQIEHF----DFEN-----------
DPSSPIVMTSKINVVDLAGSEKLSKT-----------------------
NATGETAKEGCNINLSLSALATVIDTIVKG-
AKHIPYRGSPLTMLLKDSLGGNAKTVMFANVGPSDKNLSETISTLRFALRAK---------
>XP_001455773_(Pt_Kinesin-2)
--CVRVVIRCRPLNDTEKKDGHVCIVNMDTKNGQVTVRNPKV---ADEV------
PKQFTFDQIFDTQSLQENVYNQTAHPIVESVLEGYNGTIFAYGQTGTGKTHTMEGKDDPPTLRGIIPRT
FDHIFQRIE--NMAK-NKQFLVKVSFLELYNEEIRDLLSKN--IKNKLE---IRENPETGIYIK-
DLSKFMIENPQEMREKLLHGRENRAVGATAMNQDSSRSHSLFQITVETN----EIVQ-----------
GQSHVT-VGKLNLVDLAGSERQSKT-----------------------
HATGDRLKEAININQSLTTLGNVISALVDNKSQHIPYRDSKLTRLLQDSLGGNTKTVMIANIGPADYNF
DETLSTLRYANRAKQI-------
>XP_001426973_(Pt_Kinesin-2)
-ECVKVIVRMRPFNQREKENGSKPCVIVYEDTNTVELRNTQD----NDV-------
KSYTYDYVFGAETPQLSIYQKTAFNLVESVADGYNGTIFAYGQTGCGKTFTMIGDPSNETMKGIIPRTF
DQIISIIN--NNSDTNKKFLLRCSYIEIYNEEIHDLLSKD--VKQKYE---LKEG-QQGVFIK-
DLNIAVVRTTQEMDRYMQLGTQNRSVGATAMNKESSRSHCIFTVYIECS----VTDP-----------
```

```
KGNERIT-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNF
DETLSSLRYASRAKMI-------
>XP_001427404_(Pt_Kinesin-2)
-ECVKVIVRVRPFNQKERDNGSKPCVNVYESTNSVELFRSQD----NDK-------
KQFTYDYVFGPETPQIQIYQQTAFNLVESVAEGYNGTIFAYGQTGCGKTFTMIGDPLNDNMKGIIPRTF
EQIISIIN--NNSDSNKKFLLRCSYIEIYNEEIHDLLSKD--VKQRYE---LKEG-QQGLYVK-
DLNIPIVKTLQDMDKYMALGAQNRSVGATAMNKESSRSHCIFTVYMECS----MTDD-----------
KGNERII-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNY
DETLSSLRYASRAKMI-------
>XP_00142818_(Pt_Kinesin-2)
-ECVKVIVRMRPFNSREKENGSKPCVTVHEDTNSVELRSSQD----NEV-------
KNFSYDYVFGAETPQLQIYQKTAFNLVESVADGYNGTIFAYGQTGCGKTFTMIGDPTNENMKGIIPRTF
DQIISIIN--NNSDSNKKFLLRCSYIEIYNEEIHDLLSKD--AKQKYE---LKEG-QQGVFIK-
DLNIAVVRTTQEMDKYMQLGTQNRSVGATAMNKESSRSHCIFTVYIECS----ITDS-----------
KGNERIT-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNY
DETLSSLRYASRAKMI-------
>XP_001428184_(Pt_Kinesin-2)
-ECVKVIVRMRPFNSREKENGSKPCVTVHEDTNSVELRSSQD----NEV-------
KNFSYDYVFGAETPQLQIYQKTAFNLVESVADGYNGTIFAYGQTGCGKTFTMIGDPTNENMKGIIPRTF
DQIISIIN--NNSDSNKKFLLRCSYIEIYNEEIHDLLSKD--AKQKYE---LKEG-QQGVFIK-
DLNIAVVRTTQEMDKYMQLGTQNRSVGATAMNKESSRSHCIFTVYIECS----ITDS-----------
KGNERIT-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNY
DETLSSLRYASRAKMI-------
>XP_001429325_(Pt_Kinesin-2)
-ECVKVVVRVRPFNQKEKENNSKPCVNVDEKQNVVELLKLTD----NET-------
KQFSYDYVFGMNAKQSYIYEKTAFNLVESVIDGYNGTIFAYGQTGCGKTFTMTGVPENEELKGIIPRTF
TQIQTIID--TNTDTKKKFLVRCSFLEIYNEEIRDLLGKD--HKARLE---LKES-QGSVSVK-
DLTMVTVKTAQDMDKYMTLGQSNRSVGATAMNAQSSRSHCIFTVYVESQ----IVDA-----------
KGSEFIR-VGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTVMITALSPADYNY
DETLSSLRYASRAKMI-------
>XP_001429366_(Pt_Kinesin-2)
KECVKVVVRARPLSSKEIEEGRKRIVDVDTSRKEINIQNIKG--DNNEA------
QRTFVFDEVFDLNSQQEQVYNNTALPIVESVMDGYNGTVFAYGQTGTGKTHTMEGKNDPPHERGITPRT
FDHIIKVIE--GTP--NIQFLVRCSYLELYNEEVRDLLSPN--HLTKLE---LREKPEQGIFVK-
DLSKIVVKSVAELNEWLKAGRANRKVGETKMNQESSRSHSIFTLTIESS----EIGA-----------
DQQQHIK-SGKLNLVDLAGSERQSKT-----------------------
QAVGVRFEEAININLSLTTLGNVITTLVDGKSQHIPYRDSKLTRLLQDSLGGNTKTVMVANIGPADYNF
DETMSTLRYANRAKKI-------
>Phypa_425592_(Pp_Kinesin-2)
-ERVQVVVRCRPMLVKENAEGRNNCVLVDTVGSTIQVKNLKQ--PEQEP-------
KLFTFDKTYDATSTQKQLYDDVAHPIVHSVMCGYNGTVLAYGQTASGKTFTMDGLDDPPEMRGIIPQAF
EGIFTHIQ--DSQS-SDNFLVRASYLEIHNEEIRDLLATGSQSSSRLE---LKENVEGNVYVK-
NLTSITVQSVADISHLLTVGKKSRSVGATLMNQDSSRSHSIFTITVEAS----ARSS-------
SAETDGSMHIR-VGKLNLVDLAGSERLNKT-----------------------
GATGDRFRELTNINWSLSALGNVISALVDDKSSHVPYRDSKLTRLLQDSLGGNTRTVMIANIGPADYNY
DESVSTLRYANRAKSI-------
>Sp_KRP85_(Sp_Kinesin-2A)
-DNVRVVVRCRPLNSKETGQGFKSVVKMDEMRGTVQVTNPNA--PSGEP------
PKSFTFDTVFAPGAKQTDVYNQTARPIVDAIIEGYNGTIFAYGQTGTGKTFTMEGVRSQPELRGIIPNS
FAHIFGHIA--KEQE-NVRFLVRVSYLEIYNEEVKDLLGKD--QQHRLE---VKERPDVGVYVK-
DLSAFVVNNADDMDRIMTLGNKNRSVGATNMNESSSRSHAIFTITLERS----DMGL-----------
DKEQHVR-VGKLHMVDLAGSERQTKT-----------------------
GATGQRLKEATKINLSLSTLGNVISSLVDGKSTHIPYRNSKLTRLLQDSLGGNAKTVMCANIGPAEYNY
DETISTLRYANRAKNI-------
>Sp_KRP95_(Sp_Kinesin-2B)
-ETVKVVVRCRPMNSKEISQGHKRIVEMDNKRGLVEVTNPKG--PPGEP------
NKSFTFDTVYDWNSKQIDLYDETFRSLVESVLQGFNGTIFAYGQTGTGKTFTMEGVRSNPELRGVIPNS
FEHIFTHIA--RTQ--NQQFLVRASYLEIYQEEIRDLLAKD--QKKRLD---LKERPDTGVYVK-
DLSSFVTKSVKEIEHVMTVGNNNRSVGSTNMNEHSSRSHAIFIITIECS----ELGV-----------
```

```
DGENHIR-VGKLNLVDLAGSERQAKT-----------------------
GATGDRLKEATKINLSLSALGNVISALVDGKSSHIPYRDSKLTRLLQDSLGGNAKTVMVANMGPASYNF
DETITTLRYANRAKNI-------
>XP_001014287_(Tt_Kinesin-2)
-ECVKVMVRVRPMNDKERQNNSKECVEVDTKLNQIVLRKPNE---AGSE-------
KVFTYDAVFYQKVQQQLVYEASAFPLVESVFEGYNGTIFAYGQTGCGKTHTMMGDPSKEEEKGIIPRTF
SHIINLIE--TTS--SKEFLVRVSFLEIYNEEIHDLLSKD--PKAKFE---LKQSPEKGVFVK-
DLNQIVVKSVKEMENLMYKGNENRSVGATAMNKDSSRSHSIFTIYIETS----EIDS-----------
TGNQHFR-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEANKINLSLSALGNVISALVDGRTHHIPYRDSKLTRLLEDSLGGNTKTIMIAAISPADYSY
DETLGTLRYASRAKNI-------
>XP_001276971_(Tv_Kinesin-2)
--SVKVAVRVRPMNSKEKNEKYSNIVRADKRNSSIYVKNPQG----SEL--------
QFTYDFVYPENTTQEEIYENTAAPIVAGVLEGFNGTIFAYGQTGTGKTYTMDGIADDKERRGIVPRAFE
HIFDFAT--ANAD-THKIVISVTYVELYNEEIRDLLISSKEKPVPLK---IHEDPQKGFIIN-
GVKSRPANSFEELCKIQRVGFKRRMTRKTNMNDDSSRSHSVLTLTIETL-----TEI-----------
EGSQHVR-QGRLNLVDLAGSERIEKT-----------------------
KCDKDGVIEGISINYALMVLGNCISALTTKGTLHIPYRDSSLTKLLRDSLGGNARTAMIANIGPADFNF
NETVATLRYAERAKKIENKPSVN
>XP_001319907_(Tv_Kinesin-2)
--AVKVSVRLRPMSQKEIDSGFSKVVEIDQKNSTVRIKNPQG-----QY-------
IQFSFDFCFPEDVSQEEVYNATAMPIVNGVLEGYNGTIFAYGQTGTGKTFSMDGKPTG-
ELRGIMPRAFDHIFEYIQ--ANSA-DTEFLVTVTYVEIYNNELRDLLSEK--SNEKLK---
IREDPTHGVQIK-GVAVHKVKDVEEIHALLNYGKKNRVVRKTQMNSESSRSHSIFTVTVETL-----
KQI----------DGQDHVR-SARLNLVDLAGSERVAKT-----------------------
GAEGVGFTEGVNINYELMILGNCIAALTSKGNTHIPYRDSKLTMLLRDSLGGNARTMMIAALGPADYNF
SETMSTLRYAERAKKIENKPTVN
>XP_001579747_(Tv_Kinesin-2)
--AVKVSVRLRPMSEKEINAGFKKIVEIDKKTATVKIQNPQN-----QT-------
ITFTFDYGFPEDCTQEEVYEATAAPIVSGVLEGFNGTIFAYGQTGTGKTYSMDGKTHG-
EHRGIMPRAFDHIFEYIQ--ANQD-SHEFLVTVTYVEIYNNELRDLLAEN--HEQPLK---
IREDAQKNVYIK-GVCTHKVKSVDELHALLAYGKKNRVVRKTNMNSESSRSHSILSLVIETL-----
TKI----------DGQDHVR-SARLNMVDLAGSERAAKT-----------------------
GAEGVGFTEGVNINYELMILGNCIAALT-
SKGSHIPYRDSKLTMLLKDSLGGNARTMMIAALGPADYNFSETMSTLRYAERAKKIENKPKVN
>XP_001300992_(Tv_Kinesin-2)
-ENIKVVVRCRPLSKGEQEKGYFSIVKVLPSAGQVQLYRNQE---DNNP-------
KTFQFNSAYPPDVTQQFIYDDCARPIVDAVLEGFNGTIFAYGQTGTGKTYTMEGDISSEEDKGITLHAF
DHIFAYIS--SVK--DREFLVRASYLQIYMENVFDLLGD---PSKKLH---VRNI-DNDVAVV-
GLSTHIVKSPQEIMDVLVAGRKNRVVAATSMNSGSSRSHSVFSIIIEQH------------------
SEDRGTR-MGKLHLVDLAGSERLSKT-----------------------
EASGLTAKQGAKINQSLLELGNVISALVTNKT-
HISYRNSKLTQILQDSLGGNSKTCMCATIGPSSYSYEETNSTLLYATRARDI-------
>XP_001315568_(Tv_Kinesin-2)
-ENIKVVVRCRPISKKEVDKGFKPIVKIDNTNNMVALTHGDD---DPDP-------
KSFTFNSAYAWDCTQQDIYDDAGRPIVQAVLDGYNGTILAYGQTGTGKTYTMEGVVDNEEHKGVILHAF
DHIFAHIA--KVK--DREFLVRASFLQIYMEDVFDLLGD---PKKKLH---VRSL-ENDICVV-
GLSSHIVKTPQEITELLMRGKDNRAVAATAMNAQSSRSHSVFTVVIEQS------------------
GEECGTK-MGKLHLVDLAGSERLSKT-----------------------
EATGQQAKEGAKINQSLLSLGNVISALVAG-
AKHIAYRDSKLTQLLQDSLGGNAKTVMIATLGPASYNYDETLSTLLYATRARQI-------
>Tb11.01.5490_(Tb_Kinesin-2D)
-ENIRVVIRCRNLLGFETERGDKALVRLDLATNQVIVQHPIG-----DA-------
DVFAFDAVYNNTYTQRDLFLQEVQPLVEAVLQGYNATVFAYGQSGSGKTHTMTGKLNDQEMWGMMPQVV
NHLFNEIK--KLTSATRTYKVKVSYIELYNGKSRDLLSA---KQGNLE---IKQNMAKNFYVK-
GAEMPEVTNFGEALRWFNAGTDRRQTASTDLNDNSSRSHSLFTLQVEQF----DFEQ-----------
DPSAPIVLTSKINLVDLAGSEKLSKT-----------------------
NATGETAKEGCNINLSLSALATVIDTIVKG-
GKHIPYRGSPLTMLLKDSLGGNAKTVMFANIGPSDKNISETISTLRFALRAK---------
>Tb927.5.2090_(Tb_Kinesin-2D)
-ENIKVLVRCRPLNDKEKSQGYKTSVDLDLTENTVTVQSVVG-----EP-------
DRWTFDAVINNTFTQKDVFQQFIMPLVDSVLDGFNATVFAYGQSGSGKTHTMTGKLGDEDLKGLTPRSF
EHVFDRISSMKATEPNKQFSLYVSFIELYNGKVHDLLAR---QQVPLA---LKENKDKSFFVQ-
GAHIPQVKCIDDIFHQMEEGTERRRVAATELNADSSRSHSVFTLIIECT----EVSE-----------
```

```
DGDSRSV-TSKLNLVDLAGSERQSKT-----------------------
GALGDTLKEGCNINLSLSALGTVIDTIVKGKG-
HVPFRSSPLTMILKDSLGGSSKTVMFANINPSEHNVSETISTLRFADRAKQI-------
>Hs_KIF5B_(Hs_Kinesin-1)
ECNIKVMCRFRPLNESEVNRGDKYIAKFQGEDTVVIASKP-----------------
YAFDRVFQSSTSQEQVYNDCAKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPEGMGIIPRIVQD
IFNYIY--SMDE-NLEFHIKVSYFEIYLDKIRDLLDV---SKTNLS---VHEDKNRVPYVK-
GCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNEHSSRSHSIFLINVKQE-----------------
NTQTEQKLSGKLYLVDLAGSEKVSKT-----------------------
GAEGAVLDEAKNINKSLSALGNVISALAEG-
STYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTI-------
```

Appendix I-4

MSA of kinesin-9 motor domain sequences used to a build phylogenetic tree.

```
>Mv_c21605_g1_i1_(Mv_Kinesin-2)
-ERVQVVVRCRPISERELTAGHKCCISIDTERRTVEVHNVGGRFAADNI------
PKSFTFDRVYNEKSTQRQIYKDVAYSIVHSVMCGYNGTVLAYGQTASGKTYTMEGCDHSPDLWGIIPNA
FEHIFQHIR--QSQS-SDSFLVRISYLEIYNEEIRDLLSPA--TSKKLE---LKESVETGVYVK-
NLTSLTVNSFADIRQLLMLGKKNRAIGATAMNQDSSRSHSIFTITVESS----TCDP----------
TGGKTHVR-VGKLNLVDLAGSERLSKT-----------------------
GATGERFKEMTKINWSLSALGNVISALVDGKSSHIPYRDSKLTRLLQDSLGGNTRTVMVANIGPADYNY
EESISTLRYANRAKNI-------
>XP_396164_(Am_Kinesin-2A)
-----------PXNGKELD-GHCKTXYVWIHKXXITVENPNA--AQGEP------
PKVFSFDAVFDTDSTQVDIYNETARPIVDKVLQGYNGTIFAYGQTGTGKTYTMSGAKTSPQLRGIIPNT
FAHIFGHIA--KAHD-NQKFLVRATYLEIYNEEVRDLLGKD--QNTRLE---VKERPDIGVFVK-
DLSGYVVNNADDLDRIMSLGNKNRVVGATAMNVSSSRSHAIFTITVESS----QLGE-----------
DGEQHVK-MGKLHLVDLAGSERQSKT-----------------------
KASGVRLREATKINLSLSTLGNVISALVDGQSSHVPYRNSKLTRLLQDSLGGNSKTLMCANVSPADINY
DETISTLRYANRAKNI-------
>XP_393174_(Am_Kinesin-2B)
--------RCRPMDEKELARGYMRVVDVFPSRGVVEIRHPRDDPSSDNV-------
KVFTFDAVYDWNSSQQDLYEETVRPLVSSVLDGFNGTIFAYGQTGTGKTYTMEGLKTDHERRGVIPRSF
EHIFNHIG--RSE--NMQYLVRASYLEIYQEEIRDLLQPD--QSLRFE---LKEKPDIGVFVK-
DLSTSVCKSAAEIQQLMNTGNQNRTIGATNMNEHSSRSHAIFLITIEMG----SIGD-----------
TGG--IR-VGRLNLVDLAGSERQSKT-----------------------
GSSGERLKEASKINLSLSALGNVISALVDGKTTHVPYRDSKLTRLLQDSLGGNSKTIMVANIGPASYNY
EETLTTLRYANRAKNI-------
>XP_395281_(Am_Kinesin-2C)
--SVKVAVRCRPMNARELQQGCRNVVTIDPPSKCCTLECPAT--GTGNG-------
KVYQFDAAFNPEATTESVYDNVGSVIVEAVLDGYNGTVFAYGQTGCGKSHTM---------
RGFIERTLDHIFEATS--TASA-EMRYLALLSYLEIYNERLRDLLQDG--MSNMLT---
LKEDPNRGTYVAGGLKEVTVKDAAECARLVEQGDRRRAAAATKMNAASSRSHAVLTLSLETL----
AINE--------EDSKAENTVK-RGRLHLVDLAGSERQART-----------------------
GATGDRLKEAASINLSLSALGNVISALAAGHGRHVPYRDSKLTRLLRDSLGGNARTLMIACISPSDVDA
EETLSTLRYAARARCIKNKPVIN
>Ce_KLP-11_(Ce_Kinesin-2B)
-ETVKVIVRCRPLSSQEIANNYSKIVHMRPQRGQIELKNPKE---QDEP------
SKDFTFDAIYDENSTQSDLYEETFRDLVDSVLNGYNATIFAYGQTGTGKTHTMEGKSSDPEQRGVIYKC
IDHIFEHMA--ASH--NQEYLVRASYLEIYQEELRDLLEAE--SNKKLE---IKERPDGGVYVK-
DLTSKLTRTVGEIHEVMIRGNGHRSVGRTNMNEHSSRSHAIFIITVECS----RIGE-----------
DGESHIT-VGRLNLVDLAGSERQSKT-----------------------
GATGERFKEATKINLSLSALGNVISALVDAKSAHIPYRDSKLTRLLQDSLGGNSKT------------
-ETLGTLRYANRAKNI-------
>Ce_KLP-20_(Ce_Kinesin-2A)
-EKVKVVVRCRPISTTEKLQGHKIAVTCNDEEKAVNIKSLSQ----EDP------
PRTFYFDAVFSPNTDQMTVYNVAARPIVENVLKGYNGTIFAYGQTGTGKTFTMAGDLEPVEMRGIIPNS
FAHIFDHIA--KCQH-DTTFLVRVSYLEIYNEEIRDLLSKD--HNGNLE---IKERPDVGVYVR-
NLSNPTVENASKMQALMEFGSKNRKVGATAMNLESSRSHAMFTVTIESC------------------
RNGLVT-QGKLQLVDLAGSERQSKT-----------------------
GAQGERLKEAAKINLSLSTLGNVISSLVDGKSTHIPYRNSKLTRLLQDSLGGNSKTVMIANVGPATYNY
DETLSTLRYANRAKNI-------
>Ce_OSM-3_(Ce_Kinesin-2C)
-ESVRVAVRCRPFNQREKDLNTTLCVGMTPNVGQVNLNAPDG-----AA-------
KDFTFDGAYFMDSTGEQIYNDIVFPLVENVIEGYNGTVFAYGQTGSGKTFSMQGIETIPAQRGVIPRAF
DHIFTATA--TTE--NVKFLVHCSYLEIYNEEVRDLLGAD--NKQKLE---IKEQPDRGVYVA-
GLSMHVCHDVPACKELMTRGFNNRHVGATLMNKDSSRSHSIFTVYVEGM------------------
TETGSIR-MGKLNLVDLAGSERQSKT-----------------------
GATGDRLKEATKINLSLSALGNVISALVDGKSKHIPYRDSKLTRLLQDSLGGNTKTIMIACVSPSSDNY
DETLSTLRYANRAKNI-------
>Cr_FLA10_(Cr_Kinesin-2)
```

```
-ESVKVVVRCRPLNGKEKADGRSRIVDMDVDAGQVKVRNPKA--DASEP------
PKAFTFDQVYDWNCQQRDVFDITARPLIDSCIEGYNGTIFAYGQTGTGKSHTMEGKDEPPELRGLIPNT
FRYVFEIIA--RDSG-TKEFLVRSSYLEIYNEEVRDLLGKD--HSKKME---LKESPDRGVYVK-
DLSQFVCKNYEEMNKVLLAGKDNRQVGATLMNQDSSRSHSIFTITIECI----
EKLESAAAQKPGAKKDDSNHVR-VGKLNLVDLAGSERQDKT-----------------------
GATGDRLKEGIKINLSLTALGNVISALVDGKSGHIPYRDSKLTRLLQDSLGGNTKTVMVANIGPADWNY
DETMSTLRYANRAKNI-------
>Cr_FLA8_(Cr_Kinesin-2)
-ECVKVAVRCRPLNGKEKGDNRATIVEVDNKTGQVTLNNPKG----DEP------
PKTFTFDNAFDWNVTQRDVYDVVARPIVNSVMDGYNGTIFAYGQTGTGKTHTMEGFPT-
PELQGIIPNCFDHVFETVN--SST--GKQWMVRASYLEIYNEEVRDLLSKD--PKNKLE---
LKEHKDSGVYVK-GLNAFVVKGVPELKNVLEVGKKNRSVGATLMNQDSSRSHSIFTITIETI----
EQTQ----------AQPEGHIR-VGKLNLVDLAGSERQSKT-----------------------
GATGDRLKEATKINLSLSALGNVISALVDGKSGHVPYRDSKLTRLLQDSLGGNTKTIMCANMGPADWNY
DETLSTLRYANRAKNI-------
>Dm_KIF3C_(Dm_Kinesin-2C)
-ENIKVVVRCRPMNQTEKERNCQNIVEINEF--AVSVTNPSA--RISQQ-------
KKFIFDSVYNMKTDTEVIYDEMCYSLVESTIEGYNGTIFAYGQTGCGKTHTMQGDENFSNSTGIIPKCF
DHIFERIS--MTT--NVRYLALVTYLEIYNERIRDLLNKN--ENTNVINHFLKELPGIGVSVP-
TLTTQPVVNANQCYDWLHFGNKNRVTAATLMNKNSSRSHTIFTITLEQSPFLNSIGS-----------
DAFGGIC-RGKLSLVDLAGSERQRKT-----------------------
GAQGDRLKEASQINLSLSALGNVISSLVDGKAKHVPFRDSKLTRLLQDSLGGNTKTLMISCISPTDIHY
DETISTLRYASRAKNI-------
>Dm_KLP64D_(Dm_Kinesin-2A)
-ENVRVVVRTRPMDKNELSAGALSAISVDKINRAITVMKPNA--TANEP------
PKTYYFDNVFDGGSNQMDLYVDTARPIVDKVLEGYNGTILAYGQTGTGKTYTMSGNPDSPQTKGIIPNA
FAHIFGHIA--KAKE-NQKFLVRVSYMEIYNEEVRDLLGKD--VGKSLE---VKERPDIGVFVK-
DLSGYVVHNADDLENIMRLGNKNRAVGATKMNQESSRSHAIFSITVERS----ELGE-----------
GDVQHVR-MGKLQLVDLAGSERQSKT-----------------------
QASGQRLKEATKINLSLSVLGNVISALVDGKSTHIPYRNSKLTRLLQDSLGGNSKTVMCATISPADSNY
METISTLRYASRAKNI-------
>Dm_KLP68D_(Dm_Kinesin-2B)
-ECVQVVVRCRPMSNRERSERSPEVVNVYPNRGVVELQNVVD--GNKEQ------
RKVFTYDAAYDASATQTTLYHEVVFPLVSSVLEGFNGCIFAYGQTGTGKTFTMEGVRGNDELMGIIPRT
FEQIWLHIN--RTE--NFQFLVDVSYLEIYMEELRDLLKP---NSKHLE---VRER-GSGVYVP-
NLHAINCKSVEDMIKVMQVGNKNRTVGFTNMNEHSSRSHAIFMIKIEMC------DT-----------
ETNXTIK-VGKLNLIDLAGSERQSKT-----------------------
GASAERLKEASKINLALSSLGNVISALAES-
SPHVPYRDSKLTRLLQDSLGGNSKTIMIANIGPSNYNYNETLTTLRYASRAKSI-------
>Gg_KIF3B_(Gg_Kinesin-2B)
-ESVRVVVRCRPMNSKEKSASYEKVVNVDVKLGQVSVKNPRG--TSHEL------
PKTFTFDAVYDWNSKQVELYDETFRPLVDSVLQGFNGTIFAYGQTGTGKTYTMEGVRGDPEKRGVIPNS
FDHIFTHIS--RSQ--NQQYLVRASYLEIYQEEIRDLLSKD--QSKRLE---LKERPDTGVYVK-
DLSSFVTKSVKEIEHVMNVGNQNRSVGATNMNEHSSRSHAIFVITIECS----ELGL-----------
DGENHIR-VGKLNLVDLAGSERQAKT-----------------------
GAQGERLKEATKINLSLSALGNVISALVDGKSTHIPYRDSKLTRLLQDSLGGNAKTVMVANIGPASYNV
EETLTTLRYANRAKNI-------
>XP_001708236_(Gl_Kinesin-2D)
-
DNVMVMVRVRPFNKREEQEGATEIIEMDKTLCTVTLHKPVEKGAGSATSECLPSKKVFTYDAVYPSNST
QVEVFDESVREMIDGCLEGYNATVFAYGQTGSGKTHTMMGQKDNP---GMIPLAFQRIFDFIA--QAK-
-DDQFLVRASFVEIYNEDLKDLLT----GATHLQ---LKEDPVKGVFIK-
DLSEHPVSDERHIDKLIQKGNESRAVAATLMNATSSRSHSIFQVVLERM-----TVI-----------
DGRECIR-VGKLNLVDLAGSERQEKT-----------------------
GATGDRLKEAAKINLSLTTLGCVISKLVEG-
SKHIPYRDSKLTRLLQDSLGGNSKTLMVVAVSPASTNYDETMSTLRYADRAKQI-------
>XP_001706504_(Gl_Kinesin-2D)
-DNIKVIVRCRPLNARETRENALNIIRMDEASAQVIVDPPEQEKSATQAKKV---
PRTFTFDAVYDQTSCNYGIFQASFKPLIDAVLEGFNSTIFAYGQTGAGKTWTMGGNKEEP---
GAIPNSFKHLFDAIN--SSSS-NQNFLVIGSYLELYNEEIRDLIK----NNTKLP---
LKEDKTRGIYVD-GLSMHRVTTAAELSALMDKGFANRHVAATQMNDTSSRSHSIFMVRIECS----
EVIE------------NKEVIR-VGKLNLVDLAGSERQSKT-----------------------
GATGETLVEGAKINLSLSALGLVISKLVEG-
ATHIPYRDSKLTRLLQDSLGGNSKTLMCANISPASTNYDETMSTLRYADRAKQI-------
```

```
>Hs_KIF17_(Hs_Kinesin-2C)
-EAVKVVVRCRPMNQRERELRCQPVVTVDCARAQCCIQNPGA---ADEP------
PKQFTFDGAYHVDHVTEQIYNEIAYPLVEGVTEGYNGTIFAYGQTGSGKSFTMQGLPDPPSQRGIIPRA
FEHVFESVQ--CAE--NTKFLVRASYLEIYNEDVRDLLGAD--TKQKLE---LKEHPEKGVYVK-
GLSMHTVHSVAQCEHIMETGWKNRSVGYTLMNKDSSRSHSIFTISIEMS----AVDE----------
RGKDHLR-AGKLNLVDLAGSERQSKT----------------------
GATGERLKEATKINLSLSALGNVISALVDGRCKHVPYRDSKLTRLLQDSLGGNTKTLMVACLSPADNNY
DETLSTLRYANRAKNI-------
>Hs_KIF3A_(Hs_Kinesin-2A)
-DNVKVVVRCRPLNEREKSMCYKQAVSVDEMRGTITVHKTDS---SNEP------
PKTFTFDTVFGPESKQLDVYNLTARPIIDSVLEGYNGTIFAYGQTGTGKTFTMEGVRAIPELRGIIPNS
FAHIFGHIA--KAEG-DTRFLVRVSYLEIYNEEVRDLLGKD--QTQRLE---VKERPDVGVYIK-
DLSAYVVNNADDMDRIMTLGHKNRSVGATNMNEHSSRSHAIFTITIECS----EKGI----------
DGNMHVR-MGKLHLVDLAGSERQAKT----------------------
GATGQRLKEATKINLSLSTLGNVISALVDGKSTHVPYRNSKLTRLLQDSLGGNSKTMMCANIGPADYNY
DETISTLRYANRAKNI-------
>Hs_KIF3B_(Hs_Kinesin-2B)
-ESVRVVVRCRPMNGKEKAASYDKVVDVDVKLGQVSVKNPKG--TAHEM------
PKTFTFDAVYDWNAKQFELYDETFRPLVDSVLQGFNGTIFAYGQTGTGKTYTMEGIRGDPEKRGVIPNS
FDHIFTHIS--RSQ--NQQYLVRASYLEIYQEEIRDLLSKD--QTKRLE---LKERPDTGVYVK-
DLSSFVTKSVKEIEHVMNVGNQNRSVGATNMNEHSSRSHAIFVITIECS----EVGL----------
DGENHIR-VGKLNLVDLAGSERQAKT----------------------
GAQGERLKEATKINLSLSALGNVISALVDGKSTHIPYRDSKLTRLLQDSLGGNAKTVMVANVGPASYNV
EETLTTLRYANRAKNI-------
>Hs_KIF3C_(Hs_Kinesin-2B)
-EALKVVARCRPLSRKEEAAGHEQILTMDVKLGQVTLRNPRA--APGEL------
PKTFTFDAVYDASSKQADLYDETVRPLIDSVLQGFNGTVFAYGQTGTGKTYTMQGTWVEPELRGVIPNA
FEHIFTHIS--RSQ--NQQYLVRASYLEIYQEEIRDLLSKE--PGKRLE---LKENPETGVYIK-
DLSSFVTKNVKEIEHVMNLGNQTRAVGSTHMNEVSSRSHAIFIITVECS----ERGS----------
DGQDHIR-
VGKLNLVDLAGSERQNKAGPNTAGGAATPSSGGGGGGGSGGGAGGERPKEASKINLSLSALGNVIAAL
AGNRSTHIPYRDSKLTRLLQDSLGGNAKTIMVATLGPASHSYDESLSTLRFANRAKNI-------
>XP_001682337_(Lm_Kinesin-2D)
--NIKVLVRCRPFSEKENAMGHKSCVDLDMVQNTVTVKSIIG-----EP-------
DRWTFDAVINNSFSQEDIFTQFIMPLTESVLGGFNATVFAYGQSGSGKTHTMTGVMGNSTLEGVIPRCV
KHIFDSVQKMRDEAPSTTVSMYVSFMELYNGKVRDLLAK---QQVSLD---IRENKDHTFFVK-
GAVVAQVKFPEDVIRHLEEGTDRRRVASTELNADSSRSHSVFSLILECT----ETLE----------
DGSTRAV-SSKLNLVDLAGSERQGKT----------------------
GASGDTLKEGCNINLSLSALGTVIDTIVKG-
GTHVPFRSSPLTMLLKDSLGGNSKTVMFANINPSERNVSETVSTLRFADRAK---------
>XP_001685383_(Lm_Kinesin-2D)
--NIRVVIRCRDILPYEAERGDKALVRLDLATNQVVVQHPIG-----DA-------
DVFAFDAVYNNSFTQRDIFLQEVQPLADAVLQGYNATVFAYGQSGSGKTHTMTGKLSQRNMWGMMPQVV
DYLFSEIK--KLTSSTKTFKVKVSYVELYNGKSRDLLSS---KQVNLE---IKQNTSKNFYVK-
GAEMPEVTSFEDAIKWFNAGTERRQTASTDLNDTSSRSHSLFTVQIEHF----DFEN----------
DPSSPIVMTSKINVVDLAGSEKLSKT----------------------
NATGETAKEGCNINLSLSALATVIDTIVKG-
AKHIPYRGSPLTMLLKDSLGGNAKTVMFANVGPSDKNLSETISTLRFALRAK---------
>XP_001455773_(Pt_Kinesin-2)
--CVRVVIRCRPLNDTEKKDGHVCIVNMDTKNGQVTVRNPKV---ADEV------
PKQFTFDQIFDTQSLQENVYNQTAHPIVESVLEGYNGTIFAYGQTGTGKTHTMEGKDDPPTLRGIIPRT
FDHIFQRIE--NMAK-NKQFLVKVSFLELYNEEIRDLLSKN--IKNKLE---IRENPETGIYIK-
DLSKFMIENPQEMREKLLHGRENRAVGATAMNQDSSRSHSLFQITVETN----EIVQ----------
GQSHVT-VGKLNLVDLAGSERQSKT----------------------
HATGDRLKEAININQSLTTLGNVISALVDNKSQHIPYRDSKLTRLLQDSLGGNTKTVMIANIGPADYNF
DETLSTLRYANRAKQI-------
>XP_001426973_(Pt_Kinesin-2)
-ECVKVIVRMRPFNQREKENGSKPCVIVYEDTNTVELRNTQD----NDV-------
KSYTYDYVFGAETPQLSIYQKTAFNLVESVADGYNGTIFAYGQTGCGKTFTMIGDPSNETMKGIIPRTF
DQIISIIN--NNSDTNKKFLLRCSYIEIYNEEIHDLLSKD--VKQKYE---LKEG-QQGVFIK-
DLNIAVVRTTQEMDRYMQLGTQNRSVGATAMNKESSRSHCIFTVYIECS----VTDP----------
KGNERIT-AGKLNLVDLAGSERQSKT----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNF
DETLSSLRYASRAKMI-------
```

```
>XP_001427404_(Pt_Kinesin-2)
-ECVKVIVRVRPFNQKERDNGSKPCVNVYESTNSVELFRSQD----NDK-------
KQFTYDYVFGPETPQIQIYQQTAFNLVESVAEGYNGTIFAYGQTGCGKTFTMIGDPLNDNMKGIIPRTF
EQIISIIN--NNSDSNKKFLLRCSYIEIYNEEIHDLLSKD--VKQRYE---LKEG-QQGLYVK-
DLNIPIVKTLQDMDKYMALGAQNRSVGATAMNKESSRSHCIFTVYMECS----MTDD-----------
KGNERII-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNY
DETLSSLRYASRAKMI-------
>XP_00142818_(Pt_Kinesin-2)
-ECVKVIVRMRPFNSREKENGSKPCVTVHEDTNSVELRSSQD----NEV-------
KNFSYDYVFGAETPQLQIYQKTAFNLVESVADGYNGTIFAYGQTGCGKTFTMIGDPTNENMKGIIPRTF
DQIISIIN--NNSDSNKKFLLRCSYIEIYNEEIHDLLSKD--AKQKYE---LKEG-QQGVFIK-
DLNIAVVRTTQEMDKYMQLGTQNRSVGATAMNKESSRSHCIFTVYIECS----ITDS-----------
KGNERIT-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNY
DETLSSLRYASRAKMI-------
>XP_001428184_(Pt_Kinesin-2)
-ECVKVIVRMRPFNSREKENGSKPCVTVHEDTNSVELRSSQD----NEV-------
KNFSYDYVFGAETPQLQIYQKTAFNLVESVADGYNGTIFAYGQTGCGKTFTMIGDPTNENMKGIIPRTF
DQIISIIN--NNSDSNKKFLLRCSYIEIYNEEIHDLLSKD--AKQKYE---LKEG-QQGVFIK-
DLNIAVVRTTQEMDKYMQLGTQNRSVGATAMNKESSRSHCIFTVYIECS----ITDS-----------
KGNERIT-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTIMITAISPSDFNY
DETLSSLRYASRAKMI-------
>XP_001429325_(Pt_Kinesin-2)
-ECVKVVVRVRPFNQKEKENNSKPCVNVDEKQNVVELLKLTD----NET-------
KQFSYDYVFGMNAKQSYIYEKTAFNLVESVIDGYNGTIFAYGQTGCGKTFTMTGVPENEELKGIIPRTF
TQIQTIID--TNTDTKKKFLVRCSFLEIYNEEIRDLLGKD--HKARLE---LKES-QGSVSVK-
DLTMVTVKTAQDMDKYMTLGQSNRSVGATAMNAQSSRSHCIFTVYVESQ----IVDA-----------
KGSEFIR-VGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEATKINLSLSALGNVISALVDGKTQHIPYRDSKLTRLLQDSLGGNTKTVMITALSPADYNY
DETLSSLRYASRAKMI-------
>XP_001429366_(Pt_Kinesin-2)
KECVKVVVRARPLSSKEIEEGRKRIVDVDTSRKEINIQNIKG--DNNEA------
QRTFVFDEVFDLNSQQEQVYNNTALPIVESVMDGYNGTVFAYGQTGTGKTHTMEGKNDPPHERGITPRT
FDHIIKVIE--GTP--NIQFLVRCSYLELYNEEVRDLLSPN--HLTKLE---LREKPEQGIFVK-
DLSKIVVKSVAELNEWLKAGRANRKVGETKMNQESSRSHSIFTLTIESS----EIGA-----------
DQQQHIK-SGKLNLVDLAGSERQSKT-----------------------
QAVGVRFEEAININLSLTTLGNVITTLVDGKSQHIPYRDSKLTRLLQDSLGGNTKTVMVANIGPADYNF
DETMSTLRYANRAKKI-------
>Phypa_425592_(Pp_Kinesin-2)
-ERVQVVVRCRPMLVKENAEGRNNCVLVDTVGSTIQVKNLKQ--PEQEP-------
KLFTFDKTYDATSTQKQLYDDVAHPIVHSVMCGYNGTVLAYGQTASGKTFTMDGLDDPPEMRGIIPQAF
EGIFTHIQ--DSQS-SDNFLVRASYLEIHNEEIRDLLATGSQSSSRLE---LKENVEGNVYVK-
NLTSITVQSVADISHLLTVGKKSRSVGATLMNQDSSRSHSIFTITVEAS----ARSS-------
SAETDGSMHIR-VGKLNLVDLAGSERLNKT-----------------------
GATGDRFRELTNINWSLSALGNVISALVDDKSSHVPYRDSKLTRLLQDSLGGNTRTVMIANIGPADYNY
DESVSTLRYANRAKSI-------
>Sp_KRP85_(Sp_Kinesin-2A)
-DNVRVVVRCRPLNSKETGQGFKSVVKMDEMRGTVQVTNPNA--PSGEP------
PKSFTFDTVFAPGAKQTDVYNQTARPIVDAIIEGYNGTIFAYGQTGTGKTFTMEGVRSQPELRGIIPNS
FAHIFGHIA--KEQE-NVRFLVRVSYLEIYNEEVKDLLGKD--QQHRLE---VKERPDVGVYVK-
DLSAFVVNNADDMDRIMTLGNKNRSVGATNMNESSSRSHAIFTITLERS----DMGL-----------
DKEQHVR-VGKLHMVDLAGSERQTKT-----------------------
GATGQRLKEATKINLSLSTLGNVISSLVDGKSTHIPYRNSKLTRLLQDSLGGNAKTVMCANIGPAEYNY
DETISTLRYANRAKNI-------
>Sp_KRP95_(Sp_Kinesin-2B)
-ETVKVVVRCRPMNSKEISQGHKRIVEMDNKRGLVEVTNPKG--PPGEP------
NKSFTFDTVYDWNSKQIDLYDETFRSLVESVLQGFNGTIFAYGQTGTGKTFTMEGVRSNPELRGVIPNS
FEHIFTHIA--RTQ--NQQFLVRASYLEIYQEEIRDLLAKD--QKKRLD---LKERPDTGVYVK-
DLSSFVTKSVKEIEHVMTVGNNNRSVGSTNMNEHSSRSHAIFIITIECS----ELGV-----------
DGENHIR-VGKLNLVDLAGSERQAKT-----------------------
GATGDRLKEATKINLSLSALGNVISALVDGKSSHIPYRDSKLTRLLQDSLGGNAKTVMVANMGPASYNF
DETITTLRYANRAKNI-------
```

```
>XP_001014287_(Tt_Kinesin-2)
-ECVKVMVRVRPMNDKERQNNSKECVEVDTKLNQIVLRKPNE---AGSE-------
KVFTYDAVFYQKVQQQLVYEASAFPLVESVFEGYNGTIFAYGQTGCGKTHTMMGDPSKEEEKGIIPRTF
SHIINLIE--TTS--SKEFLVRVSFLEIYNEEIHDLLSKD--PKAKFE---LKQSPEKGVFVK-
DLNQIVVKSVKEMENLMYKGNENRSVGATAMNKDSSRSHSIFTIYIETS----EIDS-----------
TGNQHFR-AGKLNLVDLAGSERQSKT-----------------------
QATGDRLKEANKINLSLSALGNVISALVDGRTHHIPYRDSKLTRLLEDSLGGNTKTIMIAAISPADYSY
DETLGTLRYASRAKNI-------
>XP_001276971_(Tv_Kinesin-2)
--SVKVAVRVRPMNSKEKNEKYSNIVRADKRNSSIYVKNPQG----SEL--------
QFTYDFVYPENTTQEEIYENTAAPIVAGVLEGFNGTIFAYGQTGTGKTYTMDGIADDKERRGIVPRAFE
HIFDFAT--ANAD-THKIVISVTYVELYNEEIRDLLISSKEKPVPLK---IHEDPQKGFIIN-
GVKSRPANSFEELCKIQRVGFKRRMTRKTNMNDDSSRSHSVLTLTIETL-----TEI-----------
EGSQHVR-QGRLNLVDLAGSERIEKT-----------------------
KCDKDGVIEGISINYALMVLGNCISALTTKGTLHIPYRDSSLTKLLRDSLGGNARTAMIANIGPADFNF
NETVATLRYAERAKKIENKPSVN
>XP_001319907_(Tv_Kinesin-2)
--AVKVSVRLRPMSQKEIDSGFSKVVEIDQKNSTVRIKNPQG-----QY-------
IQFSFDFCFPEDVSQEEVYNATAMPIVNGVLEGYNGTIFAYGQTGTGKTFSMDGKPTG-
ELRGIMPRAFDHIFEYIQ--ANSA-DTEFLVTVTYVEIYNNELRDLLSEK--SNEKLK---
IREDPTHGVQIK-GVAVHKVKDVEEIHALLNYGKKNRVVRKTQMNSESSRSHSIFTVTVETL-----
KQI-----------DGQDHVR-SARLNLVDLAGSERVAKT-----------------------
GAEGVGFTEGVNINYELMILGNCIAALTSKGNTHIPYRDSKLTMLLRDSLGGNARTMMIAALGPADYNF
SETMSTLRYAERAKKIENKPTVN
>XP_001579747_(Tv_Kinesin-2)
--AVKVSVRLRPMSEKEINAGFKKIVEIDKKTATVKIQNPQN-----QT-------
ITFTFDYGFPEDCTQEEVYEATAAPIVSGVLEGFNGTIFAYGQTGTGKTYSMDGKTHG-
EHRGIMPRAFDHIFEYIQ--ANQD-SHEFLVTVTYVEIYNNELRDLLAEN--HEQPLK---
IREDAQKNVYIK-GVCTHKVKSVDELHALLAYGKKNRVVRKTNMNSESSRSHSILSLVIETL-----
TKI-----------DGQDHVR-SARLNMVDLAGSERAAKT-----------------------
GAEGVGFTEGVNINYELMILGNCIAALT-
SKGSHIPYRDSKLTMLLKDSLGGNARTMMIAALGPADYNFSETMSTLRYAERAKKIENKPKVN
>XP_001300992_(Tv_Kinesin-2)
-ENIKVVVRCRPLSKGEQEKGYFSIVKVLPSAGQVQLYRNQE---DNNP-------
KTFQFNSAYPPDVTQQFIYDDCARPIVDAVLEGFNGTIFAYGQTGTGKTYTMEGDISSEEDKGITLHAF
DHIFAYIS--SVK--DREFLVRASYLQIYMENVFDLLGD---PSKKLH---VRNI-DNDVAVV-
GLSTHIVKSPQEIMDVLVAGRKNRVVAATSMNSGSSRSHSVFSIIIEQH-----------------
SEDRGTR-MGKLHLVDLAGSERLSKT-----------------------
EASGLTAKQGAKINQSLLELGNVISALVTNKT-
HISYRNSKLTQILQDSLGGNSKTCMCATIGPSSYSYEETNSTLLYATRARDI-------
>XP_001315568_(Tv_Kinesin-2)
-ENIKVVVRCRPISKKEVDKGFKPIVKIDNTNNMVALTHGDD---DPDP-------
KSFTFNSAYAWDCTQQDIYDDAGRPIVQAVLDGYNGTILAYGQTGTGKTYTMEGVVDNEEHKGVILHAF
DHIFAHIA--KVK--DREFLVRASFLQIYMEDVFDLLGD---PKKKLH---VRSL-ENDICVV-
GLSSHIVKTPQEITELLMRGKDNRAVAATAMNAQSSRSHSVFTVVIEQS-----------------
GEECGTK-MGKLHLVDLAGSERLSKT-----------------------
EATGQQAKEGAKINQSLLSLGNVISALVAG-
AKHIAYRDSKLTQLLQDSLGGNAKTVMIATLGPASYNYDETLSTLLYATRARQI-------
>Tb11.01.5490_(Tb_Kinesin-2D)
-ENIRVVIRCRNLLGFETERGDKALVRLDLATNQVIVQHPIG-----DA-------
DVFAFDAVYNNTYTQRDLFLQEVQPLVEAVLQGYNATVFAYGQSGSGKTHTMTGKLNDQEMWGMMPQVV
NHLFNEIK--KLTSATRTYKVKVSYIELYNGKSRDLLSA---KQGNLE---IKQNMAKNFYVK-
GAEMPEVTNFGEALRWFNAGTDRRQTASTDLNDNSSRSHSLFTLQVEQF----DFEQ-----------
DPSAPIVLTSKINLVDLAGSEKLSKT-----------------------
NATGETAKEGCNINLSLSALATVIDTIVKG-
GKHIPYRGSPLTMLLKDSLGGNAKTVMFANIGPSDKNISETISTLRFALRAK---------
>Tb927.5.2090_(Tb_Kinesin-2D)
-ENIKVLVRCRPLNDKEKSQGYKTSVDLDLTENTVTVQSVVG-----EP-------
DRWTFDAVINNTFTQKDVFQQFIMPLVDSVLDGFNATVFAYGQSGSGKTHTMTGKLGDEDLKGLTPRSF
EHVFDRISSMKATEPNKQFSLYVSFIELYNGKVHDLLAR---QQVPLA---LKENKDKSFFVQ-
GAHIPQVKCIDDIFHQMEEGTERRRVAATELNADSSRSHSVFTLIIECT----EVSE-----------
DGDSRSV-TSKLNLVDLAGSERQSKT-----------------------
GALGDTLKEGCNINLSLSALGTVIDTIVKGKG-
HVPFRSSPLTMILKDSLGGSSKTVMFANINPSEHNVSETISTLRFADRAKQI-------
```

```
>Hs_KIF5B_(Hs_Kinesin-1)
ECNIKVMCRFRPLNESEVNRGDKYIAKFQGEDTVVIASKP-----------------
YAFDRVFQSSTSQEQVYNDCAKKIVKDVLEGYNGTIFAYGQTSSGKTHTMEGKLHDPEGMGIIPRIVQD
IFNYIY--SMDE-NLEFHIKVSYFEIYLDKIRDLLDV---SKTNLS---VHEDKNRVPYVK-
GCTERFVCSPDEVMDTIDEGKSNRHVAVTNMNEHSSRSHSIFLINVKQE------------------
NTQTEQKLSGKLYLVDLAGSEKVSKT-----------------------
GAEGAVLDEAKNINKSLSALGNVISALAEG-
STYVPYRDSKMTRILQDSLGGNCRTTIVICCSPSSYNESETKSTLLFGQRAKTI-------
```

Appendix I-5

Video 1: control spermatozoids emerging from the microspore. (MOV 4.03 MB)

Appendix I-6

Video 2: control spermatozoids exhibiting normal swimming behavior by spinning in place. (MOV 3.20 MB)

Appendix I-7

Video 3: MvKinesin-2 knockdowns emerging from the microspore. (MOV 5.04 MB)

Appendix I-8

Video 4: MvKinesin-2 knockdowns swimming in place exhibiting multiple coils with attached cilia on one cell body (Monster). (MOV 4.01 MB)

Appendix I-9

Video 5: MvKinesin-2 knockdowns swimming in place exhibiting long cilia (Rapunzel). (MOV 4.42 MB)

Appendix I-10

Video 6: MvKinesin-9A knockdowns swimming erratically. (MOV 4.09 MB)

Appendix I-11

Video 7: MvKinesin-9B knockdowns swimming in a similar manner to control spermatozoids. (MOV 4.95 MB)

Appendix I-12

Primers used for (A) RT-PCR and for (B) the production of dsRNA.

A

MvKinesin-4 Ic	**Sequence (5'->3')**
Forward primer	ATTCGAGAGGGAGGGTCATT
Reverse primer	AGGTGGCTTAGTGTTTGTTGTC
MvKinesin-12 II	**Sequence (5'->3')**
Forward primer	GGAAACCGCTCTTACTATCC
Reverse primer	GTTGAGCTTCCTCTTAGTGT
MvKinesin-13a	**Sequence (5'->3')**
Forward primer	TTTACCATCACCTGAACTTCG
Reverse primer	CTCATTGGACTGTTCTCATTGT
MvKinesin-13b	**Sequence (5'->3')**
Forward primer	CAACACCACCTCCTACAATCTC
Reverse primer	GAAACACTGTCCCTGACACTAA
MvKinesin-13c	**Sequence (5'->3')**
Forward primer	CACTCCCACAGTTCAGAAACC
Reverse primer	GTCCGTTGCCGTGTTGA
MvKinesin-14 VIa	**Sequence (5'->3')**
Forward primer	GAGGGGTACATTTCTTCCGT
Reverse primer	AGGTGGCTTAGTGTTTGTTGTC
MvARKc	**Sequence (5'->3')**
Forward primer	GCAATGCTTGTGGTTAATGT
Reverse primer	TTAGCCTCGTCTAGTGAAGA
MvARK-LIKE	**Sequence (5'->3')**
Forward primer	AACGTACACCAAGGAAAGAG

Reverse primer	AACTTTCTTCAACCCTCGAA
MvKinesinOrphan III	**Sequence (5'->3')**
Forward primer	AAATCTATTCCTTCGGCTCC
Reverse primer	AATTTGGGCTATCTGGAAGG
MvKinesin-2	**Sequence (5'->3')**
Forward primer	AAATCAAGAATCCGAGCCAA
Reverse primer	ATTTCAGTCCTTAGAGCAGC
MvKinesin-9A	**Sequence (5'->3')**
Forward primer	GTAGGAGTGGGAGATGAGAA
Reverse primer	AAGTTGCTCTTGTTCGTGTA
MvKinesin-9B	**Sequence (5'->3')**
Forward primer	TGTCCAGCAAATTAGAACGA
Reverse primer	CTAGGAGTAGGAGCCGATAA
MvCentrin	**Sequence (5'->3')**
Forward primer	AGGCCTGAGTGAGGAACAGAAACA
Reverse primer	TCCTTTGCATCAATGGTGCCTGAC

B

MvKinesin-4 Ic	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGATTCGAGAGGGAGGGTCATT
Reverse primer	TAATACGACTCACTATAGGGAGGTGGCTTAGTGTTTGTTGTC
MvKinesin-12 II	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGGGAAACCGCTCTTACTATCC
Reverse primer	TAATACGACTCACTATAGGGGTTGAGCTTCCTCTTAGTGT
MvKinesin-13a	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGTTTACCATCACCTGAACTTCG
Reverse primer	TAATACGACTCACTATAGGGCTCATTGGACTGTTCTCATTGT
MvKinesin-13b	**Sequence (5'->3')**

Forward primer	TAATACGACTCACTATAGGGCAACACCACCTCCTACAATCTC
Reverse primer	TAATACGACTCACTATAGGGGAAACACTGTCCCTGACACTAA
MvKinesin-13c	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGGTAGCCAAGGGACCACATAAG
Reverse primer	TAATACGACTCACTATAGGGATTCTCCTCTTGCCATTCGTC
MvKinesin-14 VIa	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGGAGGGGTACATTTCTTCCGT
Reverse primer	TAATACGACTCACTATAGGGAGGTGGCTTAGTGTTTGTTGTC
MvARKc	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGTTAGCCTCGTCTAGTGAAGA
Reverse primer	TAATACGACTCACTATAGGGTTAGCCTCGTCTAGTGAAGA
MvARK-LIKE	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGAACGTACACCAAGGAAAGAG
Reverse primer	TAATACGACTCACTATAGGGAACTTTCTTCAACCCTCGAA
MvKinesinOrphan III	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGAAATCTATTCCTTCGGCTCC
Reverse primer	TAATACGACTCACTATAGGGAATTTGGGCTATCTGGAAGG
MvKinesin-2	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGAAATCAAGAATCCGAGCCAA
Reverse primer	TAATACGACTCACTATAGGGATTTCAGTCCTTAGAGCAGC
MvKinesin-9A	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGGTAGGAGTGGGAGATGAGAA
Reverse primer	TAATACGACTCACTATAGGGAAGTTGCTCTTGTTCGTGTA
MvKinesin-9B	**Sequence (5'->3')**
Forward primer	TAATACGACTCACTATAGGGTGTCCAGCAAATTAGAACGA
Reverse primer	TAATACGACTCACTATAGGGCTAGGAGTAGGAGCCGATAA

Appendix I-13

MSA of dynein heavy chain sequences used to a build phylogenetic tree.

```
>KU666434_c27157_g1_i1
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------
MRPFNDKHNNQQQSRSVGPPVKIRWPKAFYRSQPKHQIYGNYTE------PEP------------
ALANTETNSDLSSQENSKPADACFNPKYTGS---YPPKPKC-----
IRLFKELKSVPRPKLMLGLSILEEDEDKKAQPERDMDILGKAKSSRRTTFAFPLDLFYNPELELCHPSL
DLKQAHANNEPGVTGRSRY--YSTR---GECSWEPCLVLDYNEEKGDFLIE-----------------
------------------------------------------------------------------
-------------------------------------------
WVKTKKQKTVKRLNLILDREKEINFIAKVLDAKKLRTIMQTDM----RYFEYLE-----
GLPFENAELSDTALKTLLIDMAGSGLSKRYPDLVEDYMIS----------------------------
----------------LKDDYCRSVKKLVL----NHKYFVSK-----------
ENVAKLKALQIYHCDMPKP-----------------------------------------------
----------------------PVPGQGCYPVGFAVKNAMAFLKVIISQNLC---------------
-------------LAEEAFLLGLLKFQKEMEITDICLFSPQLFTLPRPLKLED---------------
-FRLVQEDYLRAVADNFRN------DWIPRVVAMI--DEL---------
APPTNELKGTTLEKLRGFVRRLSFTMTQQLLDVAL--------
SSMEAYQGLWEQYSINYTDVNHDEEDGLH-------------------------------------
---------------------LTPMFRIRLKVEGD--HFAFDPSLGLI-
ESTVLLLLDNMLASIRGIEDLQSRVTTISTIYQFREMRTLEVDD-----------
PRFLLCRKTIEEILEMNFRLPRKLEESYMKFQE-----LAAIKIDEFLANWKNE-----------
KHSIEDYEEAITKYLDVADKAKKNSPAEVLYGLLLIECFSLGEELYLKSMDIANRLSAQLLDSTNMKNK
SVCEKYTEIDARL---------------------NTIIGNPEDLDELRKFVATTKKELIQLQEE----
----INQSAKVWDMLSRKNYTISDQ-----
EMDLYWVTFGWPQKIGFTMEECEAKLAQGKTKFTSELAMDQLNLKGEIKILNEEIHDFMKLG------
DISSFEERLAFVLEIET-------KLRKLTALGELYNNREQILGLLPTEYP---
QFDQMSKEFEQYGILWHVCGDFMHALPQWVDGPFNQI----------
NAEEVVSSVDKWFRSAMKCSKQTVGE---------AKLVAEE--LKKRIERFQINIP-
MISSLRNPGMRDR-HWKKLSD-RLGFEVSP-H-----AGFSLSTALQLN--
LLQFIGIIDEVSEFASKEYSVEVALDKMQGDWIGVKFEYAMWRDT---G---TAILRS--
LDDLQTMLEDHIAKTQSMQASPYIAP---FEDRVKLWLSKLNLVQEVLDEWINCQQQWLYLEPIF-
GSEDIMQQMPTEGRRFKV--
VDTSWRKIMEKLMKNPEVLVVGTDEDLLKNLKEANKQLDIVQKGLNEYLETKRLAFPRFYFLSNDELLE
ILAETKDPLRVQPFCKKIFEGIHRLQF-------QDNMDISAMFSEEG-EKVNFIKLF-NP----
KDAQGAVEKWLIQVEDAMRMSLKDVTLRAYEAYAVT-----------E-
RVQWVLSWPGQVVLCVSQMYWTAEVADSIQ------KGTLQSYQQKC-NQQLQAIV-NKVRGK-
LTSLERKTIGALIVIDVHARDVVKSLADAN--IADETDFEWISQLRYGY--EDGEV------------
--
IVRMINASQKYAFEYLGNSSRLVITPLTDRCYRTLMGALHLNLGGAPEGPAGTGKTETTKDLAKAIAMQ
CVVFNCSDGLDYLA--MGKFFKGLASSGAWACFDEFNRIDLEVLSVIAQQILTIQRAKAAG--------
-----------LKMFEF---EGVKLTLSITCSVFITMNP---
GYAGRSELPDNLKALFRTVAMMVPDYALISEITLYSFGYLQARELARKLVATYRLCSEQLSSQNHYDYG
MRA-VISVLRAAGAVKQKF-----------P------DEK------
ESILMLKSLKDVNLPKFLAHDIPLFEGILIDLFPGV--VLPAPDYTLMREAIIAVCGKRN---
LQPTPIFLEKIFQLYEMILVRHGLMLVGYSYGAKTEAYRVLAAALSDLCN-AGKME-------
NVTKCFIINPKSVYIGQLYGQFDPISHEWTDGVLAKSFRNAAVD--TT-
PDRKWIIFDGPVDAVWIENMNTVLDDNKKLCLMSGEIVQM-
TGSMNLIFEVQDLAVASPATVSRCGMVYTEPGQIGWVPHKAS---WMAKL---------PVRFPGVAE-
------------------------------QAGIIEALLDWLVEPCLQFV---------
KKYCKELVPTSSINLVQSLLNMFESLLDEFRPPNPEVKSTD---------------------------
---------------------------------DSVVVPTGKDLETWLHCLFAMSLVWSIGGVI---
-DADSRQKFDGFFRKLLAQDD-----NLGYHLSAGLRIQKPSFPFGLPFPV---TG---
SVFNFVFLKHET-------CWKEWMES--TDKRQPG----
NDAEFTDIIVSTVDTARYSYLLKLQLTHQKHVMLVGATGTGKTAYIKRELAENLDKSLYRNI-
```

```
MMTFSAQTN-ANVTQDIID--SKLDKRRKGV-FGPPFGTKCVI---LVDDLNMPALE-
VYGAQPPIEILRQWMDHGGWYDRTDN--TFRQIVDVQFVTAMGPPGG-
GRNPITPRYLRHFHVLAVADFDDASLSTIFGTIMVWWAR-KIHLP-TDVAP-
ISSALVNATIDIYREIQRELLPTPSKSHYTYNLRDLSKVFQGVCMMGGALD----------------
SKKSLVRLWCHECMRIFHDRLVDDNDRHWFFNYLGKMVEQR-L-SLQ-CDFVLDPPEDGR--
AFNLDDA-----FKNLAFGDIMD-----TNNIP---KK-------YEEI-LDQKKLLVFIEEGLAEFN-
-AQTRTP-
LSLVLFSYVAQHILRISRIISQPFGNALLVGVGGVGRQSLTKLATYMAGFNLFQIQITKIYGMTEWQED
LKYVLKKAGGE-GS--PTVFIFSDTQLKEERFLEDINNILNTGE--------
VPNLFAKDEIITLQDMVRA--RAKKAGK-
DSSPAEIYMYFVDQCRKNLHMVICMSPFGSSFRTRLRMFPSLVNCCTIDW--
FTEWPDDALYSVGSKFLSA-IAMEDANL----
LPSIRDMCVMFHQNARNLSAVFYQEQRRHFYVTPTSYLELLSTYKDLLAQKRLEIATQKKRYEVGLEKL
FDAEGQVNVMKKELEALQPVLMKTAEETDSLLKIINRDRKE-
AEKTRSVVEEEEAVANVKAKEAEAIKADCESELAVAMPMLEAALSALDTLTKSDITEVKAMKNPPAPVK
LVMEACCIMKGVKSRRIPDPNNPGKKID-DFWGPAQQMLTDT-NFLQSLKDF----
DKDHIPQDIIDKVR-PYLGKDN--
FDPEVVKKASKAAYGLCSWIRAMESYHKVAQVVAPKKAKLEAATAEFEALQAALSKKKAILKEVEEKLQ
KLQEQLDIMEKKKAALEQDVDNCQKKLSRAEKLIGGLGGEKVRWTNVGEELGKVYRNLTGDVLLASGYV
AYLGPVTLPYRERVMSE-WIKACVTKNIPCSDN--F-
KLATILGSPVAIRDWVIDGLPNDSFSIDNGIIISSA-RRWPLLIDPQGQANKWIKTKEKA--KN--
LQVTKLSD-PDFVRKLENCITFGYPLLLENVGE-ELDPTLEPVLLKNVFK-
QAGSLQIRLGDTTLEYSENFRFYITTKLRNPHYMPEVAVKVTLLNFMITPEGLEDQILGIAVKNERPDL
EEEKTQLVLQSAENQRQLSDIEDRIIKVLSSSE-
GNILENETAINIISTSKVLSIDIAEKQKVAEKTEAKIDEARKGYLPVATHVASLFFTVSTLCSIDPMYQ
YSLSWYLNLFQSATRDSE--------------KT--------------------------------
------------------------------
NLLPKRIEILNDYFTYSLFCQLSRSLFEKDKLLLSFLVCTDSM--LRQK-
KIAPEELRFLVAGAVIIERE-K---SKPPASWLAQKLWTEMILLSKMEA----------
FQGFSNHFAENPKKWKHIYDSPEPYKEALPDDW-STKL---
TSFQKLLIIRTIRPDLLVLSVNSFVFETMGQRYVEPPTFDLGKVYRDSS-
PTIPLVFVLSPGSDPMAALLKY-AEGLRQ-----
NVQSISLGQGQGPKAAALIQAGIQVGDWVVLQNCHLASSWMP-QLERICE-NFGSE--
NINASFRLWLTSYPSEN------FPVTILQNSIKMTNESPKGLRANMLQNYQSDPISDP-KFFE--
MTSEK---------------GPAWRKMLFGLCFFHASVQERVKFG---------
PLGWNIPYQFSDSDLKISMRQLRSFLEEFP--------------E----------------------
--------------------------------------
SIPFKALVYLTGECNYGGRVTDAHDRRTLMCLLSTIYNPKIL-------DDG---FRFSSSGVYTT-
PPDG--PYQDFIGHIRQFPLIAT-PDVFGLHENATITKDQQETDMFLSSILATQSNEAQAGGRSR----
---EEVILELVQDMASRIPPAF-DIDLARYRY---
PVQYIDSMNSVLHQEMTRYNNLTEVIRTSLQVLQKTLKGLVVMSAEIEKLSNSVFNGKIPAMWASKSFP
SLK---PLSSYVADLTQRLSMFST-W----------MDK-G--
PPPRFWITGFFFTHAFLTAVLQNYARKY---KIPIDTVVFEFQCL---
PKEGAYEHGPEDGVYVEGMFLEGARWDYDTMMLAESQPKVLFSAAPMIWLKPTEASKKKEGH-------
---CYRCPLYRTTERRGVLATTGHSSNFVTNV--ELPTDKAS--EHWVRRGVAMFL--SLSD
>KU666435_c27157_g2_i1
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------MDID------PPP-------------
DRTSRKRPATEPLRDEDDILDPSVMVPYVTK---VGELP-------
RKVAIERRKRLFASQGLVDLLAAEGVTEDFQLNKPCLPLPVFDNTDFESRPPHRWVDKNKPV-------
----------PAEAAYAEP-----Q---QPCIWKECEVLDYCDDGSQYMIR-----------------
------------------------------------------------------------------
-------------------------------------------
WTDTGQTAWIPRLFLHFKAESPFTFAKRFAQACRLRDQTEALL----KYYLYVD-----
SMPKEDIKTLREEQEDRITKLTFMCKEYREKTLDLGKYIKE--------------------------
---------------VNTEYARIMNQIVL----EDCLRNQK-------
GVAQTFTVQLDVECIEKVPP-------------------------------------------------
-------------------------PVPSFGCIAIPKY-SYEERCSEFHFWSCIT------------
--------------------RSEVILATVRLRAECNKVLKLNLLNTHYNKSLRLED------------
-----FEQMQAQSIEQTSMHLRD------SWTSVLKNII--
RSSFKDVGKGWFNLQETSLDAYDASKMKRFLRMVRYMMEDSVRFLVE--------
```

```
DTLTRYCNFVERHCGNPKVTVVDVDSVDTVWPQNDHKIVK----------------------------
--------------------KPPLFALEIVNLEN-GLFDYNTPLKHF-
EDVVCKAFDNAVACTQAIQQLEPSVMEKLMWSGSPTIGSVNLVE----------
EGMVNLKNRIQVAIRKATGIAHTYLEKFKVFES-----MLQVEASKYKTELENK----------
KLPLKELLAEVHGHRSDQDNIEKRIPTTITFGPFIVTCKKVRGLLVQRKSQLSDIVVSLIAQVPRTMSE
GISRRFEEKEQQL---------------------KIKTRTPEDVAAQREYIAKLPMAINELRTE----
----IDLTEVWYEALDDLNYALSDG-----
DFNQKWALRFWPSRIEKLVEKTEKILATDMERYQREMEEEQVQFLLTLENLETTVSAFSRYT------
DLNQSEFVASEARKIEE-------QLKLAESKARLFNSRENLFGAPPTDYN---
RLKKNIENFEPYSYLWITADDWKKSFAHWMNGSFITL----------
DPEDVEKKVTSWYKGLFKVSRLLTQKDATA-----CAENCEE--IRKEVGAFKPYVP-
LIAALRQPGMRDR-HWDQLTK-ELNMDMHL-T-----EDFTLSKGLQMG--
LMDHLDAITNVADIASKEFAIENSLLKMEADWKDLEMQVMEYKET---G---TYIVKI--
DEQILQQLDDHIAMTQSISFSPFKKP---FEDKITKWEQQLVLVSEIVDEWFALQRQWMYLEPIF-
SSSDIQTQLPIESKRFNT--
INVVWRKVLNQAHLTPNILTMCSSKKLLDQLRESNKMIDMVQKGLADYLETKRLAFARFFFLSNDELLQ
ILSQAKNPLAVQPFLHKCFEAIHELDF-------RPDLEILAMNSAEH-EKVPFATTM--------
QPSGNVETWLNEVEKKMKESILTQIRLSLAAYKTS-----------P-
RTEWVQLWPGQVVICGSSVYWTEQVESAIS------KNSLQDYFDNVQVVQLLALT-DLVRKP-
LSKLARLTLGALIVIDVHARDVVQKLLDEK--VKAVTDFEWVSQLRYYW--EDEGI------------
--
KVRMVQATLSYGYEYLGNTPRLVITPLTDRCYMTLMGALHLNLGGAPAGPAGTGKTETTKDLAKAIAKQ
CVVFNCSDGLDYIA--MGKFFKGLASTGAWACFDEFNRIDLEVLSVIAQQILTIQLAIQQK--------
-----------LKRFIF---EDTEISLDPSCSVFITMNP---
GYAGRSELPDNLKALFRPCAMMVPDYALIGEISLFSFGFHRAKPLARKMVTTFKLCSEQLSSQDHYDYG
MRA-VKSVIVAAGNLKRAY----------P------DED------
EDVLLLRGLRDVNVPKFLSQDLPLFAGIITDLFPGV--QPPKISYDDLLKAIATVCSELN---
IQPVDAFTTKIIQLYETTLVRHGLMLVGPTGGGKTMCYRTLSMAMTILNN-EGSTTY------
QKVKTVCLNPKSITMGQLYGDFDENTHEWTDGVLACYMRELAGD--PS-
PDKKWLMFDGPVDAVWIENMNTVLDDNKKLCLVSGEIIQM-
SSTMTMMFEVEDLAVASPATVSRCGMVYMEPSARGVDVLFTS---WIQRV---------IPSVRAHEE-
---------------------------------KLRVMFKELVVGSVRMV---------
KKNLKQTVATTEDNLVLSLLSVMDSLLIPFQKDGG---------------------------------
-----------------------------------APLNEEEEKKLAQVIEPLFVFSLIWSAGVTC---
-DNDSRVKFDVYVRDAIVDL------------------------NLERPP---PDNL-
DVFGFYFDQNAL-------DWVEWMQT--IPKYVCN----
PEKPFSELIVPTADSVRSKFLLQSLFEIGKHVLCVGETGTGKTLVVQDKLLNDMN-QSYVPI-
FINFSARTS-ANQTQDLID--AKTEKRRKGV-YGPPPTKKFII---FVDDLNMPQRE-
KYFAQPPIEILRQWMDHKGWYERKPPC-AFRTLVDIQFVACMGPPGG-
GRNPVTNRFLRHFNFLSFNEMADESLFLIFHTILNATLS--AKFS-EVVRE-
CGKNIVAGTIEIYNSIRKELLPTPSKSHYTFNLRDLSKVIQGVLRADPGRTQ----------------
ALHSIVSLWLHEAMRIFQDRLINNEDRTWFSKTLSIQLEKQ-F-NVQ-WSDVITH-------------
------ERLIFCDFLV-----PGAEP---RI-------YEEV-KDFQKLSQIVDQYLEDYN--
NVSSAP-
MKLVMFLDAIEHAARICRVIRLPLGNALLLGVGGSGRQSLTKLATFMEDFELFQIEVVKGYGNNEWRDD
LKRMLLKAGVD-DK--EMTFLFSDTQIVMESFLEDINNILNSGE--------
VPNIWKNEDLDLIANAMRP--LLQAKGL-
PATKIALQTTFLKRVRQNLHVVICMSPIGDATRTRLRMFPSLVNCCTIDW--
FHEWPEEALRSVATNFIAA-TQLLEPDQ----
VQSVVEVCVFIHQSVEAASKEFYDETRRHVYITPTSYLELLQTYIKLLDEKREELGQLRGRLSAGLNKL
ETTAKDVEVMQKELVLLQPVLEKTVKEANDMMLTISEDKKN-
ADITKQEVAKQEAEANIQAAESKAIADSAQRDVDQAMPALENALSSLKNLTKADIVEVKSLKNPPEGVK
MVMEATCIMFQVAPKMVPDPNKLGKKIP-DYWEPSTKLLTDPQKFLDSLLQF----
DKDNITAATIEKIE-PYIQSEA--
FTPEQVARVSKACTSICMWARAMHAYYLVCLVVAPKRAQLAIAQAQLDATMQLLNNAQLKLKEVEKKIQ
RLEAELDAAVRKKDELEKKASDCQKRLDRAGKLIGGLGGERARWGETIRMLTTNITHVVGDVAVAAGSI
AYAGPFTPNYRKKLNTE-WLNKLKDKKVPHTPD--V-
DLQLVLKDPVKMRSWNIAGLPSDSLSEDNGIIVFKA-RRWSLMIDPQGQANKWIKNMYKE--TG--
LDVIKLSE-KDYLRTLENAVRFGRIVLLENVME-TLDPALEPLLLQQKIK-
QGGQEVIKIGDNIIPYHPDFRMFITTKLSNPHYPPEVSVKVTLLNFFVTQEGLEEQLLGQVVTKERPDL
AEMKNHLTISNANMKRELKEIESKILHLLSKSQ-
GDILDDEVLINTLSQSKVTSDEITIKVAEAEKTEIAIDETREVYRKVATRASQLFFCISDLATVDPMYQ
YSLTWFISLFNRAMEGAT---------------QA----------------------------------
------------------------------
```

```
SIIEKRIENLNEYFTYSLYVNVCRSLFEKHKLMFSLMLCIKIL--QELK-
AINSAEWRFLLAGATSSTMT-S---PNPASDWLTNKSWVEILNLCKLEK-----------
FKGFEQHFILNIAHYKRIFDSSEAHQEPLPEDW-NNRL---
NKLQKLLILRCLRVDKALLGIQDFVADYLGQRFIEPPAFDLSACYKDGS-
PSSPLIFVLSSGADPMADLLKL-AEDLRF---
TKKFEKVSLGQGQGPKAERLLELGMERGMWVCLQNCHLSQSWMP-HLERIVE-NIDPE--
KVHKDFRLWLTSMPSPD------FPVSILQNGVKMTLEPPKGLKSNLLRSYLRF---TD-KVLN--
DCVKP---------------DP-WKKMLFATCLFHAVIQERRKFG---------
ALGWNIRYEFTDGDLSVCQTQIRMFLNEY----------------E-----------------------
-------------------------------------
EIPYKVIRFLCGEINYGGRVTDDKDRRLMNNLLHKFIIPEVI------VSTK---YSFSESGVYYV-
PDAT--NVNTFVQYVKELPLVPK-PEIFGLHENADITCDQNESYDVFRTLLSLQPRERAAGAGSLTR--
---EQIIEKSCQEILSKLPRVF-DVEAVLQKY---
PTMYTESMNTVLTQECIRYNGLLRVMKISLQETLKALKGLVMMSSDLEMLCNSIFNNQVPDLWAGKAYP
SLK---PLSSWVVDLLQRTDFIQK-W----------IDQ-G--
APPVFWISGFFFPQAFLTGTLQNFARKY---KYPIDTVGFNFIIR-
DDVTVQTVKNPPEDGCFIRGLFLEGSRWDYQKHMLTESRPKELYSELPIVWLKPVQFRKAPESG-----
-----IYECPVYKTLLRAGTLSTTGHSTNFVMYI--ELPTDKPQ--AHWINRGVGLFT--SLAY
>KU666433_c1276_g1_i1
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
--------------------------------
MLPKRSLSMKRASMWKEEAEGGRRKAPTPPKRTAEEMLLDYFEELEESIKRRRMMGFQT---------
SELP------PIL------------STESLFTKAIPLGMQSLMFCRTPSNRIGIYN---SPE------
---
SMDLGNMDVYHPKARQPRRPVLRFELPNHVRLKQRWKHPDVNPNDLKNPYKPPILSEQELDFLQSKAFT
TEGDFDFMTPFLQNEKATAASNTRR---GDLVKDEDMADAEFKDKDKYFPR-----------------
-----------------------------------------------------------------------
-------------------------------------------------
IVQTPRSLHISTSLREPEVPVNVPEAAE------------RILKYQY-----
YIEAGIDEYFIAPIRERWIQNAVRHIGKHAIEGLSQATISHTLQSVTEE--------------------
---------------VHHDYIQSMRKAIV----
DYILQDETERERLGLTDINKILEVVTTSYFARSHTIQI-------------------------------
----------------------------------------
LLPEWRTAVLNAHGAMSGSLYRLNPLTLEM-------------------------------------
ANVWQNTFASQLFFDVDSGDFRSRMPFDLPT----------------FLSREKLCAKNTKESLEK---
---EWFTVAGNLL--
KQVVPTSPNQDLEGAYSAMGVLMTNQIRKLVEESIISLAKFISFYNQEINESPPTYTQTPVERVHPPRK
PTPSPEKYETLFENNDA-----------------------------------------------------
DDPDTVTNTVPPFTVTLKYNKG--VYSVEPSFQEI-
EASLIQLLDLCSYDGSSAEFFDANNNRIEPLTFSEIYADKALVN--------------
VAKEYISKTVRSNADKPLMVVKGFELFED-----LANMDAEVFAKEFRAQ----------
DKNLAEYEATIEAYSKRSSESEFASANDMDCGFVRVQCVEFKSKLSDKAQRVCTLLKEQILDGLNVANQ
EVCQRCERIARDI---------------------TKVPTNSEEAVNLQKLLFSCPNELLAVEKV----
----LADCRQKDEFLEKFMFQIPEL-----
EFRTMMLAYEWPKRVRAVVEVHAHRIQEEYEQYEQALKKKRVDFLELLEAYDGQVRSFEKHG------
EIEKAEAISLEAQTLSS-------NLAAAQSYSESINAEEQLFGMAQTKYN---
QIPAMVTGLDPFLKLWKIAGSFKKYEDEWMSCPFFSL----------
DIEKVENEVMNMWKVIYKLIKQFGDDYEE------PAKVANA--VKANIEHFKENLP-
LFRCLSNPGLRER-HFDKISE-IVGFEIKG-E-----DKTSFRQLLRLQ--
VDEHLLKLEEISEHASKEYSLEKALDQMKSEWAELVFEGSMYKDT---G---
TYILKGAPIEESQILLEEQIVKTQNMSSSAFAQF---
FSERISVWLKRINRMQSILSEWLKMQAAWMYLQPIF-GSEDILKQMPKEGTAFHS--
VDKVWRRIVPIVLKTKTILEAADIEGLLEDLKVGNEALDVVQKGLNQYLETKRLAFPRFFFLSNDELLE
ILSETKDPLKVQPFLKKCFEGISSLEF-------QHNLDITAMLSVEK-ERVEFSSKV-NP----
KGAGGNVERWLIEIEQVMKDSLKVLVQKATANYVIV-----------Q-
REKWILRWPGMVVLCGSQIHWTKEVADAIE----GGASALKKYEDKC-TDQLQSLV-KLVRSN-
LTTMERLTIGALVVIDVHARDVVSLLKEQG--VQTETDFEWLSQLRYYI--EDDEV------------
--
IVRMVNASRKYGYEYLGNSTRLVITPLTDRCYRTLMGALHLNLGGAPEGPAGTGKTETTKDLAKAIAMQ
CVVFNCSDGLDYLA--MGKFFKGLASSGAWACFDEFNRIDLEVLSVIAQQILTIQRAIMTG--------
-----------VKTFMF---EGTELRIIPTCSVFITMNP---
GYAGRSELPDNLKALFRPVAMMVPNYAMIGEITLYSFGYGQARDMARKLVATYKLCSEQLSSQDHYDYG
```

```
MRA-VIAVLRAAGNLKRKY----------V------EQE------
ESVLVLRAIRDVNLPKFLAHDIPLFEGIMSDLFPGV--VLPKPDYEAFITAIKSNCEKMG---
LIAVDTFITKVLELYEMIVVRHGLMLVGHSFGAKTSMYKVLAAVLTDLYE-KKLE--------
MRTKYDILNPKSITMGQLYGQFDPVSHEWTDGVLAKLFRNQASE--PT-
PDRKWLIFDGPVDAIWIENMNTVLDDNKKLCLMSGEIIQM-
STTMNLIFEVQDLAAASPATVSRCGMVYVEPSAIGWRPVLDS---WQLAL---------PEGFDAKSR-
--------------------------------EQIVKLCDWLLPPCLYCV---------
QKHCKRMMPMQDQNLVKSMLRIILSLSGPELTDL-----------------------------------
-----------------------------------ASSEKMDANVKVLWIDCIFLFALVWSLGASI---
-DIADRSKFDLLLRKLIANQP--PEEYKAYIIAPA-------RKVAAPFP----DGK--
LVYDFVFRKDRG-------KWAIWMDL--VDDSALP----
SDSEFYKILVPTPDSTRYTYIMEALVMNQLPILLVGPTGTGKSVCVKQYLN-KLDASRFQSV-
FFNFSAQTS-ANQTQDIID--SKLVKRKQGV-FGPQKGKQCLI---FVDDLSMPALE-
KYGAQPPIELLRQWMDHKGWYDRTDL--QFRQNEDIQFLSAMAPPGG-
GKNPVTNRYLRHFNVVCITPFSDSTMSRIFSILVDFWMK-RSRYP-QDVLK-
LRVPMVAATLEIYQTVQRELLPTPEKSHYTFNLRDLGKVFLGLQFAPPEIE-----------------
NQYKIIRLWVHECLRVFYDRLINDKDRTWLCDLIAEMLEKH-F-KER-
FVKVFAGFSHSEVKKGDVGPSM----LKYLVAGDFMI-----PGAEP---AL-------YDEI-
GDEDALMKVVQNYLEDYN--AVSNKQ-
MNLVLFQFALHHISRICRVVKQPGGNSLLVGVGGSGRQSLARLAAFIQGFDIFQVEITKNYGINEWRED
LGKMLRKAGEL-DK--QVMFLFVDTQIKLEGFVEDINSLLNTGE--------
VPNLFDSADIGAVCEAVRP--RAKKFRR-
DGSRAELYGFFVDECRKNLRIALAFSPVGDAFRDRLRKFPSLVNCCTIDW--
FSAWPVEALKSVASRFLAD-INVE-QDL----
IPRIVDTTVYFHSSIQDLAQQYFSTMRRYCYVTPTSFLELISAFSKLLEIKQKEVRGQKQRYETGLLKL
ENSSKEVNTMQEDLEKLQPKLVQSTNEVNDLLKVIDKETTE-
ANKVRDVVLAEEAAASVKAEAARSIKEETEAELEEALPMLMAALKALDTLTKQDISELKGMKSPPGPVK
LVIEAVCIMKNIKPTRMKDPSGTGKMID-DYWESSKKMLADA-EFLKSLRDF----
DKDNIPAATIQKIK-PYLANPE--
MDPNKILTVSKAAYGLCSWIIAMEAYDRVAKVVAPKKEALRIAEAEYNEVMEALLIKQAALQKVEERLA
ELSSRLYEAQEKKKAVQDEADLCEKKIIRANQLMSGLGGERARWTSQAEMFGNLYILLTGNILLGAGAI
TYLGVFTPSFRDQCLKD-WVSACKAKNIPCSQN--F-
ELSTILADPVSIRQWNIFGLPRDQSSIDNGVIISNA-RRWPLMIDPQGQANKWIKNMEAS--NG--
LLVTKLID-SDFIRKLEIGIQFGKPVLIENVGT-ELDPALDPILLQQTFK-
QSGSLCIRLGDSVLEYSPSFRLYITTKLRNPHYSPELSAKLNLINFMITIEGLFDQLLGISVAKERPDL
EEQKNQLIVQGANNQKQLKEIEDKTLQVLSQ-E-
GNILEDETGIQVLSQSKLLAEDISRKQQIAEETGVRIDEARNGYKPAARHAANLFFCVSDLANIEPMYQ
YSLPWFINLFGMSIEKSE--------------KS---------------------------------
------------------------------
ADLVLRIQNLNDHFTYSLYSNVSRSLFEKDKILFAFIMAIRIQ--LDKG-
LVILDELSFLMTGGIVVEKQ-PPKPYQEDLGWLTEKSWNELYRLSKLDN----------
FRELDDSIQKQAKDWCVIFDSQEPHMEPLPMPW-NFEL---
DVFQKLLVLRCLRPDKLVLGIIDYVMATLGEKFVEASLLNLDACYADSS-
CTSPLVFILSPGSDPMSVLIKF-AGDHGT-----
KLQSVSLGQGQGPVAIAAMTEAAREGFWVALQNCHLAPSWMP-TLERFSETELLPD--
KTNPNFRLWLTSYPSDA------FPVSILQNAVKMTNEPPNGLKANVAGSYHMYPISDP-DFFN--
NCVKP---------------KE-WKRMLYALCFFHALVQERRKFG---------
PLGWNVPYEFNESDLRISSRQLKMFIEEHP---------------E----------------------
--------------------------------------
KIPYKALNYMTAECNYGGRVTDDHDRRTLKTILGGLYCEKIH-------EDS---YPLSESGIYVA-
PPDG--TYDDFIEFIKKLPAQQQ-
PELFGFHENANITKDLNETRTILTSLLITQPTGTGGGSSSGTKTSE--EDILFGICNDIMSKIPGDF-
DIEAAQAKY---
PVTYLESMNTVLCQELQRFNKLLTVIRYSLKELQKAVKGLIVLSADLEQLGKSLILGKIPAMWAAKSYP
SRK---PLSSYVTDLIERLAFFQD-W----------LVN-G--
SPAVYWLSGFFFTQSFLTGVKQNFARNQ---KIPIDMVDFEFKIM---
PDPEFCKMKPEIGVYVRGLFLEGARWDYPKSVLAESEPKVLYSPCPIIWLMPREASLLENYP-------
---HYLCPLYKTSDRRGVLSTTGHSTNFVMEV--KLPSDKPQ--DHWIKRGVALLS--QLDE
>KU666436_c31020_g3_i1
----------------------------------------------------------------------
----------------------------------------------------------------------
----------------------------------------------------------------------
----------------------------------------------------------------------
----------------------------------------------------------------------
```

```
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
---------------------------------------------------------------------
--------------------MNP---
GYAGRSELPDNLKALFRTVAMMVPDYALISEIILYSSGYLQARDCARKIVATYQLCSEQLSSQDHYDYG
MRA-VIAVLRAASNLKQRY-----------P------DQD------
EFVLMLRSIIDVNLCKFLSQDVPLFNGIVSDLFPGV--VLPEADYTNLTKAIKDNCKQLN---
LQPTPYFMTKIIQLYEMIIVRHGLMLVGQPFSGKTSALRILQRALTDMSE-AGLSE-------
RKVQVSTVNPKSVTMGQLYGQADPITQEWLDGVLAVRFREHAAD--QS-
DDRKWLVLDGPVDAIWIENMNTVLDDNKKLCLPNSEIIQM-
PSSMNMIFEVGDLAVASPATVSRCGMVYLEPHQLGWHPLMLS---WMETL---------PPSIFGETK-
---------------------------------EHLKGLFEWLVPPCIKFV---------
RKNIKETSATSDTNLVVGLMRIFYSMIDEFKNE-----------------------------------
-----------------------------------EISKNMGKEKRKIWIDSIFVFSLIWSIGCSG---
-DDDGRKAFEEFFRKLATGHT--PDGYDIYIMNKN-------VVLLVELMS---
SDGGMTIYDYVFDKTMA-------KWTLWTEL--ITPIEIP----
ENTTFSDIIVPTKDTIRNKFILNQCVLSGIPMLFVGPTGTGKTIYINQHLVQGLSSETYMSV-
LVCFSARTS-ANMTQSQID--GQLDRRRRGV-YGPQLGKKLII---FVDDLNMPQPE-
VYGAQPPIELLRQYMDHGGWYGRDN---VFREIVDVQFVAAMGPPGG-
GRNYVTQRYLRHFNTVAISQVGEDSLRQIFTTILKWHLTIKYAFP-HDVQM-
QIPRVIESTLDVYKEAKDHLLPTPNKSHYTFNLRDFAKVIQGMMLQQPSTVP-------------
LGPEGSRQYIRLWTHEVLRVFYDRLCDDQDRNWLLKVLNNLIKKH-F-NQD-FDKLFAHLLSES--
STTIGQT----EMRKCFFGDYIK-----EDIES---RT-------YEEI-VDVPNLITLMENYLRDYN-
-GISKRP-
MNLAMFLFAVEHVSRICRVLKQPGGHMLLVGVGGSGRQSLTRLAASVSMMPVFQVEISRSYTMVEWRDD
IKLMLSKAGASPGT--PSIFLFSDTQIKDEGFVEDINNLLNSGE--------
VPNIFPGDERATISEAVKE--
KAKKLGVELNTPSDTWRFFVNECKKNLHVIFCVSPVGQAFKDRIRQFPSLVNCCTIDW--
FQEWPEDALEAVALKFLKE-TPLD-ENI----
RNSIVRICKVFHERACFYSQELRVKMGRYNYVTPTSYLELLTTFKALLVVKQGENLKRRKGYEVGLDKL
RSSAEQVAVMQQELTDLKPKLLVTVEEVEKLMLKVEREKKEVVEPKKSIVQRDEAEASKQAAEAKGIRD
ECEAALASAMPALEEALSALDTISEKDIGYVKKLTNPPAAVKLVMEAICVVLDVKPAKQPD--
GKGGFVQ-DYWKPSVTLLNNR-DFLQTLKEF----DKDNIDPKIMSRVRQQYISNSD--
FTPERAANASAAAEGLCKWIIAMDKYDVVAKFVAPKRQKLKAAEDKYQHVMVGLRAKQEELKVILQKLA
GLEADLEAIMLKKENLQHDVKMCTIKLERAEQLIGGLGGERSRWLAVAKELETLYPNLTGDVLLASGII
AYLGAFPASYRQEIVES-WMALCVKEKLPRSPT--F-
SLKTVLGNPVQIRDWIISGLPNDSFSTDNGIIVANS-RRWPLLIDPEGQANKWIRNMERE--RN--
```

```
LQIIKLSGGGEYMRTLENAIQFGLPVLLENVGE-ELDPSLEPLLLKQVFK-
SGGMTCIRLGDATIEYNSDFRFYITTKLRNPHYLPETSVKVTLLNFMITREGLSDQLLGVVVARERPDL
EQQKNELVVQSAENKRRLKELEDQILQVLSTSE-
GNILEDETAVKIISEAKAVGNDITVKQSLAEVTEKNIDDARKAYASSGDSAAVLFFCISDLANIDPMYQ
YSLPWFVNLFINSIANSQ--------------KS--------------------------------
------------------------------
TDLTQRLAIIHNYFLVALYKNICRSLFEKDKLLFSCLLTYRVM--EHAK-
RISQEEWMFFLTGGMGSALA-K---PNPAPEWLSDKSWGEIVRLSKISA----------
FENLDLYISTYKSEWKAMYESMEPHKYKLPGKL--SQL---
TLFQRLLIVRCIRPDKVVPAIRDFVYEQLGAEFVRPPQFNLEACYKESS-
ATCPLIFVLSAGSDPTAVLLTF-AAEKEM---
ADKLAPVSLGQGQGPRAAALIMDAMAQGTWVLLQNCHLAPSWMP-ELERICE-NV-PA--
EVNPGFRLWMTSYPSKK------FPITVLQNGVKMTNEPPKGLRANMKRSYGLDPISNE-DFFE--
SCSQP---------------SV-FKSLLFGLCFFHACIQERRNFG---------
PLGWNIPYGFDDGDLRISVRQLHMFINESSP--------------S----------------------
--------------------------------------
NLPLQALKYVTGECNYGGRVTDDKDRILLNAILERCYCQKIL-------EPG---YSLSESGSYVT-
PLPG--DLNSYMSHIDNFPDMPL-PEAFGLHSNADITKEVNETNLILMSMLKASMATGVSSGGSGG---
---AGSIGEVVRSLLNSLPANF-DIEACERKY---
PILYEESMHSVLSQEMARFNKLLDRIRTTLKSMEKAIRGIVVMSADIEKAYHSIAVNKVPELWKKVSYP
SLK---PLDSYFSDLLERIKTMQT-W----------YQQ-G--
PPTIHWLSGFFFAQSFMTASLQNYARKY---KLAIDTLSFDFEII-----
DKEPTAPPAEGVYVRGLFLEGCGWDPIKKCLCESKPKVIYMPAPIIWLRPSPTAKLKTYP---------
-HYNCPVYRTAERRGVLATTGHSTNFLMKI--RMPSNDPP--AHWIQRGVALIC--SLSD
>KU666437_c31426_g1_i1
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
--------------------------------------
MADPSRLVLKQSAVCKRIKLSDPATLLSRKGQDEDADDVDMDERHGGGVKF---------SVQS-----
-MQP------------NRGNRKAATNGEGRRMPRMTSHGITKEILTH---LKSGTDG-----
NNKFQAEEKAKSSHAKEDAMGMEWQLLPGLKEVIPSDVRKS---
VLRPPKNRLSAAQRALDVIQPLQPFPKVRRSSRHGPVPPSNVRPSQQSKD---
ESSSEAKDRERKRPRDNIVMLWK----------------------------------------------
------------------------------------------------------------------------
-------RTRGTFATLSESDQHSYMWMPFLGLLREQPDYPEFIYLQPGKSSLSSFD--------
PYDLDIV-----SREEISSPYYYTMSSSGVTLFRDLDAEFIPLDEWEIDYEYYKCIMKLRVFKQ-----
--YKQWKSFAIWRR-------------FVRRKLQGLSQKSL----IKQLFSIN-------
PILQKVLLEICEMCLSMSMLHLF----------------------------------------------
-------------------------DYKQGTIYNIENFLELQEDKRSNLLEKLRE------------
----------------VSEKAATNVKMACLAVLHKIEEDLLGQQNEETESSTGIAQ------------
-----PNNKTTEQSKSSNSKLAS------IAAKWKQEKE--
KAAEAKENQERFTFAIAAAKRNEKQRLLKFLRLVDYMIAQCVRLILT-----------
RSFVVLLKDLCHPIVHGTYRFSEDSATFQVLKNHQHSTD----------------------------
-----------KKEVNIEIKPPMFEVMVIVEND--ALQLSPSITEF-
MNKFEEFWKTIPDGLRLIDRLVSHHGLNGVVEQIIPENGVVISY-----
MDLIGDQQLGDVTSVIRSSLEMYFQDAQSAIARFQSFLD-----IYIQNKNFSLSWMRTEVNEG-----
--RWDIDSFRTELDKFLKQVQDIN-
ALPDNMHEGVLHINNKMMKESLLPSPQKCLKAIYALVPDLVSQYYQIFITEIHTATKKL----------
------------LAKPSSVEEFVDVANFLQVMQDRMDNLEEQ--------
FHKIKEYISLMQEYTIPIPEI-----
ESTSYQMMGSDFNSCKDAMMTAEESKVENVKRFSADLTKQLEQLKVDVTSIRQRAQHDMILD------
DNSEMQEVLNYVKSLSK-------DMEAQKNHFQRIQQYERVFQMEETRYD---
ELLDVYDEVEMKKSLWCALEDFTHKKLSWVDTHFEKL----------
VIEEIEEVVTKYAKLAVKLERGLL-----------PNRVVSK--LRKMVDEFKQTLP-
VIQNLRNKAMKER-HWKKIEI-EMGQKIER-N-----ALFTLGKLFDLK--
VMDHKSEILVISNEATQEAALEELLLRVQEKWTSIEFITKPYKEA-----KDVYVLGS--
VDDVITALEDSMVTMNTVTSSRYVSG---IRGEVEKLENQLKHFGNVLDEWLECQKQWMYLESIF-
CAPDIQRQLPNESKAFNA--
VDKQFKDIMKRVHDRPNALQAGLTNGWLETFQKSNETLEMIQKNLEDYLETKRMAFPRFYFLSNDELLE
ILAQTRNVQAVQPHMSKCFDGIRRLDF----GDDPRSIDIFAMVSGEG-EKVSLGKNL--------
KARGNVENWLTSVEQNMISSLRRMAKAAFIDYAKR------------P-
RKDWVLKHPAQLIIMIGQVYWCHSVESALE--GGNVLSELSKNKIEC-VKQLEDLS-QLVRGD-
LTSLERRILTALITIDVHARDIVEDLIRDQ--VQKLTEFGWQMQLRYYWDDNSDDA------------
```

```
--
IVRQTNARFVYGYEYLGAQSRLVITPLTDRCYMTLTGALHLKLGGAPAGPAGTGKTETTKDLAKALGVQ
CVVFNCGENLDYKF--MGKFFAGLAQCGAWACFDEFNRIDIEVLSVVAQQLLTIQNALKSS--------
----------MARFNF---EGREIKLLPTCGVFITMNP---
GYAGRTELPDNLKVLFRPVSMMIPDYALVAEVMLFSEGFANAKVLSRKMVKLYKLSSEQLSQQDHYDFG
MRA-VKSVLVMAGSLKRAN----------P------NLN------
EDIVLIRAFKDSNIPKFLSQDVNLFLAIIADLFPGA--TTPAQDFGVLRAAVEECLIEVG---
LQPVPEFVIKIIQLYETMNVRFGVMEVGPTGGGKTTCYNILRSAMTKLRL-AGNPNPS----
FQITHAYVLNPKCIKMGELYGEYNLLTNEWTDGLASTIIRQCVID--TT-
MDRKWVVFDGPVDAIWIENMNTVLDDNCILCLPNGERIKLNATTMRMLFEVQDLAVASPATVSRCGMVY
VPPEDLGWRPYVQT---
WLQRTLTPIISGDQPTTVESNEGQEQQQQQQPPPPRKSTVEEDEGAKGVKTNVPQVMDYIWNIFNTYVD
PGLKFL---------RRECKENIPSVNMNVVTTMCFYFQALCQPSR----------------------
--------------------------------------------------
DVNFRQELETLGPIINKIFSFSFIWSVGGNI----DHLSREKFDNFLRKEMEQVA--------------
----------TF-------PGTTETVYEYFFNPKDQ-------TFTPWEEL--VPGFTYV----
PGAPFFTLMVPTQDSTKLSFLMEISLEVNRSVLFQGVSGVGKSAIVLDLLKRLQDTKNVMPI-
VLNFSAQTT-SIATQEAIE--GKLEKKRKTK-LGAPYGKTIAC---FVDDVNMPAKE-
RYGAQPPIELLRQFQDFKGFYDRKLL--FWKDIDDMTLLAACAPPGG-
GRNEVTPRFFRHFSMVCVQPPSEQVLRTIFGGILGGFLQ---LFA-SDCKA-
YLKPVVDSTIEVYVRISQELLPTPAKSHYTFNLRDVSKVFQGILMVKPGDCS----------------
TKRTLRNLWIHESMRVFHDRLVNIEDKTYFKMMLYDLLKRG-FDETGSFEDVFA--------------
------KSIMFADYLR-----PGYAVED-RR-------YEQV-VEEAKIPQLFNDYLEDYN--LN-
TTSIMKLVFFKDAIDHISRISRILRQPRGNAMLIGVGGSGKQSLTRFACFIGEQKCFQIELRKGYGIVD
FREDLKKLYKISAVE-GT--PVTFLMTDTQMINEGFVEDINNLLNSGE--------
VPGLYTPDEKDRVAAEMRE--YVSKMGL-
PETKDVLYNSFISRARDNLHVVLCMSPIGESLRARCRQFPSLINCCTIDW--
FNEWPEEALLSVSNYFLAN-VELPNKDL----
QGKLARMCVYIHVSVSQMADKFCQELRRNYYITPKSYLDCVNLYIQLLAEKRQDSFTAQDRFVNGLNKL
KETNVLIEAMKVELGKLQPVLSEKAEKSQKLLEQVSKDQEA-
AQKVKDVVEEEEVEVKRQAAETQLMKDDALKDLEAAMPALNAAVEALNSLNKNDITEIKSFAKPPPLVQ
MVMEGVCVLLDQKPD---------------WDTAKKIMGDV-SFIRRLLEY----
NKDGITSAIQKKLA-KYIEDPS--
FTPEMVQKQSNAATSLCMWVRAMDVYAKVAKVVAPKKEALRQAEEALKQAAEKLAAKHRQLHEVEAQVG
LLKRNLEQTEAEQRSLLEQVQVTENRLVRAEKLTAALGDEAVRWKETADAIGSRRDLLIGDVFLCAACI
SYYGAFSGPYRAALVSN-WVSKFQEQGIPVSAS--Y-
SLRSILSTPVEVREWNIWGLPTDDVSVDNGILVTRG-KRWPLMIDPQEQANNWIKAMEAK--NG--
LRVVKLSD-GNLLRTIESSIRIGNPVLLEDIGD-HLDPALEPVLQKQIFQ-
KNGQYMIRLGDTDVDYDPNFRLYMTTKLSNPHYMPDVCIKVTLVNFTVTMKGLEDQLLGDVVRKERADL
EEQNDRLVVSISSDKKQLKDLEDKILKLLKESQ-
GNILDDEVLINTLNNSKLTSSVIQGRVKQAEVTEKEITAAREQYRVVANRGSILYFVISDLALIDSMYQ
YSLKFFCHLFSHCIEASE---------------KT--------------------------------
------------------------------
DELDARLKILINYITEFLYLNVSRGLFEEHKLTFSLLICTSIM--RSGG-
RIRENEWNFLLHSSSAHGAN-QDKLQNPAPKLISESVWQSVCALEHVSN---------
ESFKGLTKSIVSDFKAWKTFIESVEPHNSPLPNDW-DSKL---
EPFQRLVLIKCLREEKFVYACEIFIKQSLGPQFVGSVPLQLDEVFKDTS-
NITPIIFILSTGADPTGMLQRF-GERVDR-
KPGERLHIISLGQGQGPIAEMLVSKSRKTGDWVCLQNCHLASSWMQ-TMERIIE-
RFAPEAAEIHPEFRLWLTSFPCKE------FPVPVLQNGIKITNEPPKGVRANLLQSYASY---SD-
ELLE--SCTKK---------------AP-WKKLVFAITFFHAVVQERRKFG---------
PLGWNVKYDFNNSDLDCGLQTLRMFLEEQ----------------P----------------------
--------------------------------------
EIPWPALLYVTGEITYGGRVTDDLDRRCLMSTLRRFYNPEVL-------LDD---YSFTSSRLYIS-
QPEG--RYNQYVDYIKSLPTAEG-PDIFGMHENAILTFQMRESKKILGTVASIQPRATSGGGGRSS---
---DEIVSGIAEEILKSLPDLLIDVEGHSTSALG-
PDGQISPLAVFLMQELDRFNRLLKRMKLTLSELQRAIKGLVVMSSELENMFNSFLSNQVPTLWSVVAYP
SFK---PLTSWVKDFHRRVDFLRG-W----------IKN-G--
EPKCFWLPGFFFPQGFMTGALQLHARKY---QIPIDTLNFGFRVL-
DLENEQDVLKAPEDGIYVSGLFLDGARWDFENRCLAEPFPGEMQSAFPVIHFIPIVNYKPPLNE-----
------FQCPLYKTHVRAGVLTTTGASSNFVLNVSLRIPTRSNP--DFWVLQGVALLC--QLPI
>KU666439_c38124_g1_i1
MEIIKEDSVAQPAQPPSSDPGTLQPADNPQAPSPDSVAEAVPQEPTITRDPRVQWLQRRLCAGLDIPDS
IFVSFIVDPQNQQLIFEDFMQGPASCIIMYTAM----------------------------------
```

```
-----------------GLRKSVTASTRSVNGSRLAVLELHAYTAASTSHA---------------
QTPIVRHAQRIM-------------------------
YFMKLKGGDLITCEEMDSHLEYGILPVGPSLDSLEQLLLQLFLPLLTQ----EEQAGECRFWALKDTC-
-----HVD------------RLGNDLQSNMQRFLFQVSNSKQILSQSIKLT---IPPLP-------
DNCFEK-EVDYEVVIELETAANDWLPVLESTLENETQKIPS---
GKSPLAELEFWREKVNTLSSLYEQLSSQQVKNIITVLESASGNTELVQSF---
KSQYADLTKLFLEAKDNVKFLTTLERHFKTI-
CKDPLAKVVETIAPMMNALRMVWIISRYYSDDSHMGALMERIALQIAERVSDDINLKTLFKLPASQSSQ
KIIVARNVLDSWSKIYLQVRERI-EIGGRDPRW-------
EFDRKRLFERTSYMSSICSDLLNIVEVVNDFQNFLGPELKAVTGDIEGIDSVIKKVL----VMVENVE-
----KLPFNVFDKSYAL--QWSATMSNFNMDRENIEHLTKNFIDTSFKKLRSAEGA-------
LDLLQNFRAIKSEGAINKQMMDKFTDIAAQFVREIETTRE----IFEKNANA-------
PPTTRNQPRVAGSINWARCLFGRLKKTMQKFETVGSNMMGDPA-AQEMNIY--------
YCALAKQMLTFEKTWAGQWAQSVSQQAVSYLKQPILSRDLE-GKIIINFHPNLHSIIRETKYLDAMG--
---------------------------FQLPETALNVALQEEKFTSYIESIDRMLQQYQKATGVLTA--
---------------VESKLLAYHIKRLQQVLD------PGFSHLNWNS--
LGIPDFVAFCIKAINEFKSLVNQVQNNSSSIEKAVEAIASTNLLQENIIEAEEESMEFQELYDHLEQER
QNAANEALKLYRSISPLLMKLEEIIVSTNTGRSPMMVDYYRFWEEKIFGALIEMVLRGLSTLQSIFQTR
KGPDKLSAKRRRFKISFYLNKP--DVLAQPPLIDA-
VGLLSRMVRAIATSVKKFVRWMDGTCLEAPEQR-IGEDVEPFVF--
NFYTEVSANPQIIKKMLILSNNINSAVTSVHKHLDVWRKYQH-----LWRQDKNVSLSKFVTQ------
-----NPTLAKFEEKLAKYAKVATDVL-
ETPSEKNIDFLTVVSHQLISSLRNEALSWVQCIGQNMVACDLPRVAKLHARIDNYRETI----------
------------HQKPDTSDELKVVLNTVAEIQSSSMEVELD--------
YLDLEERSRARELYGVPNKPE-----
DTEKIFGVRQRWLSLVEEAKTLSISLHETKKKFTEITKVQVLDFTREASGFRDRAVQKGPGK------
AT-DLDVGLQLLEEYQL-------ELEKFQSKREQLVLAQKLFDLPLTPFP---
MLVEVENALKGSAEIYTVYSEFKANVDKFSKTLWVEL----------
DIQKIVSATDEFFVRLKRMRHLK------------SELTYAN--LEAKVKEFQESLP-
LIQDLKSEALRPR-HWDKLMK-ETGKSFDM-D----PKSFTLANLFRME--
LHKFASVISEITNAATKELNIEMELKKMAETWKEQQFELFKYNKD---GKDRGWVLKS--
TEPIVLLLEDMSLNLQSMTNSRYVRA---
FLDDVNFWERKLSLMGEVIDIWMQVQRKWMYLESIFIGSDDIRHQLPEEAKRFDN--
IDRLWKKIMAETAKNPVILDACTAPDRFATLNSLYQQLETCQKCLSEYLDTKRNAFPRFFFISDDELLS
VLG-SSDPTTIQEHMLKMFDNCAALVF------GEKNRTVVGMRSAEG-ETYSFRIPV--------
ATDGAIEVWIKNVEAEMRESLFQITKEGVFYYAKS-----------E-
RSDWILKNLGMITLAGSQVWWTWEVEDVFHNVRKGNKNAMKQFSAKC-TDQLTDLV-AMVRSD-
LSSLQRKKVNALIIVDVHARDIIDSFVRDS--ILDHREFAWESQLRFYWDREVDDI------------
--
TIRQCTGTFRFGYEYMGLNGRLVITALTDRCYMTLTTALTYRLGGAPAGPAGTGKTETVKDLAKSMALL
CVVFNCGDGLDYKA--MGSIFSGLVQCGAWGCFDEFNRIEAEVLSVVSSQIKQIQEALKNG--------
-----------LTRFQF---EGKEIALDSRTGIFITMNP---
GYAGRTELPDNLKALFRPVTMIVPDLQQICEIMLFSEGFDMAKVLAKKMTVLYKLAKEQLSKQYHYDFG
LRA-LKSVLVMAGALKRGS----------P------DMS------
EQLVLMRALRDMNLPKFVFEDVPLFLGLINDLFPGL--
DCPRVRYPQLNDVVEEDLKENKYLVMTGPSEQVDKVIQLYETMLTRHTTMVVGPTGGGKSVIIQTLARA
QTKMGY-------------
PTKLFIINPKAQPTTELYGLMDPDTRDWTDGLLSNIFREMNKPLGSDRNDKNYLVFDGDVDALWVEDMN
SVMDDNKLLTLPNGERIRL-QNHCKLLFEVADLQYASPATVSRCGMVFVDSKNLGYDPYIWK---
WCNTR------------EGEEA---------------------------------
DWLRELMAKYVKPCINYILEGVIGDEMTTPLLQTIPLTNLNMVTQLCSLLEAVLR-------------
------------------------------------------------------------------
GEQKMTTKQAMEAAFVYCLIWSLGGSLVQNANVEDRTRFDKFVKDIAGLPL-----------------
----STGDPFPPSQLPSD--LLYDFYFDVYNL-------QWCSWRSK--VPDYQPP----
ADKLFSSILVPTIDTVRSTWILKTLIQVGKPVLFVGESGTAKSVAIQKYLA-SLNVAENLIL-
NVNFSSRTT-SMDVQLTVE--DSIEKRTKDV-YGPPLGRQMIL---FIDDLNMPKVD-
TYGTQQPIAFLKLLVERGGFYDRGKEL-NWKNVKDVKFVSAMGRPGG-
ARNNVDPRFISLFCTFEIQFPAAASLFHIYNSILESHVQ---KLT-PELRE-
SSASITDMTLQMYNYLVDSLPPTPAKFHYIFNLRDLSRVYEGLTLSTADTVK----------------
TSAQMLRLWRNECLRIFHDRLISESDKEIVQKYILGLLQQK-H-GAD-LEFVAK--------------
------NPIIFGDYRH------TINSAEARL-------YEDL-GEYSLIKRLFEEILDEYN--LTNKK-
-
MQLVMFDDALEHLTRIHRIMRLDQGNALLVGVGGSGKKSLSKLAAFTAGSTVFEITLTRGYNEDSFRED
LKKLYTMLGVE-NK--RVVFLFTDSHVVDEGFLELVNNMLTSGM--------
```

```
VPALFKEDEKDSLIGQVRD--QVTADGI-
VDTKENCWSYFIKRCRKNLHVILAMSPVGDALRTRCRNFPGMVNNCVINW--
LTPWPEEALHSVASVFLEE-VDIP-ENL----
KEGIVSHMVMVHSTVTDLSVEFKDRLRRHNYVTPKNFLDFITNYRNALGASRKKNSEMSARLAGGLGKI
MQAKSEVAEMQVTLADAKIVVDAKTAECNQLLEVISSRTVE-
VQMKQNFAQDKQTDLERQSELIGREKEEAEKALEEALPALEAAAEALNNLKKEEITEIRSFAKPHILVQ
KVCECVVILRGIKDVS---------------WKGAKAMMADN-RFLQSLLEF----
DKDGINDKQVRALK-DYFKDPK--
LSVDELMNISTAGAGLLRWVTAMMNYYNVFKTVAPKRQAVANAERNLRNAQNELEKIQEEINELSQQLH
TLRTQFETNTSEQQSLKKKADLMEKRLNAAERLIEGLGSEHERWSKEVEELSFSRERLLGDCLLTSSFL
SYVGAFTIDFRTRMMQEIWLKDIFDKNIPVTKP--F-
KLESLLTDEVEISRWVSEGLPSNELSIQNGILTTQA-SRFPLCIDPQMQAINWIKKKEGKLLEG---
RIKTFND-PDFLKQLELAVQYGFPFMFENVDE-
YIDPVIDTILEKAFTSLPGGRKVIKLGDKDVEWDDNFRLYLVTKLPNPHYGPEISGKTMIINYAVTEQG
LQAQLLNETVKHERPDLEEQREVLIRESSENKTLLKTLEDTLLRELSNAT-
GNILDNEELLTTLEGAKSKASEISHKLVLARETTQEIEQLRLRYSPAAKRGTILFFVMSSLSAINNMYE
YSLNSFLRTFQISLDTSK---------------QD---------------------------------
------------------------------
PTLEGRLRSIIDTLTYDVYNYTCTGLFEKHKLMFSFQMTISIA--DGDG-
KLNRQHLDFFLKGNLSLEKS-Q---KRKPYDWLPEQGWQDIVQMVTLGP----------
QFAALGEHFENNEAEWRDWYEYEKPEEIPLPGGF-NEKL---
KTLEQLLVLRCFRIDRITVALTRYVMENMGEKFISPPVLDYQNIHRQSS-
AFTPVVFILSPGADPAFDVFKL-GEEMGF-
KAGAKLKYMALGQGMGPKAQETIEAGAARGLWVMLQNCHLLPSWLK-TLEKILE---KLQ--
KPHADFRLWLTTEPISA------FPLGVLQRSLKVVTEPPNGLKLNMRASYAKI---SQ-EMLT--
ECPHW---------------A--FRTQVYVLAFFHAVVQERRKYG---------
RLGWNVAYDFNETDLRISLLLIGTYLKKTWDNK-----------DD----------------------
---------------------------------------
TIPWGTLRYLIGEAMYGGRVSDSFDRRILKTYLEEYLGDFLF------
DPYHPFRFFTGSDGTTYKIPENG--PKENYTSYIDSLPFVQS-
PEVFGLHANADISYYTNTTKNLWRDLVDLQPRGGANKGGIKR------EDIINNVATDLLAKLPQAF-
DLPKIRKEF-----
GTPTPIQVVLLQELERWNKLIISMEDSLHKLQRALAGEIGMSKSLDDLSTSLYNGQLPALWRKLTPQTE
K---MLSAWITWLIRRYNQYKD-W-----------VEN-G--
EPKVMWLAGLHIPETYIAALVQTACRDC---GWPLDKSTIYTKVT-
PYVTASDVKEKPKHGCYISGLYLEGAGWDLQTSELKPQEPKQLAVELPMLQIIPVEATKIKLQN-----
-----TFKTPVYVTQSRRNAMGV-----GLVFEA--DLATTKHA--SHWVLQGVALCL--NIDQ
>KU666438_c31683_g1_i2
-----------
MEANMEKPQAAPPQPQQPSQPSPSMLLQPTQPSPSILLQQSPQPFIMESELVESFESFESPEIIWFRRK
MDLINMPLEAWTPEHALVAE-----------------------------------------------------
------------------------------------------------------------------------
------------------------
EFLRTQNAGRLFAYLEKSDDEVNLRLSSTFPLQWKQVLVMVRDTTKPLNSENFHEFVQCEVVKGSPGEC
MLRLMQILYVPVMVDDGNTERNTTKDFVDELDKFMASLTESLSCTKGQTLLY---VPPVKL------
GSEFVKKPRDKYLIQRLESIVIRWTRQIKEVIRGQDRIMDA--
EVAGALEEVEFWRQRSIDLAGIRTQISNPEITSILGFLEEMKSQY--IHPF---
LNLSSRIDYEALVAAENLKFLTVLEEPCREL-
ATTRLCDIPSVLPMLLQRIRMIWQISPFYNSSEKITGLLCKVSNEIIQRCSAEIPLLEIFEGDVEKCKT
ALEESIQACESWKGLYTVMMEKYVNPSLKKARGSANTKTTELGTRSIFAPLESFLQRCQDILEVCESQI
QFSKNLELPVFGGTRGTEITKSLMEMQE----TFQKLVS-----
VLKTLPYSLLDVKVTKWHDDYNQFKDGVKDLEVMMQNIMFMAFRHAASLSES-------
IELMEAFKLMARRRPICMCVEQMGLQVLMTFSNELSVIKK----QFDQLRRR-------
PLMDYSYPRYAGAALWGLQFQRRLKAPMMLLMTAASYLLEIGDGYELSVQYNQI-----
ISSIDQFIKSQHTEWTNNIDPYAMKKLEKSLL-----DDSGNGLYLLKFDQSLLALFYEVRYWRRLG--
--------------------------WEIPNVASDILEGQNELRVLRSNTLLVIRDCNKILSAVDS--
--------------EEKGLVADRISHLTIAIN------
PGLEKLTWVASPKDLERFLGDVHLKCHILEETVMQIRVAKKKIAQICDAISMSWLVSID----
KKRIYDIGEFNAKQKEHRSYMEDQLRSHFNNIKQIITSAFQKCQGETSDVRTKWEDFKKKVEASIEASL
RATVTKSLQELSN-----AVNTDTKTGSPMFSAITVLDKESSSVMLVPSIQEL-
LEVVQTVTRELVTVISVVPRLRGSDIVQGSNGRSISVSPQGSLP--------
SFFEVIAMDQEEVLMMITAGVLGVTEKVHLYLQYWEKKYKSVWDQDKDAYIRRYERA-----------
KKPLSAFDSDIVKYQDLQDDVQ-
AEETITNVKFLRIDCGPLKNSIIGHCEGWANKFTTLLNSIARVELEDIYLYIDRNTKEL----------
```

```
------------KEPPESLDMLTEKVNNWHAVLDECATVKSR--------
FEPLQEKYRVLEKFEVPISED-----
EKELLEGLPLEWEAFEQMLQSLEGELEQAKKGFYETLSLMIKSFSKEVTDARNAFVEKIPTT------
SSTKTSDALAFIDEETN-------KVEGFQQRAQTLWAGMSIFGMEKLSNK---
ENTATLKDLELLRSMWTLKKEWEEAFDSWKDGLFSEL----------
DTSSMEASAGAFSKRVFKIGRDTK----------GWSVWTD--LKDTIDAFKSTLP-
LTVDLRNPAMRQR-HWQQLME-QVGQQFDPNS-----KDFTLAKVAELR--
LDLHAAAISELSIAASKELAIEEALNKIKEVWLTLELDMVPYREV-----PKIYKLRS--
TEDVFAILEEHRVTLSSMKSNRAHLF---
FAKDINKWEKDLARVSDTIEVVIQVQRQWTYLENIFGGSEDIRKQLPGETTLFYQ--
VNDSFITSMNRLQEVRNARGALCIDGLMDLFTSMGQKLEKIQKSLDDYLEQKRQQFPRFYFLSNDDLLD
ILGQAKDPRNVQPHLKKCFEGIKRLELRVPGEDGHRQYEATGVFSPDG-EYLPFANSV--------
LLDTPPEEWLNRVEAAMYAAVKLHLVKALEDSKLA-----------K-
KDKWVKDNAGQCVISAGQCKWTADCEKALS-GVDTSKASLRKLFKKW-ISYLNKLT-DMTRSR-
ISKIDRNKVTALITIEVHARDVIDKLMRVG--CSSPNDFEWASQLRFYW--EKNDC----------
--
AIKQVLSIFYYGYEYQGNNGRLVITPLTDRCYITLGAALFTRRGGNPLGPAGTGKTETVKDLGKALARY
VIVFNCSDGVDYKM--TATMFSGIAQTGAWICLDEFNRIEVEVLSVVATQIATIMSAIKAG--------
-----------FARFIF---QGMEIRLIPTCGIFVTMNP---
GYAGRSELPENLKAMLRPVSMMVPDFTLIAENMLFSEGFRTAKILAKKLIAIMELSQRQLSKQDHYDYG
LRSFVIPIARAAGATKRGD----------P------DMV------
EEHILMGSMRDLIMPKLIYADIPLFNALLSDLFPGV--ELPQKESETLRKALEQELVAQG---
LQVVNDFVSKIIQMYDCMLARHGNMLVGKTGSGKTVAWKTLQRAQGRLKD-QGSTDF------
EHTHVHIINPLALSNDEIYGTFNRLTNEWIDGVLSNITRKVCCD--ET-
TDKKWILFDGPVDTLWIESMNSLLDDNKILTLLNGERISM-
PSQVSLLFEVEDLSQASPATVSRAGMIYLNVEDLKWWPYAES---WLQRK----------IAAGSDQV-
------------------------------LIKTVRSLLEKYVDKVTEFQ---------
KKNCKELVPTDSLCCIITLCTLFDSLATPE-------------------------------------
----------------------------------NGVAPFEGDSYIPMIENWFLFVLIWSIGAGL---
-DEEGRTKFDILLREM---------------------------DPRYPA---AG---
IVYDYYLEPKKK-------TFVPWEEKL-SGAYKIP----
VGAPFFKIQVPTVDTVRTSYILKTLILAKKHAMVVGRVGVGKTIIVQSIVN-NLP-DTLSSM-
TINFSAQTS-SNSLQDAIE--GRFEKRTKGI-YAPAGGKRLVC---FVDDINMPKKS-
QFGFMPPLELLKLWMDNGFWYDRQKQ--ELKYLKNMQLVAAMAPPGG-
GRNNISQRVQACFSLINITTPSDNQMKRIFGAILTAKLA---NFE-DEPRL-
MGDALVAACVDVYNAVTNELLPIPGKGHYVFNMRDLAKVIQGLLQATREHYI----------------
SKDMMLQLLCHECFRVYADRMWDVNDKLWLQELLDQKLKDM-F-NTE-WKTLFKD-------------
-----GELSIFTSCMQ-----PGAD----GV-------YEPI-PSFKELREVLEENLRELS--
TQPGCFNMDLVLFRDAMEHVCRIHRVLVQPRGNLLLVGVGGSGRKSLTRLAAFIADMKVFTINVTKNYG
SSQFHDDLKALYQQAGVG-ENRLPVVFLFDDTQIVVETFLEDINNMLSTGE--------
VPNLFTKDDLSAVFDQVRA--DAKKANA-
GETDDELYTFFLERARENLHIVLCLSPVQESFHRRLMMFPGLVNCSTIDW--
FLDWPEDALLEVAIKLMADESTLNVGDT----
KAKVCKLFVTIHKSVVDTSAKMLANLKRHNYVTPTSYLDFAKGYRKLLAEKKKQLEDSAAKLKGGLHTL
NETREQVAKMQVVCQEKKEVVAVAKKECEEILVEIVQEKRV-
IDEQEKQVNEEAAKIEKEAKACNAIAFDCQQDLDKALPALQAAEEALNVLTKKDLSEVKAYAKPPALVE
MTLEAVMTVLKKPPT---------------WDEAKKFLSDA-SFLGNLMKY----
DKELLVDAVLKKIG-KYTSDPD--
FTPESVGKVSGAARGLCLWVRAMESYGYVNKEVAPKKAKLKAAQDTLHKKEAALQDARNKLEEVRKKVQ
ALKDKYDRSLASKEALQRELDDLELKLERAEKLISGLAGEKSRWETSITTFQEEIKKLPGDCLMAAAFL
SYAGPFASEYRDDLVKGIWVPEVLKTEIPSTTN--F-
GFSTFLADAGDVRDWNLQGLPADSFSTENGVLVTRT-NRWPLMIDPQEQAKKWVKNMEAS--NG--
LVVVDLQT-EGLMRTMEDCIQLGKPVLLVDILQ-EIDPSLEPILAKALIS-
KGSRIYIKLGEKEIEYNPKFRLYITTKLANPHFSPEISVKTTIINFAVKDLSLQAQLLTLVVQKERPDL
DKQRNELIVQITLGKKTQASCEDQILRLLATAE-
GPLLDNLDLIQTLDISKQTFETVKQSLEVAEVTAKSIEVASAAYKPCAERASLLYFILNDLVLIDSMYQ
FSLEAYMDLFLISITRST--------------KS--------------------------------
------------------------------
EVLTERIKSLIDYHTYAVYRYTSRALFEKHKLLLSLMICAKIL--LASN-
VITDAEWQFFLRGGTVLDKS-K-QAPNPAPEWITESAWDDITELATNLP---------
TQFDGIVTSLQQETLRWEQWFKTSEPENVDLPGDW-EDKL---
NELQHLIILRCFRQDRLMFATARYVANVLGQKFVEPPMLDLGESFGDSS-
PISPLLFILSAGVDPTTSLQQF-AVTKGV---
ADNFHAVALGQGQGPIAMKLIHESAKTGGWVFLANCHLMTKWLP-QLEKVIQ-GLEKS--
```

```
NPHEMFRLWLSSAPNNN------FPISILQRSVKMTAEPPKGLRANVLRLYTGT---TE-ESFQ--
RCRAQ---------------TK-YQKLFFALAYFHSVLLERRKFG---------
TLGLNIPYDFNDTDFQVSDDLLKTYLDAY----------------E----------------------
------------------------------------
ITPFEALKYLISEANYGGRVTDEIDRRVLSSYLTQFYCEEAL------SIPN---FTLSTISIYHI-
PDDG--TLQTHKEFIQTWPTLDR-PEAFGQHTNADIASQLASSRLMLTTSASLQKNSGGSKGGVRQ---
---EDIVMNIAGDLLLQVPEPF-DLVDVQHQ----
KAGDPSALHTVLFQEIERYNILLRQIRESCANLQKGIQGLVVMSSDLETMYKAFSETKVPAKWI-
KSYPTLK---PLGAWTRDLVQRIVELKA-W----------ADG-N--
YPIVYWLAGFTYPADFLTAVMQMTARRN---LVPIDTLTWEFSII--
NKEEKDINEPPKEGIYVKGLYLEGAGWDRENECLKEPQPMELIVQMPILHFKPVVSKKKPVKG------
----IYMCPLYLYPIRTGSRERP----SFLMYVTLKCGEKNP---DHWVKRGTALLL--ALAT
>Cr_DYNC2H1-Cytoplasmic_Dynein_2
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
--------------------------
MSSDSRKTFVVTTIACAIAPAGQQDQYAAYLGQDENGAIASFLDSTQNTLQAGVSGLGTGQLQLKLSNA
AEFPEGCEFSVVLSKL------------
RAGPIRTEDVPAGVAVATVAHSPLSSLYHTLKDVYSPLIKSQTAEGVPVLDKRLSELLAQVQAGLGTAV
RKGVPASAQEVADPNQAPLQEVVTPLDEINFWAELTNSPNAGPIKSSALQVSSALEPLRQSFEQLSSDA
PEGEGGSIGWEGAKELVDLTTNALRAAW----------------------
PVKLPIGGWAMGQKRADNFLRVVGAALATYVQRRVDRLAAAGRGDLWSAPFGEVRAVLVGASGLMTRWV
QESTALVEDWRLGVDTGGHD-------
WEGAAFADAYVRSFQERLEEIYNMREMVDELAKLVGREEAGSLGVQQVFAPFQGLQALQVSDFNTHVW-
----
KAAHEDFERRMGPIEQRISQKLKELFASVIIPSLTSAIGPGRGGDRAAAGGSLIQPQQVFAEIKRYSSL
MGRKNIASALQSEKETLAKQVDRHLDSVQA----
EFDAHRDSATGGAKVAPVGRVTASIVERIMWCMQTLQKLGKVSEVLKHMLRGGVAGDEGSGGAALRNTL
GVVSELQKEVEIFRKEQYSAWEEWMSEELGEMANWKNSKLMTFDSQNSHVKTHFNDQLVLLLREVRQLQ
SLG----------------------------
FGVRKDILNEVEIANKFYRYGMVLKQRANFYNNIATEMVQ---------------
CQKPMMLKDALDFEKVLMNPKDAQGKEITWRNA----
AALDGYVRRLNEVADRLAEKNRTLRKWHSVLSDKVVTLAGTDLVRHKDKWAAGVKEMREIFGRLEAEGY
SRESQQVWRQHWDFQLYKALEVQYLSGL----------------------------------------
---EMINKTLPEVEVKMVFRQH--
RLQYDPPLEELRIRHVKDLLNTFLGLPLRMKGVSDLSERPGFFRPIVDANPTGIAR------------
-------VYAAAESLFTQLADELKKYQDWMV-----LGTLDLDEFADANLLE-------------
VSDWELNFRMLKAASRDAE-
KLPNEIRIECYKVSLAPVKGSIDEHMKKLQDTLVASLRRKTVAEKDQIEDFMKNGRELF----------
-----------TRQANTVEDIGLAGQEAKGLTAKLAEVQAA--------
RRRIDEKNKLLRQMAGGGRDA---
AFAVVDLTEVNNSWDAFTNQLQQFDAHLEEQKGNLAVQISRQLEEFKGKVAGMNSRWQELKPKS-----
GPSGNPAVVLAKIQEYAN-------AIKELREESAKLYKEAEAFKIDVPDFE---
LMTEMETDVMATKAHWDRYADFLRERDEMANRDWLSMR--------DQVWKIEDFLAKWTKATAGKS--
-------------DDPIAVI--LTQEIDNYTLCLPHLKSCLRGAGWEDT-HWNQLFG-LLGMKTSG-
PAAVSKETVTLTHFLEKADLVVKHADVIKSLDAQAQGEAVIRKALTELKMWGMAREFTFTESTQSVAGR
QRRTPLIKE--WRDAMTEVGDNQSLVASLKQSSYYNM---
FKDEVSSWENKLSFLQEGLTLLNQIQRKWVYLEPIF-G----RGALPSQQQRFRN--
VDEEFRRTMTSLESTKKVVTFADIPGIRDKLPQMAQQLDVCQRALADFLEEKRSQFPRFYFLGDDDLLE
ILGQARNPAVIQSHLKKLFAGIQKVKF------SQDQSTIQAMQSMEG-EVVDLAPTV--------
RITEQIETWLGDLTRSMKNTLQQQNEVLCAGRMND-----------E-----
FRAAASQCLQLKEAVAFTEKAEVALK----AGSSGLAKLVTEM-RAQLMKLTGSDFTGHHLLQLKKQ--
-ALVLDFIHYCDVAEYLAKDK--IGGTTEWGWTRQLRYYH-RAEGSV--------------
KVAMAEATFDYTWEYQGNAAKLVYTPLTDKCYLTLTQGMALGYGGNPYGPAGTGKTESVKALGQALARQ
VLVFNCDEEFDFKS--MGRIFVGLVKCGAWGCFDEFNRLDEEVLSAVSQQIQTIQLALKEG--------
-----------AKTMMF---
MDKTVEVDKNAGIFVTLNPAGKGYGGRSKLPDNLKQLFRSIAMTVPNNELIAEVLLLSEGFNHAKDLAR
KLVSLFSLSRELLSPQQHYDWGLRA-LKTVLGIAGRELRDA----------RKAGQNVDAE-----
IEAEIIIRSVAATKLPTLTFDDNSRFKALINDLFPGA--KLTDARNEALEKALAEAAAACK---
MELTQQQIDRMLQLHLACEQRIGVIIVGPSGSGKSTLWELLEKAYERLGR-------------
KPIVYKMNPKAMPRQQLLGSMNMDTREWSDGVLTAAARKVVKE--PL-
EQRSWIICDGDVDPEWIESLNSVLDDNRLLTMPNGERIQF-
ANNVNFIFECHSLEFASPATVSRCGMLFMSDEAMEVERMLQR---WLKVQ------------ATDNG-
```

```
------------------------------DPGQMQSWMNDFFDKAFQWA---------
LSHPRAVETTKGGILDSGLSHL-----------------------------------------------
-----------------------------------------KLGPGSKQEFMAGLCRGLGSNM---
-NPDIRNQFYNDMARMSG-----------------------------------EG---
GIMDVGVATD---------PLIVLGDE--LRERGMD-------
EADGGLVVTPEVTQNLLMMAPWFKNRDPFLVVGPEGCGKGALLDYCFKRIMGVQVA----
VVNCSAQTSAANVVQKLVQVCGKPVTTTSGKALRPPDNTRVIL---YLKDLNLPRPD-
KYNTCQLISFLQQLIAHHGYYDENL---
DFIRVERVQIVGSMTPPGSVGRHALSTRFTALVRIVTMGYPDRENLATIYTNMAQRVLA-NSKTA-
SSVSP---AALSKAMLEVYSSVRERFTPN-DYPHYEFNARELSDWINGIQRYSLE--------------
------GGLTLVQAIAHEGLRVFRDRLVGDHQEQFTSMLYGTLTSLLGY-KPD-ATPWYTSTLGAS---
-----------AEERISGDLTK---------------------IKMLRWEQDTFAELVAEKLKGYE--
R--
EHKELNLLLFPEVLERVSRFDRVLSQQGGSLLLCGNSGVGRRSLMLLLAYMHNMDFITPKMTKNYDLKS
FRNDLKEVLRRAGVE-AK--PVMLFLEDHQLVNNAFLELVNSLLSGGE--------
VPGLFTPEELAKELAPLDK--ARDEDPL-
YTGPSNSYAFFSYRIRRNLHIVVSMDPSNEMFRSRCEANPALFTRCSVQW--
LEGWSVKGLQQIAAARLTE-LVESSPELMKLGRDKLINHMIHIHASSGSQTTR-------------
EYLALVSLYGQIYNRKRTQVLEQQNFLKGGLGKLAEAAVTVDTLSAEAEKQRVVLKAKQAEADEALVHI
QDSMLK-
AADRRKEVEVLKKRTAIEEVEMKERRVKVEEELSEVQPLIDAARKAVGNIKKDNIAEIRSLKMPPDAIR
DVLEGVLMVLGQQDTS---------------WNNMKTFLGKG-SVKDDIINY----
DAHKITPEIRARCA-
KLLAAKGNSFEDAVIRRVSVAAAPMAQWFKANLEFSKVLERVSPLESELHRLQSSLEESQRLIKQYEEE
LVQLDAAVASLKAEFSKKTSEAETLKISVDKAESVLSSARQLLDGLRGEKVRWEITVGTLGEQLKELPL
SSLLAAAFITYLPSHPEEHRLKVTKD-WCA------YLGAAE--F-
DVTRFLSSESEMLKWKAEGLPADGLSAQNAVVILNSTSRSPLIIDPSTQASEWLKSHLRV--
TGQNVEVTTMAD-QRFTTTLELAVRFGKTLVVAEVDK--VEPILYPLLRMDLDR-
QGPRFVVQIGDKATDYNDTFRLFLVTRNPDPYLPPDARSLLAVTNFTVTRSGLEGQLLGLTLQKERPEL
EEQKSTMLRQEDECKVALAELERNLLQTLATST-
GNILENKDLLDKLNETKTRSATVEKALTESKTLQASLDQQREVYRPIAARGSVMYFLLADLQALNQMYT
FSLSVFLNLFKKALDRDT--------------PPG---------------------------------
------------------------------
GDVTARIALLAESLLELVFAYVSRSLFNADRLTFGMHMARHLQ----PS-LFPEAQWAFFL-
GKPVPDSA-S---PPPKPSWVREEQAGAFSALAAAFP------------QLVAAAELADSALWAQW---
ASGATDALPGKIAGGKV---
NPFQQLLLVKAFRPDRLQSAMSSFICGTLNIKSVSPPPFSLKALIEGETRPDEPVLFITTPGADPSQEL
SEYAAQTMGK---ERWYFEVAMGQGQAEKAVTLLRECAKNGDWLVLKNVHLAVSWLP-SLEKELL----
ML--QKHDNFRIFLTSEPHPK------FPSTLLEMSLKVTFEAPPGMKKNLQRTYEAW---SA-EYLA-
-SGPPI------------------RAQLLFVLAWFHAVVQERRTYI---------
PQGWTKFYEFSFADLRSGMDVITLATRAG----------------T-----------------------
-------------------------------------
APQWPLLLGLLDDAIYGGRLDNPFDSQVLLTFLRRLFSAETV-------GAAGGKVRPLPGSKVVV-
PTTN--HRADYVSIISALPDVDT-
PGLFCMPDNIDRTAQQVNSARVISQLKAMSLRADAAGGFNRAQWQAQ-
LGPLLRLWDQLMSGASALKAAMKDIRARGT--
TDKGGAPIENFVALERYKGASLVALIDRTLGAIARVLKGTDTLSSGVQSSGAALLADVIPGSW-
DAAWEGPE---APMDYCRAVVAKALAIEGHWARCQQPGGGGLLDGSG--
GAGPLELSSVFHPGTFLNALRQQSARTL---GCSMDMLK---
AVTSWETAKLKAAAGGAPVALLGGLIMQGATFDGSRLSPVAAEAPA-
FRAVPAMSMAWLHKDSPMAYAS---------YMEAPLYMTSDRSKLLARV-----------
QLPVSGPEEMDGWVLAGLSLFL--SV--
>Cr_DHC11-IAD3
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
```

```
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
---------------------------SDTAEAINEDEGLLGWEVSEWP---
AIKDLSVELEPFASLYSITNDFGLRREEWLFGPVRDL----------
NAEEVEASVADWFKKTVRLSKALPRQD--------LRQLAED--TRGRLEQFKEYVP-
IIMAVCNPGMRGR-HWERLSS-TLGQRVYP-G---GDAEVNLQKLLTLG--
ISQHADFLQELSDTASREMSLERSLDRMVTEWGGVKFEVVAWKNT---G---
GSILKGAVVEEVQMLLDDHSIKAQAMLSSPAAGP---
FLERIENWVKKLASMQDIIDAWMLAQQKWMFLGPVY-GSEEIAKQMPKERYEFSA--
ADTRFRSVMKSCERNPEVLVFTDTQGVLSDLQSCNNSFSIIERSLAAYLESKKMLFPRFFFLSNDELIE
ILSEAKEPEHVQPFAKKIFEAVSEFEF-------NSEQEICALISIEE-ERVPLDRPVVTN----
SELTPGVEFWLLKVEEQMRVSLASIMKQSIQAYART------------P-
RARWILQWPGQVVLAGAQTYWTRGVVDALA---GGGLYGLVQFGEQC-GRELMEEV-MLVRGQ-
LRPLERATIGALVVLDVHARDVVAEMIADK--VASVDDFSWQSRLRYYW--EKDTM-------------
--
VVRMLNAQCTYGYEYLGNSGRLVITPLTDRCYRTLLGAHYMHLGGAPAGPAGTGKTETTKDLAKAVAIQ
CVVFNCSDGLDYLA--MGRFFKGLAATGAWACFDEFNRIELEVLSVVAQQVLTIQRAKSAG--------
-----------KNRFVF---EGCDMNLVATCNVFITMNP---
GYAGRSELPDNLKALFRDVAMMVPDYALISEIILYSYGYLEARSMAQKLVQTYRLCSEQLSRQDHYDYG
MRA-VMAVLRAAGNLKRRY-----------S------
SAADAAVYSEPVLMLRAINDVNQPKFLDEDVPLFKGILSDLFPGV--
VLPSIDYGDLTAALSANAVAAN---
LQPLPSFIEKAIQLYEMIVVRHGLMLVGRSFSMKTVAIKTLAAALGDLCA-AYKGE-------
HRVKMHTINPKAVTMGQLYGQDDPLSKEWTDGVLAVAFRTLARD--TS-
QDRKWVILDGPVDAIWIENMNTVLDDNKKLCLNSGEIIAM-
QGLMNMIFEVQDLAVASPATVSRCGMVYMQPSLLGWRPVARS---WLAAL---------PARVTDDLK-
---------------------------------EHLTSLMEWLLPPCLRLV---------
GKDCVQPVACQDINLVSSLTRLLQALLLPYLEPPAPPPSKPGQPPP----------------------
----------------------------------PPPPPRDPGATRSGADCLFLFCLVWTVGAAV---
-DTAGRALFSQHLRRFLKAD---YGAYEPYV-TGE------AVPVRQPLPA---AH---
SVYDWVYDTVTE-------SWKGWMDMRGAASQSIS----
PEAEYSTIIVTTADVVRYSYLLELQVAAHTPLLLVGPTGTGKSVYIKSFLANKLDRTQWTHM-
VFNFSAQTS-ANMTQDIID--GKLDKRRRGV-YGPPVGKRAIA---FIDDLNMPQLD-
RYGAQPPIELLRQAMDHGGWYDRHDN--SFRKLVDIQFLAAMGPPGG-
GRNPVTNRYLRHFHVLYATEFDAASLSQIFGALTDWWFG-RCKYK-EEVLA-
LRNPLVAASLQLHAAVSAHLLPTPAKTHYIFNLRDLSKLFQGMSFVGEALEH----------------
DPQRLQRLWVHEALRVYHDRLVDEGDREWIAATLRTTAEKH-FPGCK-
FDSLMSRLADEGAGGRVGAAS-----LRRLLFADFTV-----PGAEP---RR-------YVEV-
TDFVRLTAVVTEYLSELN--AGSKKP-
LSLVLFQFCLEHVCRVARCIRQPGGHALLVGVGGSGRQSVTQLAAFIEDFHLHSIEISSAYGMSDWHDD
LKTAMRSAGEK-HK--DTVFMFSDSQILNETMVEELSSLLNTGE--------
VPNLFDTGEMITIGEAIRQ--KARAARM-
DASRADLTNFFVATVRQHLRVVLCFSPVGDAFRDRLRRFPSLITCTTIDW--
FTVWPNDALASVAEQSLSD-LAGTELQL----
RHRLAEQCVHFHLTARQLTERYQLEAKRHTYVTPTSYLQLLDCFRGLLARQQQAVTAQRRRYEVGLEKL
AGTEEQVLIMRAELEEKQPKLIASGKETAELIALVEVQTAE-
ADKVKTLVQAEEAKAKEEADKVGAIKQECENDLAEAMPAFNAAIKALNTLTKNDISEVKGMKAPPQPVR
MVMQAVCMLKGLQPTKVKDK-E-GRWGQ-DWWETSKRMLSDM-GFLDSLMTF----
DKDNIPAEVITALQ-PLLSDPN--
FQPAKIKKVSQAAFGLCSWVRAMDTYDRVAKVVAPKRKALQEAEQQLAVVMADLATKQEQLRAVAERLA
GLQAQLEAAKGRKAELEADVALCEEKLDRATKLMAGLGGEKKRWSAKVEELGDQYVRLIGDMLLSAGVI
AYLGAFSAEYRAAAVSG-WAAQCASRGVPCSPH--F-
SLLGALGDPVSLRQWAIWGLPKDDVSAANGIIVKES-SRWPLCIDPQGQANKWIRNMESG--RH--
MLVLKPATDPTYLRSLAAALPMGFPVLLEGLGE-RLDASLEPVLLKQTFK-
```

```
SSGMQCVKLGDQVVEWGPGFRLYMTTKLRNPHYPPEVCTRVVLLNFCITPAGLEDQLLGIVVAKERPEL
EEEKSKLIITGAENARRLVEIEDQILAVLSSNQ-
GSILDDGEAVAVLQEAKRLSDEIAAKQVEAAKTEQAIDKARTAYQPIAAHASVLYFCVAELLAVDPMYA
FSLTYFVGLFLRAIEDSP--------------RH--------------------------------
------------------------------
ATVPKRLALLQEHFTFFLYVNVCRALFEKDKLLFAFSLAASIQ--VAAG-
RLAPEAVRFMVTGALSMDNP-H---ANPSPAWLSDQSWSHLCELEGVAE----------
AFEGLRGSLATHNELWKAVYDNPVPHEQVLPGGF-QDRL---
DAFQRLLLMRCLSPDKLVPAVAAYVAGSMGARYIEPQDFKLGSIFLDSV-
ASMPIVFVLSPGSDPMADLLAF-AEEKRK-----
KVEAVSLGQGQGPIAEKAISQGIREGSWVVLQNCHLAKSFLP-RLELLCEQQLAAG--
DVHPEFRLWLTSYPSDI------FPPAVLENGLKITNEPPKGLRAGMERIYKSEPLTDK-TFFE--
GALKQ---------------PDAFKHLTYALAFFHCVAVGRRNYG---------
PVGWNIPYAFNENDLRISLRQLRLFLDEA----------------D----------------------
--------------------------------------
SPPLRMLVYTAGECNYGGKVTDAKDRRTLMTLMEVYYRKEAA-------LGD---AKFGPGGNFDL-
PETG--DYAHYLSALSRLPLGEAPPEVFGLHPNAAISRDLAEARQLLDGLALTTTTLTAGPSAQPS---
---EGGLRSIAAEIAAKLPADF-DLEAASAAY---
PVTYLDSMNTVLVQELGRVNALLQVIRVSLDELARALRGEVVMSGELERVAHALSAGKVPELWLGKSFP
SLK---PLGSYVKELLERVAFFDG-W----------LHA-G--
PPTVYWISGFFFTQAFLTGAKQNYARKH---KIPIDLIDFRHTVC---
DGPADTAAAPADGVLCGGMWLEAAGWDAVHHRLCESEPRVLFVPLPPVHFQPAQRRDLPAGSTV-----
---AYVCPLYKTSERRGVLSTTGHSTNFVCDV--MLPSAEPE--SHWILRGVALLT--SLDS
>Cr_DHC2-IAD4
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
----------------------
MAPFETRSGETPRKVLIQRRRRQFAAQDVAELVHGEGVAQPPQELFPLEVFDNTNFESRMHPEWSLHGE
RPQTPTTKPAGVPLTSGRALVAHDDGTGH----------SVVDWVPCTVVDFDEATNSYGVT------
------------------------------------------------------------------------
-----------------------------------------
LHQLAHSGNGSAEADAEDMHVMWLPRVKVCFSAEDPAQFARRHAEAHRSRARAESLL----RYNLYVD-
----SMPTDDIPPLTNEQVNRMLSFALNSKKLKDKLMDTSALIAE----------------------
-------------------VNIEYARTMNKVVF----DTALTAAR----------
AEREADAEAAANGGSDFPRDPPR-----------------------------------------------
-----------------------PVPERGTVPVEGGADFPQQFSEFSFKTLLT--------------
-----------------KTEVIMAITKIKVECAKVTKMCLFNTHYTKSARLEE--------------
--LEQTQVAALDSAGNYLKD------TWCVALRNAI--
RNSFKDVGKGWFNLGEASMETYEFSKLRKFLTLTRFVMEDTMRALVE--------
DSLGKFTGFIQSCCPGRVTVHSTSSVEILDASSPVPVPAIGP--------------------------
-------------------GRKPPLLVMDLATNKEATRFVYSTQPESI-
VTKIMALFDAAIGRTQGLHTLEPAIMENLFWATAPVLSTVHQQE----------
EIVVRHRELLRAALSAALVPLEEYMAKFEKYVP-----LLQLNVESYVAALEAKGE---------
ELSLTEVRAEIKRASADLESLMESVPIGISLGLVQINLVKTRELLVKKQEKLVALLKALAARVPRRAMA
SVSTKFAEIDRAL---------------------KAKANNLEDVDEQRHYIESLPNKVYELMAD----
----VEAQRGWYELLEGMRYLLPEE-----
DLKEKFAGESWGMRLTRQAEKQLEVLAGDEARFKGEMITEQDMFRDTIGDLQVLVSNFGLYT------
DLGKMEAVVTEVRSVDE-------RLKKADRDAGVYNSREALLGLPPTDYS---
PLKKVIDTFEPFLQFWTTASNWRSLHKSWMHDSWEKL----------
HGETVEREVTNAYKVLFKTAKVFGQRGGLDK----CAENCEL--IREEVEAFKKFVP-
LVQALRNPGMRDR-HWDQLSA-LLGFKLHP-D-----KTFTMAAAEQMG--
LLQHLQAITKVADVAGKEYSIEQALDKMQREWESAEMQVLDYRET------KTFVIKV--
EEQISQMLDDHIAMTQSMAFSPYKKP---FEERIAKWEQQLSLVSEILDQWIQLQRQWMYLEPIF-
GSEDIMQQLPLEGKRFAT--
VDRMWRKTTDAAKRNPLLLKVCSSQKLLDSFIEANKLLESVQKGLADYLETKRLAFARFFFLSNDELLQ
ILSQTKNPLAVQPHLRKCFEAIESLDF-------APNGEIGAMNSREK-EKVPFDKPM--------
MPQGNVEIWLGEVERRMRFSVRHQVVLAVAAYATT-----------P-
RKQWVRDWPAMVVLAVSAIYWSREVEEAIS------EGSVPAYLDKC-SADLLDLT-DLVRGR-
LSGQERLTLGALITIDVHARDVVAELAEAK--IKNPTDFEWVSRLRYYW--RNDDV------------
--
CVDMVQASIAYGYEYLGNTPRLVITPLTDRCYMTLMSAMHMNLGGAPAGPAGTGKTETTKDLAKALAKQ
```

```
CVVFNCSDGLDYQA--MAKFFKGLASSGAWACFDEFNRIDLEVLSVVAQQILTIQLAIQAK--------
----------VKRFIF---EDTEIDLNPACSVYITMNP---
GYAGRSELPDNLKALFRPCAMMVPDYALIAEICLYSYGYKNGKDLARKMVATFKLCSEQLSSQDHYDYG
MRA-VKSVITAAGNLKREF----------P------DDD------
EEVLLLRALRDVNVPKFLSHDLPLFDGIITDLFPGV--KMPEVDYNSLLKALDESCGELG---
IQPVESFVGKVIQLYETTIVRHGLMLVGPTMGGKTCCYRSLQKAMTKLAA-AGDSKY------
ERVRVVALNPKSITMGQLYGEFDENTHEWTDGVLACYMRECSED--TK-
PDKKWIMFDGPVDAVWIENMNTVLDDNKKLCLVSGEIIQL-
SASMTMMFEVEDLAVASPATVSRCGMVYMEPTALGLEPLLTS---WLARL---------PPGGVAENS-
---------------------------------GKLGAIFNALVPDALRFL---------
RKNLKETVTTVNNNLVASCFGLMDSLTKPFVRGEGE---------------------------------
-----------------------------------DPLTADEKVKLGGFLPSLMLFSIVWSLGASC---
-DKAGRTLFDEWFRKHAAEC------------------------GLPLEGAMFPGEG-
TVYDWVYDTDGTFAEGGAPGWVGWMAT--VPEFKCD----
PDRPFSEIIVPTADTVRYTYVVDKLVANQRHVLCVGETGTGKTLNVSNKLLNDMPPE-VQPV-
FMTFSARTS-ANQTQDIID--AKMDKRRKGV-FGPPAGKRMVI---FIDDLNMPQRE-
KYFAQPPIELLRQWMDHGGWYERKPPC-PFRTIVDTQFVAAMGPPGG-
GRNPVTNRLLRHFNFISFTEMSDSSVSRIFTTILGAFFR--KYFG-DAIQA-
LTDPVVTATVRLYNSIRAELLPTPTRSHYTFNLRDLSKVVQGVMRADPRSTG----------------
DSKQVLSLWLHECSRVFEDRLINDEDHGWFRARQEALLTEN-F-GLG-YGDVVTS--------------
------ERLIFGDFMV-----PGADP---RV-------YSQI-TDMPKLVKVVEEYLEDYN--
SVSSAP-
MKLVMFLDAIEHVSRITRVIRLPLGNALLLGVGGSGRQSLTRLAAYMEEYDVVQIEIAKGYGSNEWRDD
LRKVLRKTGLD-GR--DTVFLFTDTQIVQENFLEDINNILNSGE--------
VPNLWGNDDQEAIANAMRP--LMAAAGL-
PITKMGISTFFINRVRSYLHVVLCFSPIGDAFRQRLRMFPSLVNCCTIDW--
FREWPEEALRSVADSFYGD-VDFGDDTGAI--
MAGVVDCCVGVHQSVEKKSKKFYDELRRYNYVTPTSYLELLTTFIKLLGEKRTEIAEKRRRLEVGLQKL
LNTAGQVEVMQKELQELQPVLAATAKEVEDMMVVITNDKKE-
ADETKKQVEQQEKDANEQAARAKQIAEDAQRDLDEALPALERALESLKNLSRNDIVEVKSLQNPPAGVR
TVMDATCIMFDEKPKMKDDPANVGKKVP-DYWEPAKKLLNDPTKFLESLFSY----
DKDNIPDHVIKKIE-PYIQRDD--
FTPEAISKVSKACTSICMWVRAMYVYHNVALSVAPKRAALAAAQEQLNETMEQLRAAQAKLKAVEEKIA
TLEAQYEEALAKKAQLAQQVLRCTVQLQRADKLIGGLGGERVRWQATVDQLADDLINVVGDVVISAATI
AYSGPFTPLYRSSLVHE-WSGFLEAAKVPATKG--T-
NLLSTLQDPVKVRAWTIAGLPTDTLSVENGIIVSKA-RRWPLMIDPQGQANKWIKNMERE--SG--
LDVIKLSD-KDFLRTLENGVRFGRAVLLENIGE-TLDAALEPLLLKQTFK-
QGGSEVIKIGDNIIPYHPDFRFYMTTKLRNPHYAPEVSVKVSLLNFFVTPEGLEDQLLGTVVTQERPDL
ANLKSQLVVSNAKMKKELSDIEDRILQLLSASS-
GEILDDEELINTLAQSKVTSNEISAKVAEAEATEREIDETRELYRPVALRASLLFFAISDLALVDPMYQ
YSLAWFISLFIRGIEEAP---------------KA---------------------------------
-----------------------------
ASVEERGHNLNEYFTYSLYVNICRSLFEAHKLMFSLLLTIKIL--QNRN-
MIDGREWRFLLAGPTTSELS-Q---PNPAPDWLTDKAWNELLNLSHLPT-----------
FKGFADHVAANLPHYRAIFDSNDAHELPLAPPW-EDKL---
DTFQKLSFLRCLRPDKVTGAVQAFVSQHLGQRFIEPPPFDLATCYKESS-
PSVPLIFVLSPGADPMADLLKL-AEDMKF---
SRKFEKVSLGQGQGPKAEKLLEAGMERGIWVCLQNCHLAVSWMP-TLERIVE-GIQPD--
RVHKDFRLWLTSMPSPD------FPVAILQNGVKMTLEPPKGLKSNLVRQYNRL---TD-AYLA--
ASSKP---------------ED-WRRLVFGLCLFHAVIQDRRKFG---------
PLGWNIRYDFTDGDLNVSLAQLQEYLDKY----------------E-----------------------
--------------------------------------
VIPFKVLRFLFTEINYGGRVTDDKDRRLINNLIYTFCGPSVL-------EPG---YAFSPSGTYAT-
PPEAVVSVRDHLELLRAYPIVPK-PEIFGLHENADITCDQNETYDMFSTVLSLQPRVASGAGQSQ----
---EAVIGALAADILGRLPELF-DVDAVIERY---
PTTYKESMNTVLTQECIRYNALLAVMKRSLGETIKALKGLVVMSPELEGVAYSMYDNQVPELWASRAYP
SLK---PLSAWVVDLLERCAFISG-W----------VDK-G--
TPPVYWISGFFFPQAFLTGTLQNFARKY---TYPIDTVSFGFKVM-
DALDEGAVVSGPEDGCFIRGLFMEGARWDNQTHVIAESRPKELFTEMPIVWLKPEQHRKKPEQPAEGDA
SGSIGVYDCPVYKTLTRAGTLSTTGHSTNFVMYL--ELPSDKPQ--GHWINRGVALFT--GLAF
>Cr_DHC3-IAD5
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
```

```
------------------------------------------------------------------
------------------------------------------------------------------
-----------------------------------
MPTELEKPTETPQQPLKLGLYLGAQNPTNTRPSLAPKPPYAAANGQGAAAVLRARPGLPARPPAGTANE
AAAGSAAPAVRTTHGASSLITKP---RPVLASGLHSVAAAGTA------------------------
------------------------------------------------------------------
----------------------------
AAGDKPAAAAGNGVAGDAGAAAGAEPKYDFSSAPRPCDFKAMSPDALEFIYLRHAHAEVRNPYNLVIVT
HADIKANAASRNNYYTMSVKGVTHFAEGSGEFITLEQFEREYFLYRQLLRFRTFVQ-------
FRLWKAFKIWRRF------------VSSRKARSAKEVL-----QKSLFMLN-------
PVFRVALVRMRTACHGLQQLRLH-----------------------------------------------
--------------------------AVERGQLYGLPQLVSLHAARHQDTKAALEQ------------
-----------------
FEHDIVAQAQEACVAAMQRMEDQLLGVKANAAASGNGPPAGLGGGSGGGVSLGAPGVVLGGGIGDAMFP
DGPLLPPIAEGPAGGPAEGGEG--
AAGAAEAASGEGAGAGEGEEVKAPPKDWGVDGGQFLQLAVQLQAVSPPQPSARGSGAVSRVRTMRNPDG
GAEGAGVSAHNSSSQVFITQSKSLARGL---------------------------------------
LAAGSSGSVAGSMNGTPGNLPALSHELVLVPPPGEF-
VAALEKIHSTWAGQIQSVARLLTHPNLQDYMERPVDAGFTPDME---
GFTDIVLGQAYSDSASAVLGRLRAVMEDANGFKATFEPIKSSVLA-LKPVDEAGLRLAWESG-------
----RRDLEAYRRDLTSLRDQLQTVM-
DVPQHKQLNVLRVELSELVAALRPAPERALASLSDLLPRLAFSAYNSFISAVHSATRRL----------
-----------NTMPTSADELSEFLHFLAECERNKAAMDRE--------
YDAVVAHYDLAEDFDIRIPDM-----
QAAAYATLDRDYAALRDAMWAADSSRERLVETYGADLSRAVDAIAKQLAGISMQAQHDMILD------
ERSDPDVV----------------------QADKIQQHQRTFRLEETRFP---
ELEDCYEDVALRHRLWTSVRGWAELTQGWLGERFDLL----------
QPGAMEEAIGSYSRAVFKMERGLV----------PNKIVPK--LRAGVNYWRDLVP-
LVSALRNPFLKER-HWVKVNA-AVGSYIDR-N-----EELTLQGLLDIN--
LLAAREAVSLISTEATQEAALEAMLDKVADKWRHVELSLKPYKAL-----KETYVLGG--
VDEVLAVLEDSSMVMATISASRYVAG-------VEKLDRQLRLFGDTLDEWLDVQRQWLALEPIL-
TAADIQRQLPSEARAFAA--
VDRQLKEVNRKAKDRPNALQAGTQPGLLEQLRRCNEALEGVAKNLEAYLEAKRTSFPRFYFLSNDELLQ
ILSQARNPQAVQPHLQKCFDGIRSLDF----GEEGRTNDILAMVSAEG-
ERIGLSKNLKASWASFNAARGSVEQWLGSVEAAMRMAVRGQARRAHRDYVTG-----------
PGRWDWVRVTPAQLVVVVSNIHWCRAVEACLSRANGDPLTALGELHTLC-ASQLSDLT-
SVVRAAGITPLERKVLVTLITTDVHNRDVLEGCTRAA--AAEEEGAAPFRRTRLW----------
---------------------------------------CAKSM--------------
PSTGKTETVKDLAKALGVQCVVFNCGDNLDFRF--
MGKFFSGLAQAGAWACFDEFNRIDTEVLSVVAQQLQAIQTALRAG------------------
VERFVF---EGNDIRLLPSCGVFVTMNP---
GYAGRTELPDNLQALFRPMAMMVPDYALVAEVMLFSEGFEGARSLSRKMVQLYKLASEQLSQQDHYDFG
MRA-LKSVLVTAGSLKRGS----------T------GLP------
EETVLIRAMRESNIPKFLSEDARLFDAIVCDLFPGT--DLPEQRNEELHGALVAACGVLG---
LQPVPPFVSKAIQLWDTFHVRFGAMLVGPPGGGKTCIYRTLAEAITRLSG------------
VTTFQAAISEASFFLGELYGSYNTATNEWQDGLASCIIRGAVVEGQSN-
PDLQWVVFDGPVDTVWVENMNTVLDDNCMLCLPNGERIKLDPASMRVLFEVADLVAASPATVSRCGMVY
VTPDDMGWRPYVSPGGRGPAEALGAEASGSPPLARRPSAG----------------------------
---VLQYLQGLFDKFLDPLLAHA---------
RKRGEAAVATCEVALVSATCSLLQTLLLSTQLQGHPGAEGASLRKHGAGFGLRAAATTLLGAASVAASH
AAGGLGGAAAGGQSGAAASASAFATASTPYKDPLLDLTSEEPSETTQVALNYIFAFAAVWGVGGSL---
-SPACWEAFDGAAKDLFEGTA-----------------------NY-------
PGGSGTVFDYHLDYHKNF------ALTPWEAA--VPAYAFR----
PEVRYFDILVPTADTTRYGALLSCLLAVNRPVLLTGPSGSGKSALVRSCLSRLQAGGGVIPT-
TVYFSAQTS-AATVQAQLE--GQLEKKRKRR-FGAPAGRRLLL---
FVDDVNLPARDAASGAQPPIELLRHLQD-----------------------------------------
-----------FSEVALRTIFGAIMGGFLD--LHFP-
PDVRTRMLRPLVDTSVEVYSHVCAELLPTPAKSHYTFSLRDVSKAFQGMLAIRPNQCG-----------
---PGDPRGTLMRLWVHEHMRVYHDR-------GWVEAAMRRLLVRP---------------------
----------------GDFLK-----RGGTRDE-RV-------YAEV-RDVPRLGRVLEGDLDEYN--
AGGSGPRMDLVLFMDAVEHITRIARILRQPRGNAMLVGVGGSGKQSLARFAAFLADTQVFTLELSRGYG
VVEFREDIKKLYRLAGIE-GK--PVAFLLPEQHIVLECFVEDVSNL----------------------
---------------GL-
EDTREAMWRAFVDRVRDNLHLVLALSPVGEAFRERCRRFPSLLAATSVDW--
```

```
FSEWPQEALMSVSTRFLEQ-QELGGPGV----MKALAQACVDIHTSVVAAASRYYLELRRRYY------
---------------
ETAVARERLLGGVSKLTATNTTVEEMRTALRLLQPVLADKTEATQRLLEQVTHEQYE-
AEHVRAAVAGEEADVKAQAAKLAALKDEAQADLEEVLPALEAAERALSALNKTDIIEIKTFTKPPALVQ
TTMEAVCILLQERAD----------------WDTAKRLLGEA-TFIKRLMEY----
DRDNIPDKVVRSLK-RVIDDPT--
FTPDQVAKQSKAAQSLCLWVRAMDTYARVIRVVEPKRAALATAQERLGAMNGALLAKQRQLGELEARVA
TLQEQLQSTQEELHSLQHQAELSQRRLVRAEKLTGALADESVRWAAQAERMTTTLHLLVGDVFLAAAAI
-----------------------------------------------
RDWGMQGLPTDSLSVDNGVL-------------------------------------------------
---------PVLLEDVGEGQLDPALEPLLTKATYT-
QGTRLLLKLGDVEVDYDPRFRLYLATRLPNPHYLPEVCIKVNLINFTVTPKGLEDQLLGDVVRHERPDL
EEAKDRLVVSLSADKRQLGELEDRILQLLQQADAGGLLDDEQLIATLNDAKQTSGIIATRVAEAEATER
AINEAREVYRPVPARGSLLYFVTAELSAIDPMYQTSLAAFVRMFGHCLDAAP--------------
RA------------------------------------------------------------------
DDLAARLRVLMDTTTDYVYRSVCRGLFDSHKLLYSFLISAAVE--RQAG-----------
LAGVPEGDEG-EEGGEGP----VLARSISQVL-------------------------------------
--PEGGGADVPARL--------
TAFQMLLLVKVFCEGRLLAAIRRYVSGCLGPAFTEPPPASLPELFTDSS-PATPIIFILSQGA------
-----------------------------------VKAGDWVCLQNCHLAASWMP-RLERLVE----
----ELQARY-----------------SKQIQFSNRLGLKLGSPDGVRANLLRIYGEL---SD------
-------------------------------------AERRRFG---------
PLGWNVRYEFSDGDLSAAMRTLQMFLVEGAAAASTSASPGAAGAGAAGLTGMRPAAGSRGGSVFDATPP
WDLRSGGLSAASLGGSGVGGMGGVGGGGGVGLSPGSSHGDIPWDALQYVTGDIVYGGRVTDENDRRLLS
CLLRRFYTPALQ-----------------------------------------------------
---------ESRTLLDTVLSIQPRVVASTA----------ADGMGAPATPAHAQAADPLASVGG-----
---------------
SAEMERLNALRALLAKSLDELGRALRGLAVSSAELEAMATALLNNQVPPAWRRAAYPSTK---
PLASWVNDFRRRVEHLRT-W----------LTR-G--QPPAFWLPGLFFPQGFITAVLQNHARRT---
RVPIDRLAFDFKVLSPEADKELVEAVDTHAGAAH----------------------------------
-------------------RYQCPLYKTSVRAGVLSTTGQSTNFVLHLGLGIPDATDP--
DFWVMQGVAALC--ALDD
>Cr_DHC4-IAD3
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------MHAPCAGQ-----------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
-----------------------------------------------------------------------
------------------------------DAEAITAAVDEYYRSAYKMAKAAR-----------
DDRVVAKVCLKEDIEGFRKYSQ-LFMDACNPALQAH-HWAQILQGLLGRPADD-P--
APGDPVSVAHLIEWG--AMEQAEALSNISGAASKELSLRRALSRMKEDWVGVDFKMVPYKDT---G---
SHVVGH--TDEIQMQLDEQLMKIQAMNASPFVKP---
FRGEAEAWQAALEGLQELLDSWLACQSVWMYLEPIF-
SSPDIVKQMPAEGAKFAQARVDMTFRMLVDEVVANPGAIALAKEVDRRTSLAEANRLLDEVQRGLSRYL
EAKRLAFPRFFFLSNDEMLEILSETKDPTRVQPHLRKCFEGIHRLQF-----EPNTNGVVSAMLSLEG-
```

```
EKVPFMQHI-NT----AAARGKVEQWLLE-------
AVHAVTGRGIEDYAAASAPSGGGKGGAKPKRHEWALRWPGMVVLLAAGVFWTRGVEDALA------
AQKLPVYEKQC-SAELREIV-DLVRGE-LTGLQRATLGALVVMDVHARDVVAALAADK--
VEAAADFGWQAQLRTYW--QATDV--------------
SGSGHGGSLEYGYEYLGNSSRLVITPLTDRCYRTLMGAIHLNLGGAPEGPAGTGKTETTKDLAKALARQ
CVVFNCSDTLNYQD--MAKFFKGLAAAGAWACFDEFNRINLEVLSVVAQQVLDIQRAIAAR--------
----------LTMFTF---EGSELPLKWSAWCAITMNP---
GYAGRSELPDNLKALFRSVAMMVPDYAMIAEIILYSYGYLAARPAAKKIVACYKLCSEQLSSQDHYDYG
MRA-VMAVLRAAGNLKRRFANAAPSTANQTP------EQQ---
ETEELGLVLRSIVDVNLCKFLSHDVPLFNGIVSDLFPGI--TLPKPDYGPLAAALKTACANSN---
LQPTEYFITKATQLYEMVLVRHGLMLVGMPFSGKTAAYTVRACGGGRQQP-ARQAG-------
ERASFFVLNPKSVTMGQLYGQNDPASHEWTDGVLAVLFRRCAAD--PS-
PDRKWLVLDGPVDAIWIENMNTVLDDNKKLCLNSGEIIAM-
SAPMNLIFEVGDLAVASPATVSRCGMVYLEPQQLGTGPLLL--------------------------
-------------------------------------------PTTRHV-----------------
------------------------------------------------------------------
-------------------------------------------------------------
SGRALVDQFVRRAVAGTL----------------------SGLEAP----SGGSAHVQPACLNVTR--
---------------LDSCCSG----
LLACFNSILVPTVDTAAVTFLLSTAINAKYPFLLVGPTGTGKSVTVVRCLL-
ELDPEVFAPANIIGFSAQTS-ANATQALVD--AKVDKRRKGV-YGPAGGRRGVV---FIDDLNMPQRE-
TYGAQPPIELLRQLLDHNGWYSRDN---AFRAIQDVLMVGAMGPPGG-
GRAAVTPRFSRHFATLCLTEASTPTLKHIFGTIQNWFLT-TRAFP-DPVME-
LREPVIAATLDVYSAAMTQLLPTPTKSHYTFNLRDCSRVVQGLQVRAATMRA----------------
QQEAHVRLWVHEVLRVFYDRLVDVSEQQWLLDLLRDLAHCH-F-GMR-LDALLSHLTSGAEGSITPGI-
-----IRRCLFADYMT------ESDENGYRP-------YLEV-
ARPSAAVSRLEDLLADYNGLAGPGKRQLHLAMFLYAVEHVTRIARVLRQEGGHALCVGVGGSGRQSLAR
LAAFICGMEAFQVEISRSYGLNEWRDDLRRLARRAGAE-AK--
PTVFIFGDSQAAQEAFVEDVNSLLNSGE--------
VPNLFPHDERSAICEAVRAAFKRAQPAVADPSPATLWSFFVGRCRANLHVVLAFSPAGDAFRERLRRFP
SIVNCCTIDW--FHAWPDDALDAVAAKLLAE-VDLQGPGQ----
RGAVKDLCKTFHAQVTSMSGAFRREAGRHNYVTPTSYLELISCFATLLAQRRAKVLAQQQKYENGLDKL
AFTAEQVAVMRAELQALAPVLARTVAETEQLLKEIAREKEEVVEPKRAMVDVEVKQADHAAAAARAIKE
ECEMILSEALPALEAATQALDTIKSADIKLVQTFKSPPAAVKIVMEAVCVCLDVKPTLVPDPNMAGKKI
V-DWWDASKRLLMDT-TLIQRLKDY----DRDNIPQRIIDKIRKEYTGDPD--
FTPANAAKASSAAEGLCKWVHAMDQYDKVAKVVAPKRAALAEAEAEYANVLMGLHGKQAELKELLEKLA
GLEAKLAESQARKAELEAETELCALKLERAEQLMSGLGGERERWTAEAARLRGSFGAITGDMLLAAGAV
AYLGAFTMPYRCAYLQQGLATGHVVMAVACSRDAAF-
SLTAVLGDPVKIREWVIFGLPNDSFSIDSAVIAATS-RRWPLCIDPQGQANRWIKAMHEA--QK--
IKVLKLSD-PDYLRTVEAAVSFGLPVLLENVGE-ELDPSLEPLLLKQTYR-
QGAQLVIKLGDNVVEYSPDFRMHLTTALRNPHYLPETAVKVALLNFSITPEGLADQLLGVAVAAEMPQL
EEQRQALVVSSAENAKQLVEIEARILQVLSSTE-
GNILEDATAVQVLSEAKRLSDDISAKQAVAEKTQAALDAARTRYRAVAEMSAAVFFAIADLASVDYMYQ
YSLSWFIGE-QLCIPTSA----------------G---------------------------------
------------------------------
AALAARLRQIESHFLYAAYCNVSRSLFEKDKLLLSFLLASRLA--AHRG-
NLPHEQLRFLVNGGGGAAPAGSAAGAPPAPLGIPVRAWSELLVLSALPG---------
SSFSSIPAHISRSADEWAELVESPDPHARPFPAPL-DSML---
SSFGKLLVLRCLRPDKLVPALEAWVAGELGQAFVSPPPMDLAAAFAEASGPSTPLLFVLSPGTDPWAAL
LRF-AEERGQ---AKTLQVISLGQGQGPKAAALIEAARGLGGWVLLQNCHLAPSWMP-TLERLWE-
AIRED--NTDAKFRLWMTCMPTPD------FPATLLQASVKMTSEPPQGLRANMRRSMALEPLSEA-
SFWELPPPPAPAAAPSSPSREGAGADGPPTLKRLLFGLVFLHAAVQERRRYG---------
PIGWNVPYGFDDGDLRISARQMRMYVQDCLQRS-----------N----------------------
--------------------------------------
PVPYEALRYAIGECNYGGRVTDDKDRLLLATMLRRIVAPELA------LKLA---RLMSVARRAFR-
SLSA--RARSYMSYVETLPTAAL-PDVFGLHLNADISKDLATTNGLLASLTATSGGAAAGGG-------
---DAGAAALVQGLLDRVPADF-NLEAVQAAY---
PVSYHQSLNQVLVQELARYNRLLSTLRTSLTQLGRAVAGLALLSAELEGVLQAVEAGQVPAAWKPQSFP
SLK---PLGGYMTDLVERCAFLSG-W----------AQR-G--
PPAAFWLGAFFFPPAFTTAALQNFARGH---QLPIDAVGFDFEML---
RAPSSLTNPPPSGVYVYGLYLEGAGWDRAGGCLAEPEPKQLYCPAPAIWFKTVRTADSKPTP-------
---AYACPVYRTADRRGVLATTGHSTNFLMTV--RLPLDRRS--DHWVLRGVAMLT--SLPQ
>Cr_DHC5-IAD3
```


-------------MTSPTSEL----------DPEQVQAKMDDFNRTNYKALKARK-----------
DDPVAGR--LKTSLDEFQKVLP-LLQEVANKALEKR-HWDQIMA-MLGQTEA--------
ESFNVQDLLVWG--VLDHLEAVSNIGGIATKEASMLKTLDKMEHEWEGLDFRVLPYKDS---G---
AFILGG--TDDIQTVLDDQIVKIQAMNASPFVKP---
FKERASAWESTLQNLQDMLDNWLKCQATWLYLEPIF-SSDDIVKQMPEEGDKFRQ--
VDAMWRRMMKNAHHAPAVIPIAREKERLDRLVEANQMLDAIQKGLAAYLEKKRLFFPRFFFLSNDEMLE
ILSETKDPTRVQPHLKKCFEGINTLTF-------TPQLVITAMNSVEQ-ESVPFKHPI-DT----
NKARGAVERWLVEVEEAMFASIHDVSGRGLEDYVTR------------P-
RHEWVLQWPGMVVLVVTAIFWTRGVEKALK----GEDGTLKQYEERC-TSDLLRTV-DLVRGE-
LTGLQRATLGALVVMDVHARDVVTAMVAKT--VTTAADFEWQAQLRSYWE-
ENEAALAHGGDSSGRQGPTVMMRMMSAVLEYGYEYLGNSSRLVITPLTDRCYRTLMGAIHLTLGGAPEG
PAGTGKTETTKDLAKALARQCVVFNCSDSLDYQA--
MGKFFKGLASSGAWACFDEFNRIDLEVLSVVAQQVLDIQRAIHAR-------------------
LRRFTF---EGTEMQLKWSAWCAITMNP---
GYAGRSELPDNLKALFRTVAMMVPDYAMISEIILYSNGYLMARDCAKKIVQCYKLCSEQLSSQDHYDYG
MRA-VMAVLRAAGNLKRRF-----------P------AED------
EYVLMLRSIIDVNLCKFLSHDVPLFNGIISDLFPGV--VLPKPDYAAMETALKEACVTHN---
LQPTDYFLLKSIQLYEMIVVRHGLMIVGLPFAGKTSSYRVLADALTIMET-RGEEGQ------
RKAEYHVINPKSITMGQLYGQFDPISHEWTDGVLAVTFRQVASN--PE-
PHRKWLVLDGPVDAIWIENMNTVLDDNKKLCLNSGEIIQM-
SASMNMIFEVMDLAVASPATVSRCGMVYLEPHQLGWRPLVVS---WLATL---------PPHMGESHR-
---------------------------------KHLLGLFDWLVPVSLRFL---------
RRELKESAPTLDANLVNTLMRYITASFDHLQE--------------------------------------
------------------------------------EESYKKAQDKVTLHLESLFLFALVWSVGGSG---
AENSARHHFDGFLRAAVAEQL--P-GYQVLPSSRSQAAQMMPTAIAGADPP---
PGGAYSVYDFFFDTVKD-------TWRLWTEL--IDTKPIP----
ADAKFRRIIIPSIDTVRYTYLLDKGITHSQPVLICGPTGTGKSVLVQKYLY-
GLPADKYVPPNIIGFSARTT-ANSTQHLID--AKLDRRRRGV-FGPPMGKQAIMAVVFIDDLNMPQLE-
TYGAQPPIELLRQFMDHGGWYARDN---TFRRLDDVLFVGAMGPPGG-
GRNPITPRYQRHFNLVTIVDFDEPTLGRIFSTLLGWHLD-TKSFP-ANIKE-
LRDPVISATLSVYTKVASQLLPTPTRSHYTFNLRDISRVMQGFLLLPPQLLG------------
AQPAQAKDKYLRLWAHEILRVFYDRLVDDKDRSWFLAYLRTVMKEK-L-GAD-
FDKLFAHLRQEGAASQEVNIED----VRKCFFGDYMD-----PNDEPML-RL-------YDEV-
MDVAALLSRVEELLMDHN--ATSKRP-
MNLAVFLYAVEHVSRIARVLKQPGGHLLLVGVGGSGRQSLARLAAFICGMETFQVEISKSYGPAEWRED
LKRFVRIAGGD-NK--
QAVFLFSDTQIKAEAFVEDVNNLLNSGEASTGFMLYVPNMFPYDERAAVLEQCRN--
SSKKEGLALESAVDLWAYFVDKTRDNLHIVLAFSPIGDAFRERLRQFPSLVNCCTIDW--
FTAWPDDALEAVAMKFLRD-VDLT-PDQ----
RNQVMLQCKMFHEDVRHLSDAFKAEQGRINYVTPTSYLELITCFTNLLGAKRSEVSAAKRRYEVGLEKL

```
DFTASQVSIMQDDLTALKPVLIKTVAETERLMATVSKEKTEVVEPKKAIVDEDVKKAEASAGAANAIKT
ECEDALAEAIPILEAAISALDTIKPADIKLVQSFKNPPGAIKIVMEAVCVLLDIKPQRVKDPGGSGKMI
E-DYWGSAQKLLADP-KFVQTLKEY----DKDSVPPKIIERIRRDFTSNPE--
FTPANAAKASSAAEGLCKWVCAMDSYDKVAKVVAPKRAALAEAEAEYAKVMEALKAKQADLADVMGRLA
TLEEQLEKSMAEKVRLEAETELCTAKLERAEKLISGLGGEKTRWTEAAAKMGAQYECLTGDMLIAAGVI
GYLGAFTAAYREQMVNK-WVAACAGAKIPRSAT--F-
SLSAALGDPVKVRGWTIDGLPNDSFSIDNAIMVDNG-RRWPLMIDPQSQANKWIKTMEGR--RD--
LRVIKLTD-SDYMRTLENAVQFGLPVLLENVGE-ELDPSLEPLLLKQVFK-
QGGLSYLRLGDTTVEFSDQFRFYITTALRNPHYLPETAVKVTLLNFMITPDGLADQLLGVVVAQERPDL
EEQRQQLVVESAENKKKLSEIEDRILHVLSSSQ-
GNILEDATAIQILSEAKAVSNEIQIKQEAAEVTQKAIDEARTGYAPCGAFNATLFFCIRDMAAVDPMYQ
YSLAWFLKLFVRSIQQAA---------------KA---------------------------------
-----------------------------
DNVSERLGHINDHFTYALYQNICRSLFEKDKLLFAFVLASRILMSGTES-
QLDPHAYAFLLTGGVGVAER-D-VTRPSGADWLSAKAWGELCRAPNISP----------
AFEGLPELVAGCPDEWKTLFDSVEPQNVDLPLGF-SDRL---
GPFDKVLLLRMLRPDKVVPAVQHFVASSLGPKFTEPPPFDLAGSYDESS-
CTVPLLFVLSPGSDPTAALLQF-AESRGY---
GAKISVISMGQGQGPKAAALIEAARKAGTWVLLQNCHLAPSWMT-ALEKICE-SIRPD--
NTDADFRLWMTSMPSPA------FPVSILQGSVKMTNEPPAGLRANLRRSYALEPLCNQ-DFFE--
GCRQP----------------KA-FKSLLFGLCFLHAFVQERRKFG---------
PIGWNIPYGFDDGDLRISVRQLSMYLDDAAPQPLEDGSRPQQAA-D----------------------
-------------------------------------
TVPFAALQYAIGECNYGGRVTDDKDRRLLLTALQRIYRPEAL------ALDTDKPFALSASGTYVI-
PPEG--PHASFLDYISTLPIFPL-
PEAFGLHENADITKDLQNTNLMLDTLVLTGGGAGGGAKGGGGGGGASDEEMVAAMVDDILKRLPPNF-
DIEKAQSKY---
PVRYEESLNQVLCQEMLRYNRLLAIIRSSLINLGKALSGLQVLSSELDTVLRSMALGQVPALWRGKSFP
SLK---PLASYVSDLLARLDMLQS-W----------YEH-G--
PPPVFWLSGFFFTPSFSTAALQNYARKH---KLPIDTVGFDFVMM--
GTDPAAYTEPPEDGVFVHGLFLEGCAWDASAKQLCESRPKVLFENAPVIWLQPRPADQFGEYE------
----AYDCPVYRTAERKGVLATTGHSTNFLMMI--RLPTQQPQ--HHWTLRGVCMLC--SLSD
>Cr_DHC6-IAD3
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
-------------MHPLL---VPSHQ-------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------
NLVAPADLVASFQEFAW-----LVSASVGEYVDAWAEA-----------
GHSLAETDAEVGRLAALGEAVAAKAEGLVAFRLLAVDCSGAQQWLGDKAAALRNALLTWQADTWHAANV
AAVEQFEAIVARL---------------------HQAPDSTEALDELDKFVEKVDGRMPEFEGV----
----VAESRAVADVLHGAQHELAME-----
AFETHWRLLRWPLDLAGELIEVRSQLARWRGRYLSQLKADSAALIADLAELQAEVERFVTLG------
DLKQVDDRTATVLDIEN-------RLNSYAELSELYGNREEIFGLPRSEYP---
TLEDIRKAFAPSSDLWKIASEFARSLPEWLDGPFTEI----------
DAEAVAADVDRWWRSTAKLGKQLD---------KEPLEVVTA--VRAKLEDFQVHLP-
LITALRNPGLRDR-HWERIST-AAGFPVRA-D-----AGFSLSRALQLG--
LPKHLAAIEEVAEYASKEYSLERALDKMQADWAGVVFETMTWRAT---G---TTILRA--
LDDVQMLLDDQIVKTQSMRASPYIGP---FEDRVRLWESKLGLVQETLDQWLRCQQGWLYLEPIF-
GSEDIMQQMPNEGRKFKA--
```

```
VDHTWRRTMEKLSKNAEVLVTCADDELLKALAEANKLLEQVTKGLNDYLETKRLAFPRFYFLSNDELLE
ILSETKDPTRVQPFLRKIFEGINALEF-------QPDGTVTAMSSEEG-ERVPFKDTF-NP----
RESQGQVERWLIECEIAMRTTLKHTILKSFNDYAKA-----------P-
RINWVTSWPGQVVIAVDCMYWTKEVGEAIA------KQALQDYSQQC-TDELMKVV-NKVRGK-
LSKLDRKTLSALIVIDVHARDVVAQLASTG--VTSESDFAWNSQLRYGW--EGGEV-----------
--
SVRMINAQIYYGYEYLGNGSRLVITPLTDRCYRTLMGALHLNLGGAPEGPAGTGKTETTKDLAKAIAMQ
CVVFNCSDGLDYQA--MGKFFKGLASSGAWACFDEFNRIDLEVLSVIAQQILTMQRAKSAG--------
----------VSSFEF---EGTKLSLRPTFSVFITMNP---
GYAGRSELPDNLKALFRSVAMMVPDYALISEIMLYSSGYLKARDLARKLVATYRLCSEQLSSQSHYDYG
MRA-VMSVLRAAAANKQRS----------P------DTL------
EDVLMLRSIRDVNEPKFLAPDLPLFAGILSDLFPGV--DLPATDYSALDERLVSNCTRLN---
LQPTPTFLEKAHQLYEMILVRHGLMLVGYSYGAKTCIYRTLAGALADLET-AGLMEE------
HKVQLRIMNPKSITMGQLYGQFDPVSHEWKDGVLAKLFREAAVD--TS-
TDRKWVLFDGPVDAVWIENMNTVLDDNKKLCLNSGEIIQM-
SASMNMIFEVQDLAVASPATVSRCGMVYVEPEQMGWRPLRDS---WMHTL---------PPTLGEEAR-
---------------------------------AHLIALFDWLVDPCVAFI---------
RKNCKELVPTADISLPNNLMSTMMSLMDELKDP-----------------------------------
---------------------------------KQAAAMAARDVLKQLDGMFVFSLIWSVGATT---
-DSDGRIAFSDFLRKLLDGGVDRKMTRTDFDLGPGLEIADPGFKLSTQLPK---DG---
LVYDYVWDKPRA-------AWRHWMDS--
GSGESAAAAIPESANFNEIIVPTVDTVRYTWLLTLLATHGRHVLFAGPTGTGKTAYVKSAIE-
ALDKSLFTNQ-QTAFSAQTS-ANMIQDIID--ARLDKRRKGI-FGPPVGMRCVV---FVDDVNMPALE-
VYGAQPPVELLRQFLDHSGWYDRADN--TWRQLADMQLVCAMGPPGG-
GRNPVTPRFVRHFNLVAITEFDDATYTRIYGAICDWWFR-RARLP-EDVRG-
KGGALVKATLEIYNTIRAQLLPTPAKSHYTYNMRDLSKVFQGVQSIGVPVP-----------------
DTRSLTRLWAHETLRVFHDRLVDDGDRTWYCTLLKDMVNKH-L-GLK-FDNVFEPPAGSGLAKGDVAV-
-----LRNLLYCDFQV-----PGADP---AK-------YDEV-TDLPKLMTVVQDYLADYN--
AQSKNR-
MDLVLFLFAAEHICRISRIIKQPYGNALLVGVGGSGRQSLTRIATFMADYKLFTIEISKSYTVNEWRED
LKRVLRQAGGA-GQ--STVFLFSDSQLKDESFLEDINNILNTGE--------
VPNLFAKDEVVGIMEQVTA--RAKRAGK-
PLTPASLFAFFVDACRANLHMVLAMSPVGGAFRERLRKFPSLVNCTTIDW--
FSVWPSDALKSVASKFLHD-VEMTDDAT----
RSAVEDMCMEFHVNVRALAVEFKAELGRHYYTTPTSYLELIQTYKELLGSKRKQVHGLKRRYEVGLEKL
LAAEQDVGAMKEELIALQPKLIETGKEVEETLKIVDRQTQE-
AEAKKVVVQGEEAVANEKAAAAKAIKDECEADLAVALPLLESALKALDTLTKADITEVKAMKNPPKAVK
VVMEAVCQMLSIKPNKINDPANPSKKIN-DYWGPSQGLLADT-KFLDTLRAY----
DKDNIPQPIIAAVR-PYLDVEE--
FDPPVVKKASNAAYGLCCWVRAMESYDKVAKVVAPKKAKLAEAEAEFNELMVGLNAKKAELAELEAKLA
ELNKKLAEMQARKAQLEAEVDLCEKKLDRATKLIGGLGGEKSRWTEVAHKLGGDYINLTGDVLLASGFI
AYLGAFTAAYRERATSS-WVALCRERHIPCSDS--F-
KLVTVLGEPVKIRDWTIDGLPNDSFSIDNGIIVSKA-RRWPLLIDPQGQANKWIKNMEKK--NK--
LEVMKLSD-GDYIRRLENCIQFGTPVLLENVGE-ELDPTLEPLLLKSVFK-
QGGGLCIRLGDATIEYSESFRFYMTTKLRNPHYLPEVSVKVTLLNFMITPEGLEDQLLGIVVRLERPEL
EEQKTKLVLAGAENARQLKEIEDRIIEVLSASE-
GNILEDETAINVISSSKLLSNDIAQKQQIADKTERTIDETRLGYKPVARHVSVLFFCISDLAAIEPMYQ
YSLLWFVNLFEDTIARAE--------------KS---------------------------------
-----------------------------
KDLTRRIEALISHFTHSLYINICRSLFEKDKLLFSFALTVSIR--
AHIKQQLDLNLFRFLLTGGIGTAEP-P---PNP-SDWLADKCWSEMCRLAEHFE----------
AFKALPDSFRDDPGPWRAMYDSADPTKFKLPEPW-HSKL---
DGFQKLLVVRLIRPDKLVGAIQAYVHEVMGQKFIEPPPFDLDRCYQDST-
CLTPLIFVLSPGSDPMSGLLKY-ADAMRI-----
NVESISLGQGQGPKASKLIEAAQEAGGWVVLQNCHLAVSWMP-TLERLCE-GLTLE--
NTAPAFRLWLTSYPSPS------FPVSVLQNGIKMTNDPPKGLRANLLGSYLSDPVNDP-SFFE--
GCNRP---------------AP-FKKLLFGLCFFHAFVQERLKFG---------
PLGWNVPYQFSAPDFVISARQLQMFLNEMPAGDPAA-----------------------------------
---------------------------------------
PVPLPALRYLTGECNYGGRVTDAHDRRTLMSILDIFYTPAAL-------GEG---YAFSPSGRYYA-
PPEG--HAESYLGYIRGLPILAD-PEVFGLHANADITKDQQETDLMLSSLLAASSGGGGGGGAGHGR--
---DALLAEVAADIMARVPQPF-DIEAVRFKY---
PVDYFESMNTVLCQELVRFNRLLEVVHESLAGLQKALRGLVLMSGDLEALGNAMYDGRVPKLWMDKSYP
SLK---PLASYVADLIERCRMMSD-W----------VEH-G--
```

```
PPPVFWISGFYFTHAFLTGVKQNYARRQ---RIPIDTITFTYTCM--
QGHADDYKTAPEDGALISGMFVEGARWDPESCKLQESLPKVLFSPAPLIKLSPCDAAEQATFP------
----HYECPLYRTPERRGVLATTGHSTNFVMEL--MIPSDQPQ--DHWTRRGVAFLL--SLAD
>Cr_DHC7-IAD5
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
----------------------LQVVP-------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
FAVIDQRDFYTMSVRGITHYLDGTSADFATLDQWERELHLFTRLKQLKMFRL-------
YKLWKAFRLWKR-------------AVNQHKFGKARAAL----ERNLFALS-------
PVFQDPLRRFHGLCFELSHMRMQ-----------------------------------------------
--------------------------NLAPGAVYTLDEFVSEQSEHKVEVEGRLMQ------------
----------------FHQDTFNTVLQACRDDLDKLEERLAEFNKKHDENETQRAA------------
----------DASKLGHINFLAKAE----------------
AEKEREQGQSDFAYTIAAARRSEQRRLLNFIRLADYMVCDTLHSVLL-----------
ESLRELLAGTRPKRPVLVRRRLWRLW----RRRRHCRGW-----------------------------
------------------RGDALFQLELLLADNQEDLLYDPHPEEF-
FEEMQRIMANYLESLCGITRLYGDGTLMGVVMGDKAGEVEPGTE----------
LQELITNENYDDLVSQQWRGGGRAEARVWAEARVCEPGLRTRAEARAAAAAAAAAQ----------
RVTLSTFRQLMDLLAEQKAAME-
ALQPHRDVSIVRVAVGHFKDAVIPSPTACLEEMHAMLPALAADLFHEFMSTVQNALSRL----------
------------QAPSSAVEEYVEKLQFLGEVREAEKRLDAA--------
CNEIHDLYGLVDEYDIPVSAM-----
DRAAYATMDSTYNTLKTTMEEVEGARDDHVAKYSANLENGIEAISKEVVEIRNEAQHEMVLS------
DESEHDKVVAYLQQLKE-------AVDRLIAESSRINKYQRLFKVRAL----------------------
--------------------------------DMAVLEDTTTRFHKMVYKMERGLP-----------
PNRLVPQ--FRTAVDDFRNLLP-VVAALRNRALKDR-HWSKIFD-TIGQQLVR-D-----GSFTL----
------------------------------------------------------------------------
--------------------------------QRQLTLFADTLDEWVAVQKAWMYLEPIF-
SAVDIQRQLPVEAKAFFQ--
VDKQLREIMRRTKDRPNALMAGTHPGVLETFQKANETLEKIQKNLEDYLETKRMAFPRFYFLSNDELLE
ILAQTKNVQAVQPHMGKCFDGIRRLDF----GEDPKSIDIFAMISGEG-ERVSLGKNT--------
KARGNVEKWLGDVESAMIANLKKLAKAGWVSYPEE-----------S-
RSGWVLKQPAQLVIAVSQVYWCHGVEDTLR--SPDPHQSLGDYRTIN-VRQLADLT-RLVRGD-
LSGLHRRIIAALITIDVHARDI-----------------------------------------------
--------------------
PRLVVTPMTDRCYLTLTGALHLKLGGAPAGPAGTGKTETTKDLGKALGVNCVVFNCGDNLDYKF--
MGKFFSGLAQCGAWACFDEFNRIDIEVLSVVAQQLLTIQNALKAG------------------
MSSFNF---EGRMIRLVPTCGVFITMNP---
GYAGRTELPDNLKALFRPMAMMIPDYALVAEVMLFSEGFEDSKTLSRKMVKLYKLSSEQLSQQDHYDFG
MRA-VKSVLVMAGSLKRAN----------P------DLS------
EDVVLIRAMRDSNLPKFLVDDVELFQNIISDLFPGL--VVPDQDFGELEQSIRAVLGTRG---
LQQPASFVTKVIQLYDTMNVRFGVMLVGPTGGGKTECYRTLQGACTRLRAEVRHPSEK----
YQVTHTYTFNPKCIKMGELYGEYNLLTNEWSDGLGSTLIRSAVAD--TT-
PDRKWVVFDGPVDAIWIENMNTVLDDNCTLCLPNGERIKLNPTTMRMLFEVQDLAVASPATVSRCGMVY
IALEELGWRPYIQT---WAAQK--------LPAAIPQTAG----------------------------
--AVAEAIYAMFDTYVDAGLEWV---------RRKGKEYIATVDNNLTTSLAYLLQSMLSPSR------
------------------------------------------------------------------
GFQWSAKPEEVTMALNRIFGFCYVWALGGNL----VHSAKEEFDEFARDHLQTLC--------------
------------AFPG---SG---SVFDHHLDCQKRFPP----EFKHWTEV--VPVFSYR----
RDLPYFQMMVPTVDTVRFSYLLEACLDVQRSVLFTGVTGVGKSVITVAALEDLRERKHVVPY-
TINFSAQTQ-AVDTQLLIE--SKLEKKRKTR-YGAPVNKRIVF---FVDDVNMPARE-
KYGAQPPIELLRQFQDFKGFYDRKKL--FWKDVEDTTLCAACAPPGG-
GRQEITPRFARHFTMLCVPPPSDAATKTILVCVLIFVCV---RVC-GCLAQ-
LRAGVLAASRRHEYESMVELLPTPAKSHYTFNLRDLSKVFQGLLMITPTTCQ----------------
SKDTLMRLWLHESCRVFHDRLINNEDKEYFKKMLVELSGKHGL-GSASYEELFGH--------------
-----GKYVMFGDWIK-----MGLDREE-RR-------YEEIGQDVSKVVAVLEEYLDEYN--
LSNTNA-
```

```
LNLVFFMDAVEHITRIARILRQPRGNAMLVGVGGSGKSSLTRFAAFMGGFKTISIELTRGYGSNEFRED
LKKLYRTAGID-GE--SVVFLFSDTQIVQEGFLEDINNMLNSGE--------
VPGMFAQDEKDRIVADIRE--WVTATGG-
NPSKDGCYTAFVNRVRDNLHLVLAMSPVGEAFRARCRQFPSLINCTTIDW--
FSAWPEEALLSVSQKFLAG-TDLGGDTV----
RDALANMCVTIHTSVSTASDRFYAELRRRYYTTPKSYLDLINLYLQLLNDKREELATAKDRLLNGLNKL
NDTNALVDRMKLELAGLQPILEEKAQATSELLVKVAADQEQ-
AEKVKKVVAAEERDVKAMQVDTQAMADSAKADLDEALPALNAAMAALKALDKNDIVEIKSFSKPPAAVQ
TTMEAVCVLKGEKAD---------------WDTAKRLLGDS-NFMRSLEEF----
DKDNIPDAYIKKLQ-KYVEDPT--
YTPDLVAKVSKAARSLCMWTLAMNTYHRVAKVVEPKKMALRTAETQLAEANAKLEDKQAALREVEERVE
GLRNQLANAQREQRELADQADLTRKRLERAGKLTSGLADEGVRWKATAETIGEQLIKLVGDVFLSSACI
AYYGAFTGAYRQQLVSG-WIAECKERGIPVSDN--A-
TLRTTLGNPVEIREWNIWGLPTDDVSVDNGILVTRG-KRWPLMIDPQGQANSWIKAMEAR--NG--
LRCIKLTD-GNFLRTLENSIRIGNPVLIEDVGE-TLDPALEPVLQKAIFK-
QGGRTLIRLGDSDVDYDPNFRFYVTTKMSNPHYLPEVCIKVTIINFTVTMKGLEDQLLGEVVRKERPDL
EEAKDRLVLSISADKKMLGELEDKILKLLKESS-
GNILDDEQLINTLNHSKTTSSVISARVQEAEVTERSINEAREHYRPAATRGSILYFVVADLSLISPMYQ
FSLSYFAKMFSYCIDKSE--------------KA---------------------------------
------------------------------
DDVPTRLHLLSDFVTRFIYNNVSRGLFEEHKLLYSFLLCTSILRHESAGPEITAPEWSFFVRGAGAAAA
E-P-PRANPHPEWLTQAMWTALFHLEHVLP---------
DPFVGLVENVVAAGPEWRAWCEADEPHQLPLPAGW-
EDRVAQKRPFCKLLLIKIFREEKLIFACAQYVSGKLGAEFTEPPPWSLDDVFPDTS-
CRTPIIFILSTGADPTAMLQRF-AEKNGY-
VTGERLHMISLGQGQGPIAEMLINQAVKTGDWVCLQNCHLASSWML-RLEEKVE-
ELSKDSSSVHPEFRLWLTSMPSKV------FPVLVLQNGIKLTNEPPKGVKANVNRTYNDL---TL-
EALA--ACPAK---------------PGPFKKLLFALSFFHAVVQERRKFG---------
PLGWNIRYEFNTSDLECSAMTLRMFLSEQ----------------E-----------------------
--------------------------------------
NIPWPALEYVVGQINYGGRVTDDLDRRCLMSILRQYITPRVL-------DEA---
YKFTPSGLYYSPNAEG--SLDDYRDYIRGLPVTEA-
PEVFGMHANANISFQLQETRKLVDAVLSIQPRLSGGSSGKGS------
DEVVSELAAELQAALPPLLTREEAALGLFDRTEAGQLNSLSVVLGQEMDRFNRLSSAMASSLVELQKAI
KGLVVMSGELEAMYNSMLNNQVPEAWARYAYPSLK---PLASWVKDYHARITFM---------------
------------------------------------------------------------------------
------------------------------------------------------------------------
-----------------------------------------------
>Cr_DHC8-IAD3
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
------------------------------------------------------------------------
```

----------MP-LVEELANPALKTR-HWEQIFK-
LIDADIPPNDNGVGYAPFSVRMLLQYN--
ALDRYEPIQSISGVASKEYSLEKVLEKMSKDWEGVEFRCIEYKDT---G---TYILGG--
TDEIQALLDDQIVKTQAMRASPYIKP---LEEQATKWEGMLTTLQDMLDNWLTCQATWQYLEPIF-
SSPDILKQMPEEGDKFQV--
VDASWRELMETTSEAPSCTKVAEERDKLMSLQEANRLLEEIQKGLAAYLELKRLAFPRFFFLSNDEMLE
ILSETKDPTRVQPHLKKCFEGIEKLRF------EGPHAEITGMVSCEG-ELVPLKTRIKPA-----
DAHGSVEKWLVQVEAGMVESVQESCRRGVLSYPAA-----------Q-
RSNWVLEWPGQVVLVVTAIFWTRQVVAAIS---SPDKAALKECAEQN-TSQLGEIV-SLVRGE-
LSKLNRATLSALVVMDVHARDVVVALAAEKAVAGDPNHFSWLSQLRMYWEADEGTI----------
--
MVRMMNAEVEYGYEYLGNSMRLVVTPLTDRCYRTLISAIHLNLGGAPEGPAGTGKTETTKDLAKALARQ
CVVFNCSDTLDYQARTMAKFFKGLASSGAWACFDEFNRIDLEVLSVVAQQVLEIQLAVKQR-------
----------VKSFFF---EGTELPLRPTCNVFITMNP---
GYAGRSELPDNLKALFRTVAMMVPDYALISEIMLYSNGYLQARECARKIVATYKLCSEQLSSQDHYDYG
MRA-VMAVLRAAGNLKRRF----------P------DSD------
EFVLMLRSIIDVNLCKFLSHDVPLFNGIVSDLFPGV--VLPEPDYGHLMEAMKRQCALHN---
LQPTEYFLMKTIQLYEMVVVRHGLMTVGQPFSGKTASLKVLAGALSDLHD-RGITGAL----
YSRVQLRAINPKSVTMGQLYGENDKATQEWKDGVLAVTFRSLAAD--PS-
EDRKWLVLDGPVDAIWIENMNTVLDDNKKLCLPNSEIIQM-
SSTMSMIFEVGDLAVASPATVSRCGMVYLEPHQLGWQPLLAS---WINTL---------PRTLGPKIR-
---------------------------------THIEKLFEWLVPPCLRFV---------
RHDAKEVSPTEDIGLARTTMRLMAALLTEDFGAPLSIAAMAA-------------------------
-----------------------------------ADKAEKAAADKTVLVESVFLFALVWSLGCTG---
-DGESRRKFDAFLRSMMSGVI--PDGYADYTTAPK-------VHMAVPLPS---
PDNGHTVFDYCLHKRSAAKGLVPGRWQLWTDT--IPELDIP----
ADAQFADIIIPTKDSARYTFLMDLALQHNQPLLMVGPTGTGKSTYINRHLVSGLPREKWTPI-
FITFSARTT-ANMAQDQVD--GRLDKRRKGV-YGPPPGRRAVV---FIDDLNMPAKE-
TYGAQPPIELLRQMVDGGGWYGRDN---AFRSMVDVQVVAAMGPPGG-
GRTFVTNRFLRHFNVLALAQVSEDSLVHIFRTILDWHLS-KSAFP-GPVAA-
LAPGLINATLEVYTQSMAKLLPTPTKSHYVFNLRDFARVVQGVMMLPRESLPRPADPMDDSAGGGAAHE
GVALYRRLWVHEVFRVFYDRLVDDTDRQWLMGQVKATVSAH-L-DTG-FDGLMEGLLSAE--
DKKAGRAITHEDMRRCFFGDYAD-----TNEPEPSLRK-------YAEV-ADVATLLTTMEEYLHDHN-
-GTSKRP-
MNLAMFLFAVEHISRICRLLKQPGGNMLLVGVGGSGRQSLTRLAGFICGMEVLQVEIAKSYGRTEWRED
LKKVLRKAGAD-MK--NVVFLFSDTQIKDESFLEDINNILNSGE--------
VPNMFPQDERMTIMEAVRP--RAAKRGL--
ETPLELWGYFVETCRRNLHVVLCFSPIGDAFRERLRANPSIVNCCTIDW---
QTWPRDALEAVAFKFLRE-MDLD-ETT----
RKQLVQLCQAFHSRIRAASEDFKHQLGRHNYVTPTSYLELISTFRTLLDSKRAANRKAHSRYSVGLQKL
QSSSEQVAGMQAELQALQPQLVRTVAEVENLMNVIAREKAEVVEPKALIVKGEEAKAQSKADAAKAIKD
ECEADLAEALPILNEALAALDTIDEKDINYIKKLGNPPMIIKLVLEAVCVILDVKPAKIKD--
DTGKTIP-DFWKPSVGLMNER-DFLQRLKTY----DKDNIPPRIIGEIREKYVKNEA--
FTPAAARNASPAAEGMCKWVHAMSSYDRVAKVVAPKKAKLAEAEGQYEEVMVGLRAKQAELADLRAKLA
AMESDLRTNTKKKERLEQEVALCSIKLERAEKLIGGLGGEKLRWTESAQALKDAAVSLTGDMLVAAGII
AYCGAFTASFRLDVIES-FVDMVQSAGIPRSPR--F-
SLATALGDPVKTREWLIAGLPNDSFSIENGIIVAHA-RRWPLMIDPQGQANKWVKNLEKE--HK--
LQVIKLSEGGDFLRVLENAIQFGLPVLLENVGE-ELDPSLEPLLLKQTFK-
SMGVTCIRLGDATIEYSSDFRFYITTKLRNPHYLPEVAVKVTLLNFMITPAGLADQLLGVAVATERPDL
EEQKAQLVLQGAENTRRLAEIEDKILEVLSNST-
GNILEDETAISIITEAKTLGNEIQEKQRAAEVTEKEIDLARTGYKPCGDYTSILFFCISDLANIDPMYQ
YSLPWFVNLFVASMHAAE---------------PS---------------------------------
----
NDLQRRLENIYDHFTYSLYRNVCRSLFEKDKLLFAFLLCTRIL--ETKG-
RVDMDEYMFLLTGGLGAADH-R---ANPAPDWLVERGWRELCRLDKLPT-----------
FMGIADAFADDPEAWRPLYDGMEPHRVTLPGLY--NSL---
DAFRKLLIVRCIRPDKVVPAVQDFVDANLGKKYVEPPPFDLAACYADSA-
PTTPLIFVLSPGSDPTAALLQF-AGERKM---
ASRLMAISLGQGQGPKAAAMIAEGAKAGSWVVLQNCHLAPSWMT-ALDKICE-ELQPD--
TTSPEFRLWMTSYPSPK------FPVNILQNGVKMTNEPPKGIKANMRRSFMIEPICQDKDFFE--
ACGKP---------------AP-FKKLLFGLVFFHALVQERRKFG---------
PLGWNIPYGFDDGDQRISVRQLKMFLDESAP-------------GA----------------------
----
APPFAALRYVTGECNYGGRVTDDKDRLLLNTMLEKCYCPDII------NYED---YKLSSSGLYYA-

```
PAEG--DRASYLAYIDTLPINPL-
PEAFGLHANADIAKDQNDTAAMFASLLSMTGGSGGSGGGGGGGAAGAAEERVAGIVSQCLARLPPQY-
DIEAIQRRW---
PVKYEESMNTVLAQEASRFNKLLAVLHESLANIQLAIQGLLVMSSELEEAFASIAINQVPELWKRRSYP
SLK---PLGSYLDDLYERLNMFTA-W----------AAG-G--
PPPSFWLPGFFFVQSFLTASLQNYARRR---KVPIDTVGFGFEML--
GMEHAAYKTAPAEGVYVHGLYLEGCGWDAGPQRLCESQPKVLFVNAPVMWLRPQSADKKFDYP------
----HYDCPLYRTADRRGVLATTGHSTNFVMFV--KLPTDQPA--SHWIMRGVALLT--QLSD
>Cr_DHC9-IAD3
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
--------------------------------
MDFSMALADAWRDPYVKALPEAHPLVWSLKAGKSGQADVPQGGTDPYTATLPEVLDNFARRHEAKMAA-
---EITSQRRKLPPVLNDRSLFTRAAPF--------------------------------------
------------------------------------------------------------------
---------------------------------
GNKDDPFYHSPSNRIGNNFSPESMRAAAILFEEFPQDPNAPRTRGKAAGAALTDP-------------
----AAKRPADELAAGDGGERAAKAARAALADVPVDALLDAIL-------------------------
-------------------------------------RYHWYVEA-------GVDGRHVAPFR-----
------------------------------------------------------------------
-----------EEWAGNALGMAYYDALLRSSLEEMHAEYVG---------------------------
---------------------AMRRCMVDYVVRSPVERQR-----------------
LALTPLEPLLALPGPTQRSWRAVVDRTLSPGWHADVDTAREEVAWTLQTLSANALELSKLWHASRFSSA
LLVDVSSSDFAARL----------------------------------------------------
-----------------------------------------PLYADAFYQQQT-----------
EVMELVKGQLWSSWAPKSLEVFNRLPPVYING-----------------------------------
--------DADAYYRSIATLQSNQLRGLVARSLESYVSLFAAH------------------
PPRQEVDPQGDTLLWSAPPVFVLDLVELEGKP---
AFKPTFQECEDAVRAVLDNAVLAVCGLPRVGSNTEESVV----------------------
AAKKAEIERLRAAAAAIMTGEVGKPSTTAE----------------ELLALKKLPIPDD-----
DLDVAIKAYEWPRKMVEVMRDANAKVASEHKDFEAQLKARRKDFGELLEAYGREVEGYAHKN------
EIVKRDQIASEVAELAE-------KLKAAQAEADLINGRERLFGWAATKYG---
GIAKLTSTLEPYVILWTTVSQFYDKFASWMNGPFYKL----------
NPEEVEADTNDAFRRLYKLTKVFSGSGGATELREAPLAVAEE--AKGRVQSFQEHLP-
LITAICNPGLRER-HWTMLAD-IVGFEIKR-D-----EVTSLKRLLDYD--
IAMHISKITDISDSASREWSIEKALDKMISDWQGLSFELGPWKET-------GACVRL-----------
------------------------------CVRVADILDQWLKCQGKWQYLEPIF-
GAEEIMKQIPREGQAFRD--MDAIWRRIMNT-------------------------------------
---KMAFPRFFFLSNDEVLEILSEAKDPLKVQPFVKKCFEAVKEFTF-------EKSGEISGMLSVEG-
EKIQWIETV------NPAATGAVEKWLLDSESAIRRTLHKVAGDALEGYAKT-----------E-
RSRWILEWPGQMVLNCSQVYWTREVTEAIT---TGGSRGLAAYADRC-TLELNKIV-NLVRGQ-
LASLERATCGSLVVIDVHARDVVAAMAREG--VEDARDFKWESQMRYYWEFNEQPP------
SGVHPQETLMVRMINAEALYGYEYLGNSGRLVITPLTD------------------------------
---------------------------------
SGAWACFDEFNRIELEVLSVVAQQVLTIIRAKALK------------------VKTFNF---
EGSEIRLVPTCNAFITMNP---
GYAGRSELPDNLKALFRDVAMMVPDYAMISEIILYSYGYLEARAMARKLVQTYRLCSEQLSSQDHYDYG
MRA-VMSVLRAAGNLKRVF----------P------DSA------
EDVLMLRAINDVNLPKFLDQDVPLFNGILSDLFPGV--ELPVVDYDNLVAAIKENCVKSN---
LQPLDSFIVKIIQLYEMIIVRHGLMLVGYSYGMKTAAYRVLAAALSDLHA-KGLNKE------
FHTKYYVLNPKSITMGQLYGAEDPVSKEWTDGVLAVVFRSTARD--TS-
PDRKWVIFDGPVDAIWIENMNTVLDDNKKLCLNSGEIIAM-
QGLMNMIFEVQDLAVASPATVSRCGMVYVQPALLGWRPVMLS---WLNTL---------PAAFGPALR-
--------------------------------QQLTNLFDWLVPPMLRVA---------
LKLVRSPQPMQDINLVASLMRLLECHLDEYRSDLPADAPPPV--------------------------
---------------------------------VNIKELTDPQQASLLQGCFLFSLVWSLGALA---
-DEEGRRKVDANLRKLLGHDP--LPEVASYVAAGT------PHKVTVPFPE---GR---
TVYEYVFDKTRL-------KWTTWMET--IESRALD----
VEAEYTSIIVPTVDTVRYTYLLDKLVTHNMHCLFVGPTGTGKTAYVKRHLQEGLP-ERFTSM-
LMTFSAQTS-ANMTQDIID--GKLDKRRRGI-YGPPQGKRMVI---FVDDVNMPQVE-
EYGAQPPIELLRQFMDHSGWYDRKEL--TMRKLVDV-----------------------
```

```
HVVSLTAFDTDNLSTIFSALVDWWLK-KYNYQ-
AGGVARFAKPLVAASLEVYEAAQRELLPTPAKSHYTFNLRDVSKVFQGITKAAGNVE-----------
------DGLSITKLWVHEVLRVFYDRLVDESDRRWMGATLAGLVERH-F-KEK-
MSKVLNLDSSGDVAPEQMVTA-----LRGLVFVDFMV-----PGADP---RV-------YAEV-
KDPGAMQRVVTEYLSDFN--ATSKKP-
MNLVLFRFALEHIARICRIITSPGGNALLVGVGGSGRQSLTRLAAFIQEYEVYQIEISKTYCKTEWHED
IKKVLRMAGES-NK--RVVFLFSDTQIKEEGFVEDISNLLNTYE--------
VPNLMQNSDLAAIFENIRA--RAKVAGM-
DGGRDQLYNFFVQEVRRNMHIVLSFSPVGDSFRERLRKFPSLVNCTTIDW--
FTKWPTDALHTVAESFLSS-LDGMEAAV----
AAQLPSLCVMFHQSVQELTDRFKSEARRHYYVTPTSYLELLLSYKSLLGRRQSEVMTVKRRYEIGLDKL
QVTEESVTGMKEELIALQPQLEESTRQTEAAMEVISKESVE-
ADKVKQVVSKEEATASAEAATVKAIKDECEADLAEALPLLEAALKALDTLKPADITEVKGMKSPPAGPA
RVKDTAS----------------GRMVD-DYWEASKKMLMEF-DFLDSLRKF----
DKDHIPPEVIVKIR-PFAQDPE--FQP--------------------------KVIEKHKVTLARSE-
------------------------------------------------------------------
---ETTTDNFRRLVREI---SQLLTYEAT-------------------------------------
---------RDLPMETIAIKT---------------------------------------------
------------------------------------------------------------------
-PIAPTQS-----------------------------PVLAGKK-----------------------
-------------------------------------------------------------LC-------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
---------------------------------LVSILRAGNGFLDGMLEL------------------
------------------------------------------------------------------
------LPAA--------------------------------------------------------
----------------------RVG---------HIGL-----------------------------
------------------------------------------------------------------
----------------------------------------YRDPETL----------------------
------------------------------------------------------------------
------------------------------------------------------------------
------------------------------------------------------------------
-------------EPVEYYF--------------------------------------------------
------------------------------------------------------------------
-------------------------KMPEDIQE---------------RI--
>Cr_DYH1A-IAD1a
-----------------------------------------------
MDRRLEWVKEKACLGLGVEPNLFEAAIANPESRARV-
TAFLDGTVTSSALLFALEEATIYVEEYQEVLAEEQAPEAEDGEGEEHDGQEPGEAGGEGAEGSTAPGDS
GDGQPEDAPAAAAEANGANPEDEAAAPADGAADGAAGEGGEEGDGAEGDEPPAPPAPKYVRRVISVPKV
VSKLNVALGSLPEELSVFPVFYFILNRSGHVAAEELDSAVEFGLLSEGPSLRILEQMLSSVFVPILVQM
SGGDVASGGVLMQSMTDNS------HRE-------------LLG-----
NMQKFHSQVTQALQQLTGDVTLQ---LPDFP-------
LEDMDRAAADTDLVMQLEQYMAEWSQVLASVLQRESQKHPT---
GKGPLAEIEFWRERNAVLSSLYEQLNLPQVKRMILVVEKGSDDRNLMAGF---
KSQLGELTKLATEARDNVKFLTTLERHFKNI-
ATGPLGGILDTLPPMMNALRMVWIISRHYSDDQRMGSLFQRIAREIGDRVEAAVDLRHIFRMTSADAVE
LLKVCKSVLEHWLQTYMAMREKI-ELSGRDARW-------
EFPKQLLFARTNYMAEICTDLIEMVEIVDDFFRFLGPELKTVTGDTAGIDRVVHRVV----AMYEPIE-
----SISFNVFDYGNQH--EWKAAKQQFYADNEDIKEATRELIDTSFRKLRSAEGA-------
CELLQSFKSIKSKGAIQKQVMNKFNDILEQFAREIEQTAD----IFERNKDA-------
PPVTKNQPPVAGAIKWVRSLLERLKRTMAKLLSTEEEIIRTTELGQAVESK--------
FKSFARSVMLTEKKWFSSWSDSINGVAMQHLKQTIFRRSAATNRVEVNFHPDLVRLIRETRYLDRMG--
--------------------------FPIPEIALNVALQEDKFLQWLEGLNSMLFKYYESIDQLTP--
---------------VERELMERKLEELESCLQ------PGFTILNWNS--
LGITEFIGTCDKAIATFQQLVKQVQKNSGIIEQVVYAIAGAQLVTEP--
EEGAEVMDLQEFYEDIERQRLAALESLVKKYRTISPLLGKIEEVVAGTNSGKSPALSSYYSFWERAIFN
ALNTMVLCAMTKLQDMIEQRSKHAEGGRKPPLFKVTVSLQSV--DVVVQPPMTEV-
NKALGRLVRSLVESTKAFVRWMDGTCVETPEQRGATDDDEPIVF--
TFYWDVAANPQVIKTMLNLNQSIQRAITSVNKYAESWRRHQA-----LWKTDKNSVLDKFKAR------
-----DPSAAQFEDKLSKYAKMATEIS-
AQAKDFDQDFIRVSCHALASSVCDEAQAWVRAIAQTMRELDAVTESQLRDKIAKYQTAL----------
```

```
------------HRPPDTLEELKQVLNTVNTIRGESMVMELR--------
YADLEERYRTRLLYATNPEEESQCAHELASASQVRALWTELLNEAEAVDWSLEETKKKFSETTRSQVSD
FAAITAELWEKFRTTGPGL------PTVELASGLDELHKYES-------
NLADALRQREQLVLAEKLFGMEITAYP---ELAQLESEIRKLAQVYGVYAEHAEAVRQYGGQLWSEL--
--------DVGKMMAGTEAILTKLRKLKSLK------------LLPVYEL--VEKEIQGFYNSLP-
LMKELKSEALRKR-HWTRLME-VTGQEFDM-D----PKTFTLGNMFAMQ--
LHKYAEEIGKITNAAVKELTIESEIRKLADVWREQRFELGKYMKG---PEDRGWVLRS--
TEDILVLLEDMGLNLQSMMASPFVRP---
FLTEVRAWEQKLSLIGECIEVWMHVQRKWMYLESIFVGSDDIRHQLPAEAKRFDN--
IDRQWQKIMNDTAKNTVVLDACMADGRLDLLKSLSEQLEVCQKSLSEYLDTKRCAFPRFYFISDDELLS
ILG-TSDPTSVQEHMLKLFDNCAALVF------GRGNKTITGMVSSEK-EGFEFRNVV--------
PIEGAVELWMTNVEAEMRKTLYQITKEGIFFYAKT------------P-
RTKWISENLGMVTLVGSQIWWTWETEDVFRRVRDGNKHSMKEFAAKL-TGQLSELT-SMVRSD-
LSNEVRKKVNTLIIIDVHARDIIDTYVRDS--IVDAREFAWESQLRFYWDRQQDDI-------------
--
LIRQCTGLFKYGYEYMGLNGRLVITALTDRCYMTLTTALTYRLGGAPAGPAGTGKTETTKDLAKSMALL
CVVFNCGEGLDYKA--MGSIFSGLVQCGAWGCFDEFNRIEAEVLSVVSSQIKNIQEALKND--------
-----------LTRFQF---EGKEISIDPRTGIFITMNP---
GYAGRTELPDNLKALFRPVTMVVPDLEQICEIMLFSEGFDSAKVLAKKMTVLYKLSREQLSKQHHYDFG
LRA-LKSVLVMAGSLKRGA----------P------DMS------
EQLVLMRALRDMNLPKFIFDDVPLFLGLINDLFPGM--
DCPRVRYPQFNDVVEADLADQGFKVLTEPSEQVDKVVQLYEVMMTRHTTMVVGQTGGGKTVILNTLARA
QTKLGK-------------
KTHLYTINPKAISVAELYGVLDKDTRDWTDGLLSNIFREMNKPLPAERDEARYLVFDGDVDAVWVENMN
SVMDDNKLLTLPNGERIRL-QNHCKLLFEVFDLQYASPATISRCGMVYVDSRNLGYKPYIYT---
WLNSR-----------AKQAEV----------------------------------
DILRGLFEKYAVPSVDWILEGIDGEELVRRPKQAVPVTNLNMITQLCNLLNATIT-------------
------------------------------------------------------------------
DHPRMSDPQILEAIFIFCTIWSLGAAIVQRPESPDRDRFDAFVKHIASMGL-----------------
----VDGERVAATQLPAR--SLYEYCFDTNEG-------VWKSWRSY--LQPYEPP----
ADGAFAKILVPTVDVVRSTWLLNTVVAAGKPCLFVGESGTAKSVTIANYLA-HLDSTINIVL-
NVNFSSRTS-SLDVQRAIE--DSTEKRTKDT-YGPPMGKRLLM---FIDDLNMPRVD-
TYGTQQPIALLKLFIERKGLYDRGKEL-SWKNMKDVQVVGAMGPPGG-
ARNPVDPRFISLFSVFEIQFPSNENLRTIYQAILSRHLA---
KLPTDEIRDQLGERLTDVTLELYNFIIDKLPPTPSRFHYIFNLRDLSRIYEGLLLTVGDVFK-------
----------TPEQFLRLWRNECLRVLHDRLISTDDKRVMTERLEALVQQK-F-PNL-AAHTLA-----
---------------SPVLFGDFKNVINELQGEGEVAPRM-------YDDL-
GDYNSIKPLFEDVMTNFY--NRKRKP-
MNLVFFEDALEHLTRIHRTLRLPQGNCLLVGVGGSGKQSLSKLAAFTAGCEVFEITLTRGYDELAFRED
LKRLYAMLGSD-NK--RVMFLFTDAHVADEGFLELINNMLTSGM--------
VPALYDGAEKDGLIGSVRA--EVEKKGL-
LATKESCWSYYVDKCRNNLHVVLAMSPVGETLRSRCRNFPGMVNNTVIDW--
FEPWPEQALTSVASVFLAE-EALP-EAL----
RPQIVEHMVTVHQSVRTFSTRFLEELRRYNYVTPKNYLDFINNYKRALATNRRTIEDTVTRLSGGLEKL
IQAAVEVDAMQKELSQAQVVVAQATKECNELLEVISTNTVD-
VETKAKAAAIKEAQLKVDSEQIAIEKAEAEAALEEAIPALEEAAAALQDLSKDHITEIRSYAKPPEQVQ
KVCECVVILRNIKDVS---------------WLGAKSMMADG-NFLRSLVEF----
DKDSLTDKQVKKVK-
EYFKDPKAPLTYDSLRAISTAGAGLLKWVLAMVNYNNVARTVEPKRKKVAESEKNLRIAQKDLASTKLE
LQSLNDQLGKLRTQFEEKTAEQQDLKAKADLMERRLIAASKLIAGLGSERERWTRDIADLESRRDRLIG
DCLLTSSFLSYTGAFTATYRHAMVYEMWQDDVKARGVPVTQP--F-
RLEALLTSDVETTGWASEGLPSDELSIQNGILTVRA-NRWPLCIDPQMQAVNWIKSREGKMLEG---
KVKTFND-SDFLKQLELSIQYGFPFLFENLDE-YIDPVIDPVLEKNLVP-
GDGKFVIKLGDKEVEWDSNFRLYMTSKLSNPHYGPEISGKTMIINYGVTQQGLTEQLLNVTLRHERSDL
EEAREALIKQMSENKATLQALEDTLLRELSNAQ-
GNILDNSELIATLESAKLKAVEIAEKLEASKVTAAEIEETRVRYSPAAKRGAILFFVIAGLSAITNMYE
YSLASFLVVFNGSLHSSR--------------RD----------------------------------
------------------------------
ASIEGRLRNIIDTLTYDVYAYTCLGLFERHKLMFSFQMTCKIL--EGDT-
PLDPQLLDFFLKGNLSLEKA-A---
RRKPFDWFPDAGWQDLMRLVELGQKKIGADGRMHALGSLANDVESDEAAWRTWYDLEAPEEAELPCGY-
QSFL---SDFEKLCLMRCLRMDRVTVGITRFVIGVMGEKYVQPPVLEYRSIYKQST-
ETTPIVFVLSPGADPAFDVFKL-GEEMGF-
RPGAKLKYMALGQGMGPKAQELIETGATRGLWIMLQNCHLLPTWLK-TLEKILE---KIT--
```

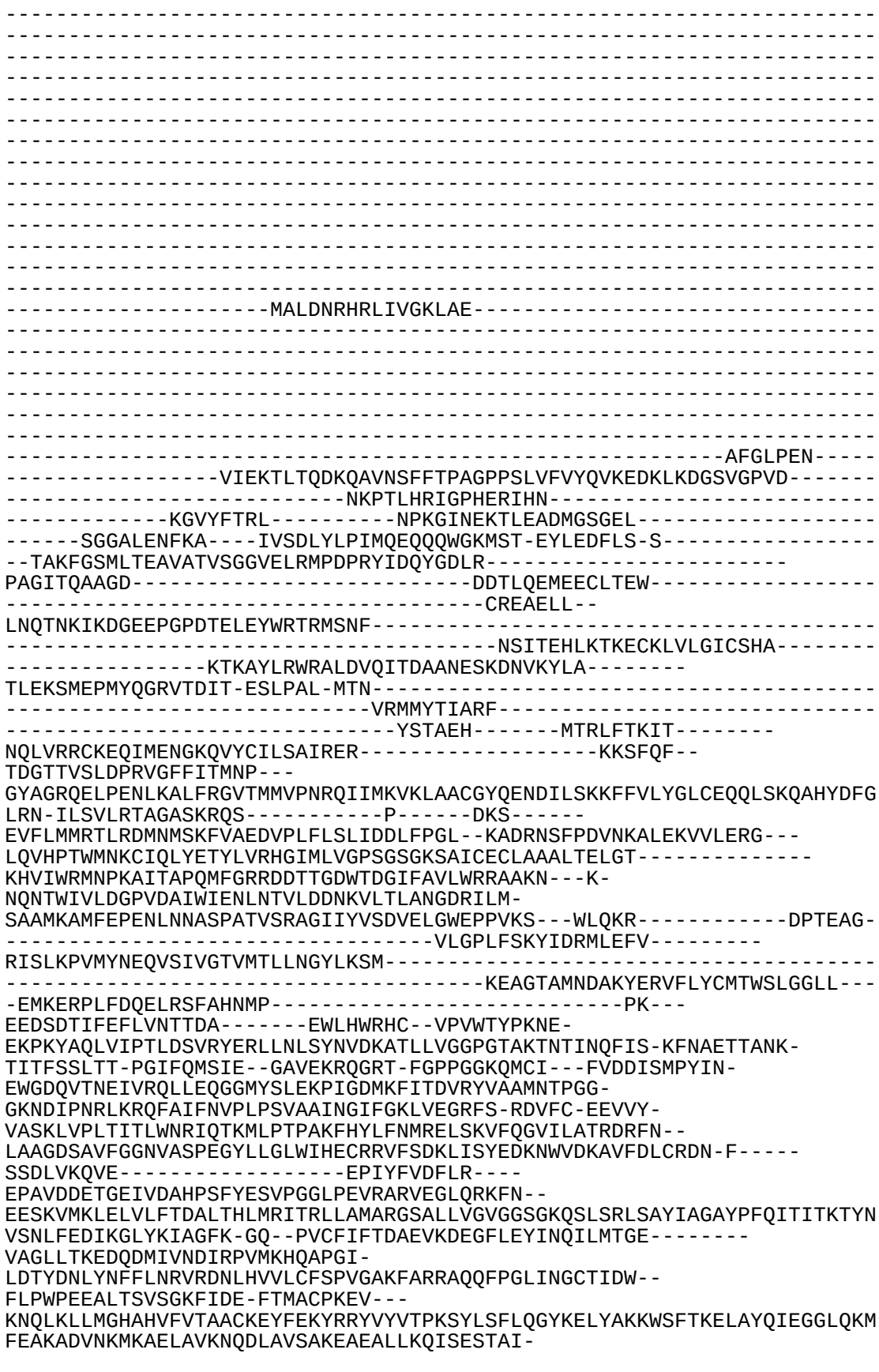
```
KPHADFRLWLTTEPTDR------FPLGVLQRSLKVVTEPPNGLKLNMRQSYSKI---TE-EVLA--
DCPHQ---------------A--FRPLVYVLGFFHAVVQERRKYG---------
KLGWNVPYDFNETDFRISMALISTYLTKAWDAQ----------DD----------------------
-------------------------------------
LIPWGTLRYLIGEAMYGGRVSDSYDRRILTTYLDEYLGDFLF------DTFQPFRFYACKDYEIAI-
PQTG--SRDTYLKAVEALPLVQS-PEAFGLNANADISYYTSATKAIWTDLVDLQPRTGGGGGGVAR---
---EEFIGGVARDIAAKIPEPF-DLPQLRKEL-----
GTPSPTQVVLLQELERWNSVLGVMVSSLRDLQRALSGEIGFSSRLEELASSLYNGKLPAMWARLNPATE
K---ALGAWMLWFGRRYRQYKD-W----------TEH-G--
EPKVIWLSGLHIPETYIAALVQAACRDK---GWPLDKSTLYTKVT-
KFTDPYQVSERPKYGCYMSGLYLEGAAWDLEASQLRKQDPKVLVNELPILQVIPIEANKLKLAN-----
-----TFRAPVYVTQARRNAMGV-----GLVFDA--DLASAEHS--SHWVLQGVALVL--NIDQ
>Cr_DYH1B-IAD1b
----------------------------
MEPGDEGKGHQLTADATCIAWVRSKLQLLKPESLGDSDGAEWLSSVWHNDVHTPVVSTFLMSVKATRMF
AA-----------------------------------------------------------------
-------------------------------------------------
LDGGHEGGSSPKLVLALEVPKQFEQMVYFVRDPSKFVTRENVGSVIFFGVMRGGDPLHSLLNIMHGLYV
PVVVANTT----------------------WPE------------
TVKSDFTAQMHKFMANLTETVYEVKGKTILY---IPQED-------
LRDPKAAAKQKDLVQRLESTIIHWTRQVKELLNQQDSVDAS--
EQAGPLAEIEFWRERSVDLSGIRAQLDDGAVSSIVSVLEYAKSSY--LAPF---
LSLRNLIHREAVAAEDNLKFLLCLEEPCQQL-
ASAHPQTIPSLLPPILNCIRMVWNLSRFYNTPERLSVLLRKLSNEIINRCCSVISLPDVWSGDVDNVMV
ALRQSMEAGERWKELYKRTAAAV--AVRSPKPW-------
DFDISSIFAHIDAFLQRCNDLLEVCEAQLQFAPRTPLPVFGGTYGPEVKKSILDIQE----SFQGLVQ-
----GLQALKYDILDVKATRWHDDFNGFKGGVKDLEVMMANVIQRAFDTQPCLAAR-------
GELLEGFQTMAKRDYIRRFVEKKTVEFFALFNAEINTVKK----LFDAVKRS-------
QPKSPILPRYAGLAKYAMNLMRRLEQSHKVVDSVRYTLPQVSEAADVMQQYELA-----
HQAIEQYISNTHNDWFSTIESSIAKELQACLLTQ---DKASGGLLSMNFHKDLLSMGQEVHFWERMR--
---------------------------LAVPLVAMEINAQREKYRVLRDNILMVVRDYNKILTALDK--
--------------EERKLFHDRIRYLDRRIM------
PGVTKLQWTADKHALEFYYREARKFCRDADMAVGDYKTANSRLDAICRSISELVLVDVE----
KKKIYQHAEFANLQESHHAKIKDRLVSAVDEIRDIMASIHRVFEQDSEEVQREWVRFTQKVDRKLEDAL
RHVIKKSLQELSRLL----NGDNKTEVMPIFHVTMVLERT-NRVELRPTIQAL-
FDTINSVARNLILVLQSVPR----
VALQLTDKQRRDMEDAGLPLPKPLPTLYETISADEDAVLRTIMQITSGITSIIDKVQAFLTYWEKKYRQ
VWEADRDAYIRRYEKA----------QKPLSSFEADISRYLQCIDEIR-
GEDGATNMRFLRIDCGPLKLTLVGHCEAWVSKFTGLLGQLAATELRTLHTYFRENKDSL----------
-----------MLAPSTLEQLAELVGLHRRLADERRRTEAR--------
FEPLRDKYKLLERYEVGAKEE-----
EAALLEGLEPAWTQFQALLDETAGKLERYKDNFREKVKSLLDTFLKDVAQLCEDFSRDAPYS------
SEVPTPDALDFIQASKQ-------ADEDTRKRAAEIKNGMDIFNIPQPQYK---
DLAAMEKDLDFLDRIWGLKDEWEQLYYGWKDGSFTDIKARAARWEPEQVEEMEEAAVRIGKNVAKLGRD
IR----------QWTVWSS--LKDTLDAFKRTMP-LITDLRNPAMRPR-HWQNLQD-HIGVRFDP-H-
---SRDFTLDSLVALR--LDQHVEFVAELSVNATKELAIENNIKAIAATWSALGLDMAEYKST------
--FKLRS--TEEIFTSLEENIVTLSTMKASKYFIV---
FEKDIAYWEKTLSHISETIEIILQVQRNWMYLENIFIGSEDIRKQLPQESQMFDA--
VHNNFMRLMKQLYSTANCLKACTAQGLLESFQDMNNKLERIQKSLDNYLENKRQQFPRFYFLSSDDLLE
ILGQAKDPLNVQPHLKKCFEGIKKLDM-HLPGEDRKQTISVGITSPDG-EYLPFANPV--------
ITEGRPEEWLNRVEDAMFLTTKKHLYKVLEESKAQ-----------K-
KEKWVKENQGQMIITAGQIVWTHECEKALA-DADSARKNLKLLKKKW-ISYLNKLT-AVTRSK-
LNKIERNKVVALITIEVHARDVIEKLGKSN--CSSTNDFEWVSQLRFYWDREKNDC------------
--
IVKQVLSVFYYGYEYQGNNGRLVITPLTDRCYMTLGAAMFTRRGGNPLGPAGTGKTETVKDFGKALARY
VIVFNCSDGVDYKM--TGKMFSGLAQTGAWACLDEFNRIEVEVLSVVATQIAAVMQAIKES--------
----------KKRFLF---LGQEIRLNPSCGIFVTMNP---
GYAGRSELPDNLKAMLRPVSMMVPDFTLIAEIMMFSEGFSSAKVLAKKMIAIMELSQQQLSKQDHYDYG
LRSFVIPIARAAGSLKRLD----------P------EGS------
EEVILYRTMLDLIKPKLVYLDLPLFMALLSDLFPGV--ELPPADGGSLRRAIEAELRESN---
LQIVPEFVTKIIQVFDCKVARHGNMIVGRTGSGKSEAWKCLQRALGRLRK-EEPDDDR----
FQKVHVHTINPLALSNDELYGCFEAATHEWQDGVLARIMRTVCKD--ET-
HEQKWILFDGPVDTLWIESMNTTLDDNKLLTLLSGERIAM-
TPAVSLLFEVEDLSQASPATVSRAGMIYLNVEDLGWRPFITS---WLAAK---------QAAPGADAA-
```

```
------------------------------IIDQVSKLVDKYMEAALEHK---------
RLHCRELVPTDRLSCVRAFTRLWDALAVPENGVGTMPVDESAGPPGSKAAAAAAAAAAA---------
---------------------------------APPEETSGGTGGNLVEMWFLFCLIWGIGGPL---
-DEEGRKKFDAFMREM-----------------------------DTRYPS---SD---
TVFEYFVEPKAK-------SWLAWETKL-TGAFKPA----
MDQPFFKILVPTVDTVRNRFVGSALVRVSQHTLIVGNVGVGKTMIVGSLLE-GLPGDRMSSM-
TINFSAQTS-SNSLQDTIE--GKLEKRTKGV-FAPAGGKRLVC---FIDDLNMPQKS-
KFGFIPPLELLKLWVDNGFWYDRAKC--EVKHIKDMQLLAAMAPPGG-
GRNAFSQRVQACFATLNVTAPNDNQLKRIFGTILNAKLA---DFD-DEVKP-
LSEPITMATIGIYRAVSKELLPTPSKSHYLFNTRDLAKIIQGMMQATKAFYN-----------------
SKEEVLQLWCHECMRIIADRMWDHADKEWLVRQLDEKLGTT-F-STS-FGTLFEAYNE-----------
------TVPPFVTFMR-----QNVDV---PV-------YEAV-RDMVALKDLLTERLEDYA--
LEPGHSAMDLVLFRDALSHVCRIHRILGQPRGNALLVGVGGSGRKSLARLAAFVAELKCFTIEITKNYR
QTEFREDLKGLYRQAGVA-NK--PTVFLFDETQIVYETFLEDVNNILTSGE--------
VPNLFPKDELGSVLDELRP--AAKAAGA-
GETADALYGFLLERVRTNLHVVLCLSPVGEAFRERCRMFPGLVNCTTIDWWVFTEWPADALFEVAQKQL
MD-VDLGSTEV----
KTAVCKVFVTAHQSVENTSAKMFAALKRRNYVTPTNYLETVRGYKGLLAEKRTELGEKAAKLQGGLHKL
DETSVQVAAMKKVAEEKKVVVAQAKADCEELLVEIVQDKRV-
ADEQEKQVNAEAQKIGKEAEEANIIAAQVQQELDKALPALREAEAALDVLTKKDMSELKAYAKPPEKVE
MTLNAVLTVLRRPPN----------------WDEAKKRLSDA-NFMQSLKEF----
DKDKLDDSLLKKIG-KFTANPD--
FTYEKINTVSAAASGMCKWVHAMETYGYVAKDVAPKRAKLKSAQDTLARKQAALALAQEQLAVVLAKVQ
ALKDKYDTSIARKQALEEELADLEGKLERAEKLVTGLAGERVRWEASISEYNIALGCLPGDVVVAAAFM
SYAGPFPSEYRDELVKHTWLPQVKALNIPASEH--F-
DFALFLANPAMVRDWNIQGLPSDSFSTENGVMVTRG-RRWPLMIDPQGQANKWIKNMEGR--GGR-
LKVLNLQM-SDMARQIENAIQFGQPVLMQDILQ-EIDPILEPVLAKSFIK-
RGNQTLIKLGDKEVDYNFDFRLYLTTKLANPLYTPEISTKVMIVNFAVKEQGLEAQLLATVVKNERPDL
DKQKNDLVVKVAAGKRTQAELEDTILHLLSTAT-
GSLLDNVTLINTLDQSKTTWEEVNASLAVAEETQKKIEAASQLYRPCSVRASVLYFVLNDLSTIDPMYQ
FSLDAYNDLFLLSIKNSP---------------KN---------------------------------
-----------------------------
DNLAERIKSLNDFHTYAVYKYTSRGLFERHKLLLSLQMCVRIL--QTAN-
QVNTEEWQFFLRGGTVLDRS-S-QPNNPSQEWISEEAWDNITELDALPN-----------
FKGVVSSFESNLGEWEAWYRKGDPEASELPAEW-ESKC---
NELQRLILVRCLRPDRVIFAATTYVSNALGRKYVEPPVLDLAETLKDST-
ALSPLIFVLSAGVDPTDNLRKL-ATEKGM---
TSRFFTVALGQGQAPTATRLIEDGLREGNWVFLANCHLMTSWLP-TLDKIIE-GFETK--
QPHENFRLWLSSNPSPS------FPIAILQRGIKMTTEPPKGLRANLLRLYNSV---SD-ASYA--
QCKTQ---------------IK-YQKLLFALTYFHSVLLERRKFR---------
TLGFNIPYDFNDTDFSVSDDLLKSYLDSY----------------E-----------------------
-------------------------------------
QTPWDALKYLIAEANYGGRVTDELDRRVLASYLNKFYCEDAL------AVPG---YLLSPLSTYYV-
PENG--PLQSFKDYILTLPAGDR-PEAFGQHPNAEISYLIEDSKVLLDSLLSLQPRTEGAAGGAGTRR-
---EDVVMAIATDLLDQVPQPF-NLEEVMKA----
KADDPSALHVVLFQEVERYNALLVAVRRSCVELQRGIKGLVVMSADLDLIFESLYAAKVPAAWL-
KTYPSLK---PLGPWTRDLLQRIEQLAT-W----------VEE-T--
YPRVYWLSGFTYPTGFLTAVLQTTARKA---SVPIDTLSFEFSII--
NLDEREINAPPKEGVYIKGLFLEGAGWDFENGCLCEPNPMELIVPMPILLFRPVENKKRTAKG------
----IYTCPLYLYPLRTGTRERP----SFMINVDLRSGSADP---DHWIMRGTALLL--SLAT
>Cr_DYHA-OADb
------------------------------------------------------------
MQSIFWEVPNAQGEAPCPRSGHSFTVLGE-----------------------------------------
------------------------------------------------------------------------
------------------------------------------------
RFVLFGGCGRKDGKAAAFNDLYELDTSDPDEYKWKELVVANAPPPRARHAAIALDDKRLLVFGGLNKR-
-----IRY------------NDVWLFNYDDKSWTCMEVEGAAPEPRAHFTA---
TRFGSRVFIFGGYGGSGQVYNEMWVLHFGEDGFRWQNITESIEGTGPAPRFDH-----
SAFIYPVTPNSDTYDKLLIMGGRDLSQMYQDSHMLDLNKMAWENETQP---
PTLPYEICNNVCDGIESVPYHKVFSFGGRKGMMQYLNTVEVMDCGSQMWSTPPVDHGVAPVGREDTAWV
FDVKTCSLLIFGGWANRWLGDLHKLNVSPIIGPPYACTAI----------------------------
---QPEMGPVFGSTELVIRGLRFRDGKVQVKFGLSEKNEVVVEGTYVDQETIRVQTPNYE----
QFGALTV-----DVRVS------INGEGWTVNKIKYAYFANTAARNCIAYGPGLLAETISGVEV-----
--PFIIQAKDTLNDKRTSGGDVFKVTVVSADGKNEGVSRVRDLQNGQYEVQYAA-------
```

```
PTAGPYLIHVAFNELGTSDFVPIRGSPFTVKCTDSWTKHRVMGAAPAKRKGATI-----
CTMGNELVLYGGDKSGVTVLNTEGAEWRWSPA-----TVSGSTPPDRTAHSTVVLSDGELVVFGGIN--
-------------------------LADQNDLNDIYYLRKQGEGWVWSCPSESRPYIRHPKGAAA--
--------------VSAEPSAEPAAEPAAEPAA-----EPDADAPAAE--
PAAEGEEGAVPAEGEEGAEGASGSRPVSAKPAPAAAAPAAEALPELP------
VSARNSHVAVAIDKDLYVMMGDHDGDLMTELAMVDTSDRTCAHW------------------------
-----------------------LEPILKGDVPVPRK-------ACAAAA-
TGNTIVLFGGQTQNADGEATVTGDLVIMEVTGPNSIQCAVNPAAPGASGSPAARYGAVMQEFSNGKLFL
HGGMDAASKPLNDGWLFDVPSK--TWQCVYVGSSDVVLPT-----------
GSLATLWRNRIVLVSAAVGSPKLDSVQSLDFQELR-
DSVAFTPKMRASTETLLKGLEDWVDTQAHGMELARSPE----------------------------
KLSKDFENGLRKVMDALFQVKSQRSQTDLL--------IDQLHEAFAQLAEEKVPGINK-----
MEKRLEAAAHKWDEIKKAQPQVKTDVEPIQAAKGEDIKKEIETFAAKVRNYRADFRRRGFFK------
YATGFDGAYPLLDAAAH-------
ELAELKKECDRLSELASVFEFPQAIEPVTVAIKETVEDLVMVKDVWDTAVLCELQFQDWRQTLWSDI--
--------RTDIMEEGAKQFVKEVKSLHKKVR----------DEDVFRG--VDQVVKNFLVSVP-
LVADLRSPAMRDR-HWEQLMA-TTKMTFNV-K----DPNFKLDDLLALE--
LHKFEEEVGEIVDRAQKEEKMEIAIRKLNDTWTRVEFQFHRHKDY---D---VHTVKM--
AEEDFEALEDNQVQVQGMIANRYMAT---
FKDEILGWQKKLNDVADVNQIMAEIQRTWAYLESLFIHSEEVKKELPQATERFAA--
IDTEVKKVLREFQQLKNCVSCCNREGLYANLETQERELEICKKALNDYMESKRRAFPRFYFVSSADLLD
ILSNGNNPMRVQIHMNKCFQAIDKLRLDSEEVVPGRRPKALGMESCVGIEYVPFSSPL--------
PLENKVEQYMNDIIAKMRNELRMVLKASVEDYPSK-----------P-
RDKWLFDWPSQIILVVNQIYWCLEVEQAFTEMARGDKGAMSKYNEFQ-VKQLTKLI-EVTRTD-
LSKPDRQKIMNMITIDAHSRDMVLAGADQP------DSFQWVSQLRSYWDRDISDC------------
--
RIRICDASFPYGYEYLGNGPRLVITPLTDRIYITATQACWLSLGTAPAGPAGTGKTETTKDLSAQLGKS
VYVFNCSPEMDYRT--
MGDIFKGLAASGSWGCFDEFNRLVPEVLSVCSVQYKCVTDSQKKKTMLPGRGLEYIKDGVKHPAVEHWS
FIAADGVEMPLEEGTSAFITMNP---
GYIGRAELPESLKALFRPITVMVPDRQLIMENMLMAEGFVEAKMLAKKFASLYYLLEDLLSPQKHYDWG
LRA-IKSVLVVAGSLLRAE----------A------GQV------
EADVLFRALRDFNIPKILAQDMVIFMGLLNDLFPGI--DPPRKRDMEFEDVIVSTIKDLG---
LTPEDDFVLRVVQFSELLAIRHCVFLMGPTGTGRTECYRVLAKAITKGCN-
NPVNDYLKMTNKKKVVIRDINPKSISTYELYGQVNQATREWKDGLLSYYMRELANM--PG-
DDPKWLLLDGDLDANWIESMNSVMDDNRLLTLPSNERIRV-
LPHMKLIFEIRDLKFATPATATRAGILYISEGQ-QWHNMAMS---WINRV---------
VKPYAERAKWKDPQ-------------------------LPCTWLREMFDKYIPPTLLEM--------
-KKSYSHITPLAQMNFISTLVNIMEGVLKPE-------------------------------------
---------------------------------------
NLSNKADQAMFEMYFVFAMIWAFGGGLVEKDGIPYRRNFDKWFKQTWT--------------------
------TVKIPG---KG---TVYDYFVNPKTQ-------KFQPWAEL--VTDIDYD----
GSRPMSTVFVPTAETSSLRFFLDMMVDLRKPIMFVGGAGVGKTQLVKGKLG-SLNEEQISL--
SISFNYFTD-VVSFQKVLE--SPLEKKAGIN-YGPPGTKQLIY---FVDDLNMPKLD-
LYETAMPISLIRQHLGWGHWFDRAKL--TPKNINNTQYVACMNPTAG-SFI-
INPRLQRLFMTLAVDFPGQDSLMKIYGTFLQGHLK---KFS-ESIQD-
MGTKILQAALALHDRVSQTFRKTAINFHYEFTVRHLANVFQGLLMSTPEAFN----------------
SPTKWGKLWLHESERVYADRLVSLYDLDAYNKAATAIAKKY-FSVAD-IDDYYKKKDP-----------
------KPLIFCHFAR------GLAD---KA-------YDEV-ADYTSLYKTLTEALNEYN--E--
TNAAMDLVLFEDAMKHVCRISRIVSNPSGHALLVGVGGSGKQSLARLAAHICGYATQMIVISGSYSMNN
FKEDIQKMYKRTGVK-GE--GVMFLFTDSQIVDERMLVYINDLLSSGE--------
IPDLFPQEDRDEIVNALRS--ETKSLGL-
LDTAENCWATFIQKVKTNLHMVFTASPVGENFRVRSQRFLATVTSTVIDW--
FQPWPESSLFSVAKRFLDE-VDLGEDAV----
RNAVVEFMPYSFQLVNKVSIKFREQERRYNYTTPKTFLELIKLYKNVLAAKRKANQDNTERLENGLHKL
HKVQADVDILVEEAKVKAVEVEHKVASANIFAEQVGVEKEK-
VNAENAAAQVEAEKCAVIAKEVSEKQASCEKDLAAAEPLVAEAMAALETVTKKDLGEAKSLKKPPPGVD
DITAVVIILLENNPKD------------
KSWQAAQKLMNNVDKFLERVKSFKSVIDAGQVARKTVDACR-PYLALEW--
FNREAIGKKSAAAAGLCEWAVNIIKYYDVVQEVEPKRQELAAANAKLEEANVTLAAVEEKVALLNAKVQ
ELEQQYKEANDDKEAAIRESERCQRKLELANRLINALASEGERWALTVEQLRKSYEVLTGDMLLAAAFV
SYAGPFTAKFRAQLIDD-WILFLRERHMPMTEG--
ITDPLKVLVDDALVAGWIREGLPSDPTSVQNGTILTNS-ERWSLMMDPQLQGILWIKERESK--NN--
LQVTRMGA-SNMLQVMERAIEAGHSVLVENMGE-TIDAVLNPIITRSTFK-
```

```
KGRSLYVKLGDKECEYNKNFRLFLHTKLSNPHYPPEIQAETTLINFTVTEAGLEDQLLALVVNKERPDL
EETKTQLIIQNTEFTIKLKELEDGLLLKLSTAE-
GDITEDVALIESLEDAKRVSTEISEKVKESRETEAAINENRNKYRTVAARGAMLFFLLNSLNKIHAFYQ
FSLNAFVTVFSRGLDLAPGGRKKGKGLKKTPSLRDQPMDHQSLMEKARRSSGVGDRRPSQEGLPGPEAS
QASLAESQGGRGSQVGDAEDEDDESFAMAPEALEQRLVNLLETCTFTVYNYTRRGLFDRDKLIVLSLLT
FTIL--LRSQ-AVDASEYEALCRGMRNPTPP-P--ITDDLSRWMAESQWAALDVLTTLPC---------
--FAHLAKDMEKNSDDWFNWCNNEAAERAPMPGEW-G-KL---
TEFRQLLIIRALRPDRITNALQNFCEHMMGSDYVNQDAFSPAAMMDESS-
SATPIFFILFPGYSPSKEIEVY-
ANKCGYSVANGRLCLISMGQGQEAPAEAVLDKYTREGGWVFLDNVHLMQGWIP-KLERKLE--IAAE--
SAHPDFRCFFSAEPINGAPHANIIPESILQTCIKISNEPPSDMKSNMRRAFAAF---TP-
EQCDRPSTPAK----------------
RVAFRAILFGLCFYHSLLLGRKKFGVGIGTGSGSGLGFCRGYSFNIGDLTTCGDVLYNYLEAY------
----------E-----------------------------------------------------------
----
QIPWRDLQYMFGEVFYGGHITDSMDRRCCTTYLEVLIRNEILPKGNPDEVEAWEAPTLELAPGFFA-
PKPV--DYPTLKEYIETSLPAES-PVVYGMHPNAELSLLTSLGETLFKTVVEVAGGGGGGGGGGGG--
---ENAVRQALETFKERLPEPF-NMVEVEL-----
RVKEKTPFVVVALQEATRMNALLSEMKRSMEELQLGLDGALNMSDNMEKLAKGIASNTVPELWMSCMST
RVQEVYTLTAWYQDVVKRHDQLSA-W----------TAG-
DIITPHSVWLPGLFNPKAFLTAVMQTFARAN---KLPLDVMKFMTEVT-
RMTSPEQVTEAAPLGVYVHGLVLEGARWDREDGCLRDSKPNELHPAMPVLQVKPVTADQFNLEG-----
-----YYECPVYTNMQRANVYSPVVSTF--------TLRTQDMP--AKWVLASVALLLQDDLAG
>Cr_DYHB-OADb
--------------------------------
MAEDEGMTAAAPSSSVKDIRFDYIRDRVCSCLKVPDSAYDKLVSGDGRTSLVQFMEEAHTKRLLIMLDG
K-----------------------------------------------------------------
-----------------------------------------D-------------------------
----
LSATVKPPPKFKKKTVYFLKLQETKLDNDNIKKLVIHGEISENPLETLAAISQDVFMPVLTAPANQQG-
-----WPD------------VVAKEVTENLHKFVSNVFVTIGQMKGQTLLP---
LPPQNTVPTLQPEQSMHSLKDQDKIHILESAIVTWTKQIKNVLKADPDAPLKEPGAYPGPLTELNFWSE
RAANLNSIHEQLTSEKTQKVVKVLELAKSTY--YPAF---
QRLFREVEAAQQEANDNVKFLKPLRKYLDKLNMMDDFPMLVDLFKPIMHTLMLIWKHSKSYNSSTRFVT
LMQEICNDLIMQACKYVPGSDLIQMEPSEAVDKLRMTLRVLGTFKNYYFEYRALSMQDTPENPW-----
---KFQNNSLFARLDSFLERCHDMMDLMSTCMQFNRLERVEI-GGTKGKVLTNGVKAIHQ----
DFTSAVE-----KFQQVTYDVMDVDAKQFDEDFFGFRVVIKELERRLAAIIIQAFDDCTTIGTT-----
--FKLLDSFEGLLDREVIAHDLEKKHTDLLHSYARDLKDVAD----LFHQYKDR-------
PIVAKNSAPYSGAAYWVRGLMERIKDPMDRLLTMNKMVLESELFREIQRTYDHL-----
WEEMTEYRTRAVDAWCAQVAATSDEKLNLPLLSLIEETADGIRVLGVNFDPALVRLLRETKYFLLLETS
TQDKNADRNAEKAAEGGEVEVVKKAPKLSVPDSAKDLFASADTFRQQISALDLICSIYNKVQRTILA--
--------------VEKPLVQQKLDAVEQALN------RGLAELNWKC--
AEIDTYIKECMELVKDVDLVLNTIKDNVKATQGILAMWEKNLMFERK----
DGKTYTFDELNDAFNQLIQQRHSEIRDAGKEITKLLSSSNRVLKVS--
KGAASWRAYVDYFSNIVIDGFSAAIISTVRYLLSQID--PDILAKTESSPLLEIQVELVAP--
DIVWKPDLGEG-GAK--------PGLRDMIKKWLQSFLEIGQLMKRLDVGEGNYAK------
ELEEDFEVYDALNQVMMVTLANESRCEDFKNQFAKFDY-----
LWKQDLQATLQQFITDNGVTLPDGTRDDPPLAKFEEQIVKYKNVASEIA-
SFKDTMTMGYVKVNAKPLRQALSTWASKWVYLFTHYLQEKVVNSITELYTFMDTSNSTLDLKVMGEGVE
EEPEYHPDQDPEEAAAKKAAEEEEKRKALYAIMACMRDIRRRTERGTDTMFEPLKETVTALHTFGIQLS
DT-----
VLHQLDNAEFNWRTLKKKMLNRREQLAPLQQAEAVEIRRKSDAFNERVEDFRTFFQRKAPFAVSGGELK
LEQVKPAYKLLDEFRSGSLEGYPSVLGIIAESKQLQEAQDLFELYQPGYL---
QLQRCSEELGHLKSLWDTVGTVMFTFRDWYKTPWDKI----------
DVDFLVEETKKLSKDIKMLNKAVR----------NYDVYRM--LEEAIKAVLTSLP-
LVQDLHHPAMRER-HWKLLMQ-TTGKHFVM-D-----DKFCLGDLLALE--
LHNYVDACSEIVDRAQKELNIEKQLKKIEDTWAGLSLAFSTYQDS---D---VMALLV--
DDAVNEALEADNLQLQNLSGQKYVQSNPMFLETVSKWQNNMGRVSAVLETWQNVQKKWQNLESIFIGSA
DIRVQLPEDSKRFDA--
VNADFQELMRTAPDITNVVEACTLDGRQERLENMQSMLEQCEKALQEYLETKRVAFPRFYFVSPADLLD
ILSKGSNPQLILRHLQKCFDNIDNLSFRKDERGDP-TKIATHMHSKEG-EVVEFVEDC--------
SCDGPVEVWLQNVVDSMKLALQVEFRKAIPTYDEL------------P-
RTKWIYVYSAQNTVVVSRTFFTQEINEAFDDLEEGNEEALKVELDRQ-VQQLADLI-DEINKE-
QTSLDRKKLITLCTIDVHSRDLVQKLIDER--VEDQMCFQWQSQLRYIQSEKTKTC------------
```

```
--
QVNICDAEIAYSYEYIGNCGCLCITPLTDRCFITLTQAQRLVLGGAPAGPAGTGKTETTKDLARALGIQ
CYVFNCSDQMDYKA--MGHTYKGLAQTGAWGCFDEFNRIPVAVLSVCSTQYKTVLDAIRAK--------
----------KERFTF---EDADISLKSTVMAFITMNP---
GYPGRAELPESLKALFRPVSMVVPDLALICEIMLMAEGFQMSKILSRKFVILYKLCEDLLSKSRHYDWK
LRA-IKTTLYVAGGMKRAA----------P------ELS------
EDKVLLRALRDFNLGKLTADDTSIFMGLLNDLFPKTLELVPRALDKAFDEAAHKAATELG---
YQPDDQFLLKISQLRELFVVRWSVFLLGAAGCGKTAVWRTLLRAQNSSGE-------------
KTIYQAVNPKAVTRNELYGYLHPATREWKEGLMSVTFRNMANN--KT-
NKHQWIVLDGDIDAEWIESMNTVMDDNKMLTLASNERIPL-
TPSMRLLLEINHMVHCSPATVSRGGVIFINADDVGWQPVVAS---WIDKL-----------EAAEYR-
---------------------------------PLLTALFTKYVDPCLEHC---------
RRNFKTVVPLPAVNQAMTICKILEGILP-----------------------------------------
---------------------------------
KETVRGAPPPDKKLLHYHFVFACVWAFGGCMLVDKVTDYRTQFSKWWVSEWK----------------
----------DVQFPE---KG---LVYDYYVDEQNC-------IMVPWEDR--VTKFQY-----
IPGDFTSLFVPTVETTRLTYFLDSLVSNKHYAMFVGNTGTGKSAIMVNKLR-NMDTETMSFY-
TINMNSLSE-APALQVILE--QPLEKKSGVR-YGPPGSRRMVY---FVDDMNMPLVD-
KYDTQSSIELLRQMVDYHGWYDKVKI--QLKEIINCQMAACMNPTAG-SFN-
ITPRMQRHFVTFAVQMPNAEITRAMYYQIIDGHFS---SFD-VDVAK-
MSNKLVDATCELHRNVMHNFLPSAVKFHYQFNLRDLSNITQGLTRAIKEYYR----------------
EPVKVARLWVHECERVFRDRMINEADMAKFDEFRVAVTKKF-FDDCG-GMVAIEE-------------
------RPLIYASHAS-----MTYTPEDVPV-------YNAL-SSYDVLRKTLEDKLREYN--E--
SNAVMDLVLFQQAMEHVTRIARIIDLPRGNAMLVGVGGSGKQSLARLASYICGYEVYQISVSSTYGIND
FKENLLGLYRKAGTK-GT--PITFLMTDNQIVKEGFLVYINDLLSTGY--------
IADLFTPEDKEAFTNAVRN--EVKAAGI-
LDSAENCWDFFIDKVRKFLHIVLCFSPVGDKFRIRARQFPALVNCTMFDW--
FHGWPGEALVSVAQRFLVD-VPNMEEVV----
RENIAYHMAYAHQCVSEASERFKEAFRRYNYTTPKSYLELISLYKMLLQLKRDDLRRSKERLENGIDKI
AQAAAQVTDLQRVLKEEQIVVDEKKAQTDELIVSIGKEKAI-
VDQAVEAGREDEEAATALQTEVSAFQAECERDLLEAEPIIAQAEAALNSLNKKELSELKSFGSPAAEIV
QV-AAACLVLTCGGKIPKDRD----------WNAGKKMMADVNSFLSSLMNF----
DKDNVPVVCVEVVEKDYISNPG--
FTPDNIKGKSAACAGLCSWVINICKYFRIYQVVAPKRAALAEANKKLDTANKKLKVIRDEVKRLQDRVA
LLEQSLMKATEDKNAAIAQADRTARKAQMAERLINGLSGENTRWGAEIKRLESLEGRLVGDVLIASAFV
SYAGPFNMQFRKSLVDEKWLPDIIERQIPMTQG--I-
RPLDLLTDDATKAKWANEGLPTDPLSVENGAIMSNA-SRWALMIDPQLQGIKWIINKETN--NG--
LVIIQQSQ-PKYIDQVINCIENGWPLLIENLPV-DIDAVLDPVIGKMTIK-
KGRNIIMKIGDAEVQYDSRFRLYLQTKLSNPHFKPEVAAQTTLVNFCVTEKGLEDQLLALVVDHERPDL
QEQAAGLVRSLNEYNITLVELENNLLFNLANAT-
GNILENIELIEGLEETKRTAVEIEEKVKLAKQTEIQIAKAREVYRPVATRGSLTYFLIDNLNALDRVYH
YSMANFVFVLKKGMDMTPGGKDESKVPLAERLNQE---------------------------------
-----------------------------
VDLDKRVELLVETTCFVLIGYVAQGLFERHKLIVATQLCMQIL--RSRG-
ELHYAKFEYLLRGPKVMGAD-N-----PLHDWVSDSVWGSVQALKELDD-----------
YQGLPEDLIGSSKRWREWMELERPEDEPLPGDW-K-RM---
QEFDKLLLFRALRPDRLTSAMGRFVTNMLGAKYVTSQPYDLERSYQDAS-
PGTPIFVFLSPGVDVAGSVEAL-
GKKLGFTLDNGKYASVSLGQGQEPIAMDRLSAAHKNGGWVLLQNIHLTIDWTTNQLDKKVD-KL-VE--
GAHPDFRLFLSAEPPPSLERG--LPISLLQNSIKLTNEPPEGLKANLRRAWNNF---NE-EILE--
SCAKQ---------------AE-
FRAIVFALCYFHAALLERKKFGVGNLPGARSGIGWNMNYPFNTGDLLCCGQTANNYLENN---------
-------V---------------------------------------------------------
-KVPWEDLRYNFGEIMYGGHIVEDYDRRLAMCYLRKYVNEGLL------DNME---FFPG----
FAMPPNTA--NHRQVLEFIDEVMPPET-
PLAFGLHPNAEIGFKLREAESFCNSLVQLQPRESSGEGGMSA------EERAKLVLDEVVDKLPDIF-
DMEDVRSKIN--
PDDPNMPFVMVAIQESERMNMLLAEMKRSLLELDLGLKGDLTMTEPMERLLKALATDAVPGSWRNLAYP
SLR---PLGSWLGNLLARHAQLVD-W----------TAELS--
TPKAVWLSGLFNPQSFLTAVMQATARRN---DWPLDKTVIITEVT-KK-
QPDQIEANSRDGAFIHGLTLEGARWDDKIGALDDSKPKELFCPMPVILVRAVTQDKAEMKD--------
--VYKCPVYTTEARFR---------EEVFEA--QLKSKHTE--IKWVLAGVCLFL--DVV-
>Cr_DYHG-OADa
```



```
AEKEKQKVAVIVDAVTKKASEIATVKDDAERDLAAAKPALDAALEALNSIKDGDIKNLKALKKPPQIIT
RIFDCVLVLRMLPVTKAEYTDEKGRMVQVGNYPEAQKMMNQM-SFLQDLKDF----
AKEQINDETVELLE-PYFMSED--
FTFENAQKASGNVAGLCNWAESMAKYHNVAKVVEPKIAKLREAEAELKLATKEKNAAEERMAKVQAKLD
EMQAQFDAAMAHKQALEDDAAATQRKMDSANALIGALAGEEARWTAQSKEFDVQIQRLTGDCALASAFV
SYLGPFNKEFRELLLNRDFYGDCMKLNVPVTPH--L-
QITKFLVDDSEVGEWNLQGLPTDELSIQNGIMVTRA-SRYPVLVDPQGQGREWIKNREEA--NQ--
LKTTQLND-KLFRNHLEECLAFGRPLLIENIEE-ELDPLLDPVLERRLVK-
KGKTWVVPLADKEVDFTETFRLFCTTRLPNPHFTPELSAKVTVVDFTVTMAGLEDQLLGKLISKEKKEL
EDQRQQLLEEVQSYKKRIKQLEDDLLFRLSNSQ-
GNLLDDTELIDVLAVTKQTAQDVSEKLANASETNKRINEACEEYRPVAHRATLLYFLIAEFSVVNCMYQ
TSLAQFNQLYELAIDRSE---------------KA---------------------------------
-----------------------------
NMPSKRIHNIIEYMTYEIYLYVQRGLFERHKIIFALMLTNKVL--TSAG-
KVKATDLDVFLKGGAALDIN-S--VRKKPKDWIPDSVWLNIIALSAMDA-----------
FRDIPDSVFRNDGLWRQWYDQEAPEMAKVP-DY-EDRL---
NKFERMCVVKTFREDRTLIAAADYIAEALGQRFVESVPLNMEKAWQESH-
AKCPLICLLSPGADPTKLIEDL-AKKKKI-----
KTLGVSMGQGQEVIARKHMAAASLEGHWVLLQNTHLGLGYLT-EVETFLV---KEE--
NVHEDFRLWITAEPHPQ------FPIGLLQMGIKITNEAPVGIKAGLRASYQWV---NQ-DMLD--
MVSRQ---------------E--WRQLLFVMCFLHSVVQERRKFG---------
PIGWNVPYEFNQSDLSACVQFLQNHLSEMDAKK-----------AP----------------------
---------------------------------------
QPTWETVRYMISAIQYGGRITDDFDKLLMDTFAEKYFLQPVL-----
QPSYELFKDTRSSDGFSYRVPDST--DIDAFRSYIETLPGTES-
PEIFGLHPNADITFRTLQVQESIVTILDTMPKGAGSGSGLSR------EDVVDKICEDLLSKAPPLF-
DKEETKEKLKKLPGGPTLPLTVHLRQEIDRLNIVTRLTTTTLKNLRLAIAGTIALSGGLIDALDALFNA
RIPQQWLSKSWEAS----TLGNWFTGLLQRYDQLNK-W-----------LNL-G--
RPKAYWMTGFFNPQGFLTAMKQEVNRKHAADKWALDDVVMSSEVTHPPKDFESLKEGAPEGVYVYGLYL
DGCAWSGRENRLMDSDPKKLFNPLPVLHVTGVLAKDKKRSG----------LYEAPTYRVKARKGL---
-----NFITTF--SLRTEDDK--SKWILRGVGILC--SID-
```

Bibliography

Abdel-Ghany, S. E., Day, I. S., Simmons, M. P., Kugrens, P., and Reddy, A. S. N. (2005). Origin and evolution of kinesin-like calmodulin-binding protein. *Plant Physiol*., *138*(3), 1711-1722.

Absalon, S., Blisnick, T., Kohl, L., Toutirais, G., Doré, G., Julkowska, D., et al. (2008). Intraflagellar transport and functional analysis of genes required for flagellum formation in trypanosomes. *Mol. Biol. Cell, 19*(3), 929–944.

Afzelius, B. A. and Eliasson, R. (1979). Flagellar mutants in man: on the heterogeneity of the immotile-cilia syndrome. *J. Ultrastruct. Res.*, *69*(1), 43–52.

Ahmed, N. T., Gao, C., Lucker, B. F., Cole, D. G., and Mitchell, D. R. (2008). ODA16 aids axonemal outer row dynein assembly through an interaction with the intraflagellar transport machinery. *J. Cell Biol., 183*(2), 313–322.

Akera, T., Goto, Y., Sato, M., Yamamoto, M., and Watanabe, Y. (2015). Mad1 promotes chromosome congression by anchoring a kinesin motor to the kinetochore. *Nat. Cell Biol., 17*(9), 1124–1133.

Ambrose, J. C. and Cyr, R. (2007). The kinesin ATK5 functions in early spindle assembly in Arabidopsis. *Plant Cell*, *19*(1), 226-326

Ambrose, J. C., Li, W., Marcus, A., Ma, H., and Cyr, R. (2005). A minus-end-directed kinesin with plus-end tracking protein activity is involved in spindle morphogenesis. *Mol. Biol. Cell, 16*(4), 1584-1592.

Anderson, P. and Kedersha, N. (2006). RNA granules. *J. Cell Biol., 172*(6), 803–808.

Asai, D. J. and Wilkes, D. E. (2004). The dynein heavy chain family. *J. Eukaryot. Microbiol.*, *51*(1), 23–29.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., et al. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet., 25*(1), 25-29.

Awan, A., Bernstein, M., Hamasaki, T., and Satir, P. (2004). Cloning and characterization of Kin5, a novel Tetrahymena ciliary kinesin II. *Cell Motil. Cytoskeleton*, *58*(1), 1–9.

Banks, J. A., Nishiyama, T., Hasebe M., Bowman J. L., Gibskow M., dePamphilis C., et al. (2011). The *Selaginella* genome identifies genetic changes associated with the evolution of vascular plants. *Science*, 332(6023), 960-963.

Bannigan, A., Lizotte-Waniewski, M., Riley, M., and Baskin, T. I. (2008). Emerging molecular mechanisms that power and regulate the anastral mitotic spindle of flowering plants. *Cell Motil Cytoskeleton, 65*(1), 1–11.

Bannigan, A., Schieble, W. R., Lukowitz, W., Fagerstrom, C., Wadsworth, P., Sommerville, C., and Baskin, T. I. (2007). A conserved role for kinesin-5 in plant mitosis. *J. Cell Sci., 120(16)*, 2819-2827.

Barbar, E. (2008). Dynein light chain LC8 is a dimerization hub essential in diverse protein networks. *Biochemistry, 47*(2), 503–508.

Beales, P. L. (2005). Lifting the lid on Pandora's box: the Bardet-Biedl syndrome. *Curr. Opin. Genet. Dev., 15*(3), 315–323.

Berbari, N. F., Lewis, J. S., Bishop, G. A., Askwith, C. C., and Mykytyn, K. (2008). Bardet-Biedl syndrome proteins are required for the localization of G protein-coupled receptors to primary cilia. *Proc. Natl. Acad. Sci. USA, 105*(11), 4242–4246.

Bernstein, M., Beech, P. L., Katz, S. G., and Rosenbaum, J. L. (1994). A New Kinesin-like Protein (Klp1) Localized to a Single Microtubule of the Chlamydomonas Flagellum. *J. Cell Biol., 125*(6*)*, 1313–1326.

Besse, F. and Ephrussi, A. (2008). Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nat. Rev. Mol. Cell Biol., 9*(12), 971–980.

Bhogaraju, S., Taschner, M., Morawetz, M., Basquin, C., and Lorentzen, E. (2011). Crystal structure of the intraflagellar transport complex 25/27. *EMBO J., 30*(10), 1907–1918.

Bieling, P., Telley, I. A., and Surrey, T. (2010). A Minimal Midzone Protein Module Controls Formation and Length of Antiparallel Microtubule Overlaps. *Cell, 142*(3), 420–432.

Blacque, O. E., Li, C., Inglis, P. N., Esmail, M. A., Ou, G., Mah, A. K., et al. (2006). The WD repeat-containing protein IFTA-1 is required for retrograde intraflagellar transport. *Mol. Biol. Cell*, *17*(12), 5053–5062.

Blacque, O. E., Reardon, M. J., Li, C., McCarthy, J., Mahjoub, M. R., Ansley, S. J., et al. (2004). Loss of C. elegans BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev., 18*(13), 1630–1642.

Blaineau, C., Tessier, M., Dubessay, P., Tasse, L., Crobu, L., Pagès, M., and Bastien, P. (2007). A novel microtubule-depolymerizing kinesin involved in length control of a eukaryotic flagellum. Curr. Biol., 17(9), 778–782.

Bloodgood, R. A. (1977). Motility occurring in association with the surface of the *Chlamydomonas* flagellum. *J. Cell Biol., 75*(3), 983–989.

Boothby, T. C. (2013). Post-transcriptional Regulation of Spermatogenesis Through Intron Retention in the Fern *Marsilea vestita*. Ph.D. University of Maryland, College Park, MD.

Boothby, T. C., Zipper, R. S., van der Weele, C. M., and Wolniak, S. M. (2013). Removal of Retained Introns Regulates Translation in the Rapidly Developing Gametophyte of *Marsilea vestita*. *Dev. Cell, 24*(5), 517-529.

Boothby, T., and Wolniak, S. (2011). Masked mRNA is stored with aggregated nuclear speckles and its asymmetric redistribution requires a homolog of mago nashi. *BMC Cell Biol.*, *12*(1), 45.

Brazelton, W. J., Amundsen, C. D., Silflow, C. D., and Lefebvre, P. A. (2001). The bld1 mutation identifies the Chlamydomonas osm-6 homolog as a gene required for flagellar assembly. *Curr. Biol., 11*(20), 1591–1594.

Briggs, L. J., Davidge, J. A., Wickstead, B., Ginger, M. L., and Gull, K. (2004). More than one way to build a flagellum: comparative genomics of parasitic protozoa. *Curr. Biol.*, *14*(15), 611–612.

Brown, J. M., Marsala, C., Kosoy, R., and Gaertig, J. (1999). Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and normal cytokinesis in Tetrahymena. *Mol. Biol. Cell, 10*(10), 3081–3096.

Brown, R. C., Lemmon, B. E., Shimamura, M., Villarreal, J. C., and Renzaglia, K. S. (2015). Spores of relictual bryophytes: Diverse adaptations to life on land. *Rev. Palaeobot. Palynol***.**, *216*, 1–17.

Buschmann, H., Dols, J., Kopischke, S., Peña, E. J., Andrade-Navarro, M. A., Heinlein, M., et al. (2015). Arabidopsis KCBP interacts with AIR9 but stays in the cortical division zone throughout mitosis via its MyTH4-FERM domain. J. Cell Sci., 128(11), 2033-2046.

Busson, D., Dujardin, D., Moraeu, A., Dompierre, J., and De Mey, J. R. (1998). Dynein and dynactin are localized to astral microtubules and at cortical sites in mitotic epitheial cells. *Curr. Biol., 8*(9), 541-544.

Cai, S., O'Connell, C. B., Khodjakov, A., and Walczak, C. E. (2009). Chromosome congression in the absence of kinetochore fibres. *Nat. Cell Biol.*, *11*(7), 832–838.

Chan, K. Y. and Ersfeld, K. (2010). The role of the Kinesin-13 family protein TbKif13-2 in flagellar length control of Trypanosoma brucei. Mol. Biochem. Parasitol., 174(2), 137–140.

Cole, D. G. (2003). The Intraflagellar Transport Machinery of Chlamydomonas reinhardtii. *Traffic, 4*(7), 435–442.

Cole, D. G., Chinn, S. W., Wedaman, K. P., Hall, K., Vuong, T., and Scholey, J. M. (1993). Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. *Nature, 366*(6452), 268–270.

Cole, D. G., Diener, D. R., Himelblau, A. L., Beech, P. L., Fuster, J. C., and Rosenbaum, J. L. (1998). *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J. Cell Biol.*, *141*(4), 993–1008.

Cross R. A. and McAinsh A. (2014). Prime movers: the mechanochemistry of mitotic kinesins. *Nat. Rev. Mol. Cell Biol., 15*(4), 257-271.

Dagenbach, E. M. and Endow, S. A. (2004). A new kinesin tree. *J. Cell Sci. 117*(1), 3-7.

Dawe, H. R., Shaw, M. K., Farr, H., and Gull, K. (2007). The hydrocephalus inducing gene product, Hydin, positions axonemal central pair microtubules. *BMC Biol., 5*(1), 33.

Dawson, S. C., Sagolla, M. S., Mancuso, J. J., Woessner, D. J., House, S. A., Fritz-Laylin, L., and Cande, W. Z. (2007). Kinesin-13 regulates flagellar, interphase, and mitotic microtubule dynamics in *Giardia intestinalis*. *Eukaryotic Cell, 6*(12), 2354–2364.

De Rybel, B., Vassileva, V., Parizot, B., Demeulenaere, M., Grunewald, W., Audenaert, D., et al. (2010). A novel aux/IAA28 signaling cascade activates GATA23□dependent specification of lateral root founder cell identity. *Curr. Biol., 20*(19), 1697–1706.

Deane, J. A., Cole, D. G., Seeley, E. S., Diener, D. R., and Rosenbaum, J. L. (2001). Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. *Curr. Biol., 11*(20), 1586–1590.

Deavours, B. E., Reddy, A. S., and Walker, R. A. (1998). Ca2+/calmodulin regulation of the Arabidopsis kinesin-like calmodulin-binding protein. *Cell Motil. Cytoskeleton, 40*(4), 408-416.

Deeb, F. (2009). The role of spermidine in the regulation of development and differentiation in spermatids of *Marsilea vestita*. Ph.D. University of Maryland, College Park, MD.

Deeb, F., van der Weele, C. M., and Wolniak, S. M. (2010). Spermidine is a morphogenetic determinant for cell fate specification in the male gametophyte of the water fern *Marsilea vestita*. *Plant Cell, 22*(11), 3678-3691

Delgehyr, N., Rangone, H., Fu, J., Mao, G., Tom, B., Riparbelli, M. G., et al. (2012). Klp10A, a microtubule-depolymerizing kinesin-13, cooperates with CP110 to control *Drosophila* centriole length. Curr. Biol., 22(6), 502–509.

Demonchy, R., Blisnick, T., Deprez, C., Toutirais, G., Loussert, C., Marande, W., et al. (2009). Kinesin 9 family members perform separate functions in the trypanosome flagellum. *J. Cell Biol., 187*(5), 615–622.

Deng, Z. Y., Liu, L. T., Li, T., Yan, S., Kuang, B. J., Huang, S. J., et al. (2015). OsKinesin-13A is an active microtubule depolymerase involved in glume length regulation via affecting cell elongation. *Sci. Rep., 5*, 9457.

Desai, A., Verma, S., Mitchison, T. J., and Walczak, C. E. (1999). Kin I kinesins are microtubule-destabilizing enzymes. *Cell, 96*(1), 69-78.

Desai, P. B., Freshour, J. R., and Mitchell, D. R. (2015). *Chlamydomonas* axonemal dynein assembly locus ODA8 encodes a conserved flagellar protein needed for cytoplasmic maturation of outer dynein arm complexes. *Cytoskeleton (Hoboken, N.J.), 72*(1), 16–28.

Dhonukshe, P. and Gadella, T. W. J. (2003). Alteration of microtubule dynamic instability during preprophase band formation revealted by yellow fluorescent protein-CLIP170 microtubule plus-end labeling. *Plant Cell, 15*(3), 597-611.

Di Tommaso, P., Moretti, S., Xenarios, I., Orobitg, M., Montanyola, A., Chang, J.-M., et al. (2011). T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. *Nucleic Acids Res., 39*, W13–7.

DiBella, L. M., Sakato, M., Patel-King, R. S., Pazour, G. J., and King, S. M. (2004). The LC7 light chains of *Chlamydomonas* flagellar dyneins interact with components required for both motor assembly and regulation. *Mol. Biol. Cell.*, *15*(10), 4633–4646.

Dymek, E. E., Goduti, D., Kramer, T., and Smith, E. F. (2006). A kinesin-like calmodulin-binding protein in Chlamydomonas: evidence for a role in cell division and flagellar functions. *J. Cell Sci., 119*(15), 3107–3116.

Efimenko, E., Blacque, O. E., Ou, G., Haycraft, C. J., Yoder, B. K., Scholey, J. M., et al. (2006). *Caenorhabditis elegans* DYF-2, an orthologue of human WDR19, is a component of the intraflagellar transport machinery in sensory cilia. *Mol. Biol. Cell, 17*(11), 4801–4811.

Endow, S. A. (1999). Determinants of molecular motor directionality. *Nat. Cell Biol., 1*(6), E163-E167.

Eng, R. C., and Wasteneys, G. O. (2014). The microtubule plus-end tracking protein ARMADILLO-REPEAT KINESIN1 promotes microtubule catastrophe in *Arabidopsis*. *Plant Cell, 26*(8), 3372–3386.

Espindola, F. S., Suter, D. M., Partata, L. B., Cao, T., Wolenski, J. S., Cheney, R. E., et al. (2000). The light chain composition of chicken brain myosin-Va: calmodulin, myosin-II essential light chains, and 8-kDa dynein light chain. *Cell Motil. Cytoskeleton, 47*(4), 269–281.

Evans, J. E., Snow, J. J., Gunnarson, A. L., Ou, G., Stahlberg, H., McDonald, K. L., and Scholey, J. M. (2006). Functional modulation of IFT kinesins extends the sensory repertoire of ciliated neurons in Caenorhabditis elegans. *J. Cell Biol., 172*(5), 663–669.

Fan, J. and Beck, K. A. (2004). A role for the spectrin superfamily member Syne-1 and kinesin II in cytokinesis. *J. Cell Sci., 117*(4), 619–629.

Fan, Z.-C., Behal, R. H., Geimer, S., Wang, Z., Williamson, S. M., Zhang, H., et al. (2010). *Chlamydomonas* IFT70/CrDYF-1 is a core component of IFT particle complex B and is required for flagellar assembly. *Mol. Biol. Cell*, *21*(15), 2696–2706.

Fink, J. L. and Hamilton, N. (2007). DomainDraw: a macromolecular feature drawing program. *In Silico Biol., 2*, 145-150.

Frey, N., Klotz, J., and Nick, P. (2009). Dynamic bridges--a calponin-domain kinesin from rice links actin filaments and microtubules in both cycling and non-cycling cells. *Plant Cell Physiol., 50*(8), 1493-1506.

Frey, N., Klotz, J., and Nick, P. (2010). A kinesin with calponin-homology domain is involved in premitotic nuclear migration. *J. Exp Bot., 61*(12), 3423–3437.

Fujikura, U., Elsaesser, L., Breuninger, H., Sánchez-Rodríguez, C., Ivakov, A., Laux, T., et al. (2014). AtKinesin-13A modulates cell-wall synthesis and cell expansion in Arabidopsis thaliana via the THESEUS1 pathway. *PloS Genet., 10*(9), e1004627.

Goodenough, U. and Heuser, J. (1984). Structural comparison of purified dynein proteins with in situ dynein arms. *J. Mol. Biol.*, *180*(4), 1083–1118.

Goodenough, U. W. and Heuser, J. E. (1985). Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. *J. Cell Biol., 100*(6), 2008–2018.

Gorgoni, B. and Gray, N. K. (2004). The roles of cytoplasmic poly(A)-binding proteins in regulating gene expression: a developmental perspective. *Briefings in Functional Genomics Proteomics, 3*(2), 125–141.

Goshima, G. and Kimura, A. (2010). New look inside the spindle: Microtubule-dependent microtubule generation within the spindle. *Curr Opin Cell Biol., 22*(1), 44–49.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson D. A., Amit I., Adiconis X., Fan L., Raychowdhury R., Zeng Q., et al. (2011). Full-length transcriptome assembly from RNAseq data without a reference genome. *Nat. Biotechnol., 29*(7), 644-652.

Han, Y.-G., Kwok, B. H., and Kernan, M. J. (2003). Intraflagellar transport is required in *Drosophila* to differentiate sensory cilia but not sperm. *Curr. Biol., 13*(19), 1679–1686.

Haraguchi, K., Hayashi, T., Jimbo, T., Yamamoto, T., and Akiyama, T. (2006). Role of the Kinesin-2 Family Protein, KIF3, during Mitosis. *J. Biol. Chem., 281*, 4094–4099.

Harris, J. A., Liu, Y., Yang, P., Kner, P., and Lechtreck, K. F. (2016). Single-particle imaging reveals intraflagellar transport-independent transport and accumulation of EB1 in Chlamydomonas flagella. *Mol. Biol. Cell, 27*(2), 295–307.

Hart, P. E. and Wolniak, S. M. (1998). Spermiogenesis in *Marsilea vestita*: a temporal correlation between centrin expression and blepharoplast differentiation. *Cell Motil. Cytoskeleton, 41*(1), 39-48.

Hart, P. E. and Wolniak, S. M. (1999). Molecular cloning of a centrin homolog from *Marsilea vestita* and evidence for its translational control during spermiogenesis. Biochem. *Cell Biol., 7*(2), 101–108.

Hendrickson, T. W., Perrone, C. A., Griffin, P., Wuichet, K., Mueller, J., Yang, P., et al. (2004). IC138 is a WD-repeat dynein intermediate chain required for light chain assembly and regulation of flagellar bending. *Mol. Biol. Cell*, *15*(12), 5431–5442.

Henson, J. H., Cole, D. G., Terasaki, M., Rashid, D., and Scholey, J. M. (1995). Immunolocalization of the heterotrimeric kinesin-related protein KRP(85/95) in the mitotic apparatus of sea urchin embryos. *Dev. Biol., 171*(1), 182–194.

Hepler P. K. (1976). The blepharoplats of Marsilea: it's de novo formation and spindle association. *J. Cell Sci., 21*, 361-390.

Heuser, T., Raytchev, M., Krell, J., Porter, M. E., and Nicastro, D. (2009). The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. The J. Cell Biol., 187(6), 921–933.

Hirokawa, N. (1998). Kinesin and dynein sperfamily proteins and the mechanism of organelle transport. *Science, 279*(5350), 519-526.

Hiwatashi, Y., Obara, M., Sato, Y., Fujita, T., Murata, T., and Hasebe, M. (2008). Kinesins are indispensable for interdigitation of phragmoplast microtubules in the moss Physcomitrella patens. *Plant Cell, 20*(1), 3094–106.

Hiwatashi, Y., Sato, Y., and Doonan, J. H. (2014). Kinesins have a dual function in organizing microtubules during both tip growth and cytokinesis in Physcomitrella patens. *Plant Cell, 26*(3), 1256-1266.

Hodges, M. E., Scheumann, N., Wickstead, B., Langdale, J. A., and Gull, K. (2010). Reconstructing the evolutionary history of the centriole from protein components. *J. Cell Sci.*, *123*(Pt 9), 1407–1413.

Hoffman, J. C., Vaughn, K. C. and Joshi, H. C. (1994). Structural and immunocytochemical characterization of microtubule organizing centers in pteridophyte spermatogenous cells. *Protoplasma 179*, 46-60.

Höök, P. and Vallee, R. B. (2006). The dynein family at a glance. *J. Cell Sci.*, *119*(Pt 21), 4369–4371.

Hou, Y., Qin, H., Follit, J. A., Pazour, G. J., Rosenbaum, J. L., and Witman, G. B. (2007). Functional analysis of an individual IFT protein: IFT46 is required for transport of outer dynein arms into flagella. *J. Cell Biol., 176*(5), 653–665.

Howell, B. J., McEwen, B. F., Canman, J. C., Hoffmann, D. B., Farrar, E. M., Rieder, C. L., and Salmon, E. D. (2001). Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and a role in mitotic spindle checkpoint inactivation. *J. Cell Biol., 155*(7), 1159-1172.

Huang, B., Rifkin, M. R., and Luck, D. J. (1977). Temperature-sensitive mutations affecting flagellar assembly and function in Chlamydomonas reinhardtii. *J. Cell Biol., 72*(1), 67–85.

Hyams, J. S. (1985). Binding of tetrahymena dynein to axonemes of *Marsilea vestita* lacking the outer dynein arm. *J. Cell Sci., 73*(1), 299-310.

Hyams, J. S. and Campbell, C. J. (1985). Widespread absence of outer dynein arms in the spermatozoid of lower plants. *Cell Biol. Int. Rep., 9*(9), 841-848.

Imanishi, M., Endres, N. F., Gennerich, A., and Vale, R. D. (2006). Autoinhibition regulates the motility of the C. elegans intraflagellar transport motor OSM-3. *J. Cell Biol., 174*(7), 931–937.

Inoue, S. and Salmon, E. D. (1995). Force generation by microtubule assembly/disassembly in mitosis and related movements. Mol. Biol. Cell, 6(12), 1619-1640.

Iomini, C., Babaev-Khaimov, V., Sassaroli, M., and Piperno, G. (2001). Protein particles in Chlamydomonas flagella undergo a transport cycle consisting of four phases. *J. Cell Biol., 153*(1), 13–24.

Iomini, C., Li, L., Esparza, J. M., and Dutcher, S. K. (2009). Retrograde intraflagellar transport mutants identify complex A proteins with multiple genetic interactions in Chlamydomonas reinhardtii. *Genetics, 183*(3), 885–896.

Ishikawa, M., Soyano, T., Nishihama, R., and Machida, Y. (2002). The NPK1 mitogen-activated protein kinase kinase kinase contains a functional nuclear localization signal at the binding site for the NACK1 kinesin-like protein. *Plant J., 32*(5), 789-98.

Itoh, R., Fujiwara, M., and Yoshida, S. (2001). Kinesin-related proteins with a mitochondrial targeting signal. *Plant Physiol., 127*(3), 724–726.

Jaillon, O., Aury, J.-M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., et al. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature, 449*(7161), 463–467.

Janisch, K. M. and Dwyer, N. D. (2016). Imaging and quantitative analysis of cytokinesis in developing brains of Kinesin-6 mutant mice. *Methods Cell Biol., 131*, 233–252.

Janisch, K. M., Vock, V. M., Fleming, M. S., Shrestha, A., Grimsley-Myers, C. M., Rasoul, B. A., et al. (2013). The vertebrate-specific Kinesin-6, Kif20b, is required for normal cytokinesis of polarized cortical stem cells and cerebral cortex size. *Development, 140*(23), 4672–4682.

Jékely, G. and Arendt, D. (2006). Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. BioEssay, 28(2), 191–198.

Jimbo, T., Kawasaki, Y., Koyama, R., Sato, R., Takada, S., Haraguchi, K., and Akiyama, T. (2002). Identification of a link between the tumour suppressor APC and the kinesin superfamily. *Nat Cell Biol, 4*(4), 323–327.

Jin, H., White, S. R., Shida, T., Schulz, S., Aguiar, M., Gygi, S. P., et al. (2010). The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell, 141*(7), 1208–1219.

Johnson, K. A., and Wall, J. S. (1983). Structure and molecular weight of the dynein ATPase. *J. Cell Biol., 96*(3), 669–678.

Johnson, U.G. and Porter, K.R. (1968). Fine structure of cell division in Chlamydomonas reinhardi. Basal bodies and microtubules. *J. Cell Biol., 38*(2), 403–425.

Jones, M. A., Raymond, M. J., and Smirnoff, N. (2006). Analysis of the root-hair morphogenesis transcriptome reveals the molecular identity of six genes with roles in root-hair development in Arabidopsis. *Plant J., 45*(1), 83–100.

Jonsson, E., Yamada, M., Vale, R. D., and Goshima, G. (2015). Clustering of a kinesin-14 motor enables processive retrograde microtubule-based transport in plants. *Nat. Plants, 1,* 15087

Jürgens, G. (2000). Cytokinesis: the art of partitioning. *Plant Cell, 12*(6), 827–828.

Kamiya, R. (2000). Analysis of cell vibration for assessing axonemal motility in Chlamydomonas. *Methods, 22*(4), 383–387.

Kamiya, R., and Okamoto, M. (1985). A mutant of Chlamydomonas reinhardtii that lacks the flagellar outer dynein arm but can swim. *J. Cell Sci., 74*, 181–191.

Keil, R., Kiessling, C., and Hatzfeld, M. (2009). Targeting of p0071 to the midbody depends on KIF3. J. Cell Sci., 122(8), 1174–1183.

Kim, Y., Heuser, J. E., Waterman, C. M., and Cleveland, D. W. (2008). CENP-E combines a slow, processive motor and a flexible coiled coil to produce an essential motile kinetochore tether. *J. Cell Biol., 181*(3), 411–419.

King, S. J., Bonilla, M., Rodgers, M. E., and Schroer, T. A. (2002). Subunit organization in cytoplasmic dynein subcomplexes. *Protein Sci, 11*(5), 1239–1250.

King, S. M., Barbarese, E., Dillman, J. F., Patel-King, R. S., Carson, J. H., and Pfister, K. K. (1996). Brain cytoplasmic and flagellar outer arm dyneins share a highly conserved Mr 8,000 light chain. *J. Biol. Chem., 271*(32), 19358–19366.

Kline-Smith, S. L., Khodjakov, A., Hergert, P., & Walczak, C. E. (2004). Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. *Mol. Biol. Cell, 15*(3), 1146–1159.

Klink, V. P., and Wolniak, S. M. (2001). Centrin is necessary for the formation of the motile apparatus in spermatids of Marsilea. *Mol. Biol. Cell, 12*(3), 761-776.

Klink, V. P., and Wolniak, S. M. (2003). Changes in the abundance and distribution of conserved centrosomal, cytoskeletal and ciliary proteins during spermiogenesis in *Marsilea vestita*. *Cell Motil. Cytoskeleton, 56*(1), 57-73.

Klotz, J., and Nick, P. (2012). A novel actin-microtubule cross-linking kinesin, NtKCH, functions in cell expansion and division. *New Phytol., 193*(3), 576–589.

Kobayashi, T., Tsang, W. Y., Li, J., Lane, W., and Dynlacht, B. D. (2011). Centriolar kinesin Kif24 interacts with CP110 to remodel microtubules and regulate ciliogenesis. *Cell, 145*(6), 914–925.

Koonin, E. V. and Aravind, L. (2000). Dynein light chains of the Roadblock/LC7 group belong to an ancient protein superfamily implicated in NTPase regulation. *Curr. Biol., 10*(21), R774–6.

Kozminski, K. G., Beech, P. L., and Rosenbaum, J. L. (1995). The Chlamydomonas kinesin-like protein FLA10 is involved in motility associated with the flagellar membrane. *J. Cell Biol., 131*(6), 1517–1527.

Kozminski, K. G., Johnson, K. A., Forscher, P., and Rosenbaum, J. L. (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl. Acad. Sci. USA, 90*(12), 5519–5523.

Kulaga, H. M., Leitch, C. C., Eichers, E. R., Badano, J. L., Lesemann, A., Hoskins, B. E., et al. (2004). Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat Genet, 36*(9), 994–998.

Lawrence, C. J., Dawe, R. K., Christie, K. R., Cleveland, D. W., Dawson, S. C., Endow, S. A., Goldstein, L. S., Goodson, H. V., Hirokawa, N., Howard, J. et al. (2004). A standardized kinesin nomenclature. *J. Cell Biol., 167*(1), 19-22.

Lawrence, C. J., Morris, N. R., Meagher, R. B., and Dawe, R. K. (2001). Dyneins have run their course in plant lineage. *Traffic, 2*(5), 362–363.

Le Bot, N., Antony, C., White, J., Karsenti, E., and Vernos, I. (1998). Role of xklp3, a subunit of the Xenopus kinesin II heterotrimeric complex, in membrane transport between the endoplasmic reticulum and the Golgi apparatus. *J. Cell Biol., 143*(6), 1559–1573.

Lechtreck, K.-F., and Witman, G. B. (2007). Chlamydomonas reinhardtii hydin is a central pair protein required for flagellar motility. *J. Cell Biol., 176*(4), 473–482.

Lechtreck, K.-F., Delmotte, P., Robinson, M. L., Sanderson, M. J., and Witman, G. B. (2008). Mutations in Hydin impair ciliary motility in mice. *J. Cell Biol., 180*(3), 633–643.

Lechtreck, K.-F., Johnson, E. C., Sakai, T., Cochran, D., Ballif, B. A., Rush, J., et al. (2009). The Chlamydomonas reinhardtii BBSome is an IFT cargo required for export of specific signaling proteins from flagella. *J. Cell Biol.*, *187*(7), 1117–1132.

Lee, Y.-R. J. and Liu, B. (2000). Identification of a phragmoplast-associated kinesin-related protein in higher plants. *Curr. Biol., 10*(13), 797-800.

Lee, Y.-R. J. and Liu, B. (2004). Cytoskeletal Motors in Arabidopsis. Sixty-One Kinesins and Seventeen Myosins. *Plant Physiol., 136*(4), 3877-3883.

Lee, Y.-R. J., Li, Y., and Liu, B. (2007). Two Arabidopsis phragmoplast-associated kinesins play a critical role in cytokinesis during male gametogenesis. *Plant Cell, 19*(8), 2595-2605.

Lee, Y.-R. J., Qiu, W., and Liu, B. (2015). Kinesin motors in plants: from subcellular dynamics to motility regulation. *Curr. Opin. Plant Biol., 28*, 120–126.

Lipka, E. and Müller, S. (2012). Potential roles for kinesins at the cortical division site. *Front. Plant Sci., 3*, 158.

Lipka, E., Gadeyne, A., Stöckle, D., Zimmermann, S., De Jaeger, G., Ehrhardt, D. W., et al. (2014). The Phragmoplast-Orienting Kinesin-12 Class Proteins Translate the Positional Information of the Preprophase Band to Establish the Cortical Division Zone in Arabidopsis thaliana. *Plant Cell, 26*(6), 2617-2632

Lloyd, C. and Chan, J. (2006). Not so divided: The common basis of plant and animal cell division. *Nat. Rev. Mol. Cell Biol., 7*(2), 147–152.

Loktev, A. V., Zhang, Q., Beck, J. S., Searby, C. C., Scheetz, T. E., Bazan, J. F., et al. (2008). A BBSome subunit links ciliogenesis, microtubule stability, and acetylation. *Dev. Cell, 15*(6), 854–865.

López-Bautista, J. M., Waters, D. A., & Chapman, R. L. (2003). Phragmoplastin, green algae and the evolution of cytokinesis. *Int. J. Syst. Evol. Microbiol., 53*(6), 1715–1718.

Lu, L., Lee, Y. R., Pan, R., Maloof, J. N., and Liu, B. (2005). An internal motor kinesin is associated with the Golgi apparatus and plays a role in trichome morphogenesis in Arabidopsis. *Mol. Biol. Cell, 16*(2), 811–823.

Lucker, B. F., Behal, R. H., Qin, H., Siron, L. C., Taggart, W. D., Rosenbaum, J. L., and Cole, D. G. (2005). Characterization of the intraflagellar transport complex B

core: direct interaction of the IFT81 and IFT74/72 subunits. *J. Biol. Chem., 280*(30), 27688–27696.

Lucker, B. F., Miller, M. S., Dziedzic, S. A., Blackmarr, P. T., and Cole, D. G. (2010). Direct interactions of intraflagellar transport complex B proteins IFT88, IFT52, and IFT46. *J. Biol. Chem*, *285*(28), 21508–21518.

Macaskill, A. F., Rinholm, J. E., Twelvetrees, A. E., Arancibia-Carcamo, I. L., Muir, J., Fransson, A., et al. (2009). Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron, 61*(4), 541–555.

Malcos, J. L. and Cyr, R. J. (2011). An ungrouped plant kinesin accumulates at the preprophase band in a cell-cycle dependent manner. *Cytoskeleton (Hoboken), 68*(4), 247-258

Maney, T., Hunter, A. W., Wagenbach, M., and Wordeman, L. (1998). Mitotic centromere-associated kinesin is important for anaphase chromosome segregation. *J. Cell Biol., 142*(3), 787–801.

Manning, A. L., Ganem, N. J., Bakhoum, S. F., Wagenbach, M., Wordeman, L., and Compton, D. A. (2007). The kinesin-13 proteins Kif2a, Kif2b, and Kif2c/MCAK have distinct roles during mitosis in human cells. *Mol. Biol. Cell, 18*(8), 2970–2979.

Manton, I., and Clarke, B. (1951). Demonstration of compound cilia in a fern spermatozoid with the electron microscope. *J. Exp. Bot., 2*, 125-128.

Marande, W., and Kohl, L. (2011). Flagellar kinesins in protists. *Future Microbiol., 6*(2), 231-246.

Marc, J. and Gunning, B. E. S. (1986). Immunofluorescent localization of cytoskeletal tubnulin and actin during spermatogenesis in Pteridium aquilinum (L.) Kuhn. *Protoplasma, 134*, 163-177.

Marcus, A. L., Li, W., and Cyr, R. J. (2003). A kinesin with an atypical bipolar spindle undergoes normal mitosis. *Mol. Bio. Cell, 14*(4), 1717-1726.

Martin, K. C. and Ephrussi, A. (2009). mRNA localization: gene expression in the spatial dimension. *Cell, 136*(4), 719–730.

Matsuura, K., Lefebvre, P. A., Kamiya, R., and Hirono, M. (2002). Kinesin-II is not essential for mitosis and cell growth in Chlamydomonas. *Cell Motil. Cytoskeleton, 52*(4), 195–201.

Mayr, M. I., Hümmer, S., Bormann, J., Grüner, T., Adio, S., Woehlke, G., and Mayer, T. U. (2007). The human kinesin Kif18A is a motile microtubule depolymerase essential for chromosome congression. *Curr. Biol., 17*(6), 488–498.

McGrail, M. and Hays, T. S. (1997). The microtubule motor cytoplasmic dynein is required for spindle oriengation during germline cell division and oocyte differentaiton in *Drosophila. Development, 124*(12), 2409-2419.

Merdes, A., Heald, R., Samejima, K., Earnshaw, W. C., and Cleveland, D. W. (2000). Formation of spindle poles by dynein/dynactin-dependent transport of NuMA. J. *Cell Biol., 149*(4), 851-862.

Miki, H., Okada, Y., and Hirokawa, N. (2005). Analysis of the kinesin superfamily: insights into structure and function. Trends in Cell Biol., 15(9), 467–476.

Miki, H., Setou, M., Kaneshiro, K., and Hirokawa, N. (2001). All kinesin superfamily proteins, KIF, genes in mouse and humans. *Proc. Natl. Acad. Sci. USA, 98*(13), 7004-7011.

Miki, T., Natio, H., Nishina, M., and Goshima, G. (2014). Endogenous localizone identifies 43 mitotic kinesins in a plant cell. *Proc. Natl. Acad. Sci. USA, 111*(11), E1053-E1061.

Miki, T., Nishina, M., and Goshima, G. (2015). RNAi Screening Identifies the Armadillo Repeat-Containing Kinesins Responsible for Microtubule-Dependent Nuclear Positioning in Physcomitrella patens. *Plant Cell Physiol., 56*(4), 737-749.

Miller, M. S., Esparza, J. M., Lippa, A. M., Lux, F. G., III, Cole, D. G., and Dutcher, S. K. (2005). Mutant Kinesin-2 Motor Subunits Increase Chromosome Loss. *Mol. Biol. Cell, 16*, 3810–3820.

Mitchell, D. R. and Kang, Y. (1991). Identification of oda6 as a Chlamydomonas dynein mutant by rescue with the wild-type gene. *J. Cell Biol.*, *113*(4), 835–842.

Mitchell, D. R. and Sale, W. S. (1999). Characterization of a Chlamydomonas insertional mutant that disrupts flagellar central pair microtubule-associated structures. *J. Cell Biol., 144*(2), 293–304.

Mizukami, I. and Gall J. (1966). Centriole replication. II. Sperm formation in the fern, Marsilea, and the cycad, Zamia. *J. Cell Biol., 29*(1), 97-111.

Molk, J. N. and Wolniak, S. M. (2000). Spermatid formation in *Marsilea vestita* involves Myosin Light Chain Kinase (MLCK). Mol. Biol. of the Cell. 11s: 352a.

Moores, C. A. and Milligan, R. A. (2006). Lucky 13-microtubule depolymerisation by kinesin-13 motors. *J. Cell Sci., 119*(19), 3905–3913.

Moran, R. C. 2004. A Natural History of Ferns. Portland: Timber Press.

Morlino, G., Barreiro, O., Baixauli, F., Robles-Valero, J., González-Granado, J. M., Villa-Bellosta, R., et al. (2014). Miro-1 links mitochondria and microtubule Dynein motors to control lymphocyte migration and polarity. *Mol. Cell. Biol., 34*(8), 1412–1426.

Morris, R. L., and Scholey, J. M. (1997). Heterotrimeric kinesin-II is required for the assembly of motile 9+2 ciliary axonemes on sea urchin embryos. *J. Cell Biol., 138*(5), 1009–1022.

Morsci, N. S. and Barr, M. M. (2011). Kinesin-3 KLP-6 regulates intraflagellar transport in male-specific cilia of *Caenorhabditis elegans*. *Curr. Biol., 21*(14), 1239–1244.

Mukhopadhyay, S., Lu, Y., Qin, H., Lanjuin, A., Shaham, S., and Sengupta, P. (2007). Distinct IFT mechanisms contribute to the generation of ciliary structural diversity in *C. elegans*. EMBO J., 26(12), 2966–2980.

Mukhopadhyay, S., Lu, Y., Shaham, S., and Sengupta, P. (2008). Sensory signaling-dependent remodeling of olfactory cilia architecture in *C. elegans*. *Dev. Cell, 14*(5), 762–774.

Müller, S., Wright, A. J., and Smith, L. G. (2009). Division plane control in plants: New players in the band. *Trends Cell Biol., 19*(4), 180–188.

Mykytyn, K., Mullins, R. F., Andrews, M., Chiang, A. P., Swiderski, R. E., Yang, B., et al. (2004). Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc. Natl. Acad. Sci. USA, 101*(23), 8664–8669.

Myles, D. G. and Bell, P. R. (1975). An untrastructural study of the spermatazoid of the fern, *Marsilea vestita*. *J. Cell Sci., 17*(3), 633-645.

Myles, D. G. and Hepler, P. K. (1977). Spermiogenesis in the fern *Marsilea vestita*: microtubules, nuclear shaping, and cytomorphogenesis. *J. Cell Sci., 23*, 57-83.

Myles, D. G. and Hepler, P. K. (1982). Shaping of the sperm nucleus in Marsilea: a distinction between factors responsible for shape generation and shape determination. *Dev. Biol., 90*, 238-252.

Myles, D. G., Southworth D., and Hepler P. K. (1978). Cell surface topography during Marsilea spermiogenesis: flagellar reorientation and membrane particle arrays. *Protoplasma, 93*, 405-417.

Myster, S. H., Knott, J. A., O'Toole, E., and Porter, M. E. (1997). The Chlamydomonas Dhc1 gene encodes a dynein heavy chain subunit required for assembly of the I1 inner arm complex. *Mol. Biol. Cell*, 8(4), 607–620.

Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peränen, J., Merdes, A., et al. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell, 129*(6), 1201–1213.

Naito, H. and Goshima, G. (2015). NACK Kinesin is required for metaphase chromosome alignment and cytokinesis in the moss Physcomitrella patens. Cell *Struct. Funct., 40*(1), 31-41.

Narasimhulu, S. B., Kao, Y. L., and Reddy, A. S. (1997). Interaction of Arabidopsis kinesin-like calmodulin-binding protein with tubulin subunits: modulation by Ca(2+)-calmodulin. *Plant J., 12*(5), 1139-1149.

Nekrasova, O. E., Amargo, E. V., Smith, W. O., Chen, J., Kreitzer, G. E., and Green, K. J. (2011). Desmosomal cadherins utilize distinct kinesins for assembly into desmosomes. *J. Cell Biol., 195*(7), 1185–1203.

Neuwald, A. F., Aravind, L., Spouge, J. L., and Koonin, E. V. (1999). AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Res., 9*(1), 27–43.

Ni, C. Z., Wang, H. Q., Xu, T., Qu, Z., and Liu, G. Q. (2005). AtKP1, a kinesin-like protein, mainly localizes to mitochondria in Arabidopsis thaliana. *Cell Res., 15*(9), 725–733.

Nicastro, D., McIntosh, J. R., and Baumeister, W. (2005). 3D structure of eukaryotic flagella in a quiescent state revealed by cryo-electron tomography. *Proc. Natl. Acad. Sci. USA, 102*(44), 15889–15894.

Nicastro, D., Schwartz, C., Pierson, J., Gaudette, R., Porter, M. E., and McIntosh, J. R. (2006). The molecular architecture of axonemes revealed by cryoelectron tomography. *Science, 313*(5789), 944–948.

Nishihama, R., Soyano, T., Ishikawa, M., Araki, S., Tanaka, H., Asada, T., et al. (2002). Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/MAPKKK complex. *Cell, 109*(1), 87-99.

Niwa, S., Nakajima, K., Miki, H., Minato, Y., Wang, D., and Hirokawa, N. (2012). KIF19A is a microtubule-depolymerizing kinesin for ciliary length control. *Dev. Cell, 23*(6), 1167–1175.

Notredame, C., Higgins, D. G., and Heringa, J. (2000). T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol., 302*(1), 205–217.

Nott, A., Le Hir, H., and Moore, M. J. (2004). Splicing enhances translation in mammalian cells: an additional function of the exon junction complex. *Genes Dev., 18*(2), 210–222.

Oda, Y., and Fukuda, H. (2013a). Rho of plant GTPase signaling regulates the behavior of Arabidopsis kinesin-13A to establish secondary cell wall patterns. Plant *Cell, 25*(11), 4439–4450.

Oda, Y., and Fukuda, H. (2013b). The dynamic interplay of plasma membrane domains and cortical microtubules in secondary cell wall patterning. *Front. Plant Sci., 4*, 511.

Ogawa, K., Kamiya, R., Wilkerson, C. G., and Witman, G. B. (1995). Interspecies conservation of outer arm dynein intermediate chain sequences defines two intermediate chain subclasses. *Mol Biol. Cell, 6*(6), 685–696.

Ogawa, T., Nitta, R., Okada, Y., and Hirokawa, N. (2004). A Common Mechanism for Microtubule Destabilizers—M Type Kinesins Stabilize Curling of the Protofilament Using the Class-Specific Neck and Loops. *Cell, 116*(4), 591–602.

Oh, S. A., Allen, T., Kim, G. J., Sidorova, A., Berg, M., Park, S. K., and Twell, D. (2012). Arabidopsis Fused kinase and the kinesin-12 subfamily constitute a signaling module required for phragmoplast expansion. *Plant J., 72*(2), 308-319.

Oliver, T. N., Berg, J. S., and Cheney, R. E. (1999). Tails of unconventional myosins. *Cell. Mol. Life Sci., 56*(3-4), 243-257.

Omoto, C. K., Gibbons, I. R., Kamiya, R., Shingyoji, C., Takahashi, K., and Witman, G. B. (1999). Rotation of the central pair microtubules in eukaryotic flagella. *Mol. Biol. Cell, 10*(1), 1–4.

Oppenheimer, D. G., Pollock, M. A., Vacik, J., Szymanski, D. B., Ericson, B., Feldmann, K., and Marks, M. D. (1997). Essential role of a kinesin-like protein in Arabidopsis trichome morphogenesis. *Proc. Natl. Acad. Sci. USA, 94*(12), 6261-6266.

Ou, G., Blacque, O. E., Snow, J. J., Leroux, M. R., and Scholey, J. M. (2005). Functional coordination of intraflagellar transport motors. *Nature, 436*(7050), 583–587.

Ovechkina, Y., Wagenbach, M., and Wordeman, L. (2002). K-loop insertion restores microtubule depolymerizing activity of a “neckless” MCAK mutant. *J. Cell Biol., 159*(4), 557–562.

Pan, R., Lee, Y. R., and Liu, B. (2004). Localization of two homologous Arabidopsis kinesin-related proteins in the phragmoplast. *Planta, 220*(1), 156-164.

Pan, X., Ou, G., Civelekoglu-Scholey, G., Blacque, O. E., Endres, N. F., Tao, L., et al. (2006). Mechanism of transport of IFT particles in *C. elegans* cilia by the concerted action of kinesin-II and OSM-3 motors. *J. Cell Biol., 174*(7), 1035–1045.

Paschal, B. M. and Vallee, R. B. (1987). Retrograde transport by the microtubule-associated protein MAP 1C. *Nature, 330*(6144), 181–183.

Paschal, B. M., Mikami, A., Pfister, K. K., and Vallee, R. B. (1992). Homology of the 74-kD cytoplasmic dynein subunit with a flagellar dynein polypeptide suggests an intracellular targeting function. *J. Cell Biol.*, 118(5), 1133–1143.

Patel-King, R. S., Gorbatyuk, O., Takebe, S., and King, S. M. (2004). Flagellar radial spokes contain a Ca2+-stimulated nucleoside diphosphate kinase. *Mol. Biol. Cell, 15*(8), 3891–3902.

Pazour, G. J., Dickert, B. L., and Witman, G. B. (1999). The DHC1b (DHC2) isoform of cytoplasmic dynein is required for flagellar assembly. *J. Cell Biol., 144*(3), 473–481.

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B., and Cole, D. G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J. Cell Biol*, *151*(3), 709–718.

Pazour, G. J., Wilkerson, C. G., and Witman, G. B. (1998). A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). *J. Cell Biol.*, *141*(4), 979–992.

Pedersen, L. B., Veland, I. R., Schrøder, J. M., and Christensen, S. T. (2008). Assembly of primary cilia. *Dev. Dyn., 237*(8), 1993–2006.

Pennell, R. I., Hyams, J. S., and Bell, P. R. (1986). The blepharoplast of Marsilea: a structure concerned with basal body assembly lacking tubulin. *Eur. J. Cell Biol., 40*(2), 238–241.

Pennell, R. I., Vondy, K. P., Bell, P. R., and Hyams, J. S. (1988). Composition and function of the blepharoplast of *Marsilea vestita*. *Eur. J. Cell Biol., 46*(1), 51–60.

Perkins, L. A., Hedgecock, E. M., Thomson, J. N., and Culotti, J. G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol., 117*(2), 456–487.

Perrone, C. A., Yang, P., O'Toole, E., Sale, W. S., and Porter, M. E. (1998). The Chlamydomonas IDA7 locus encodes a 140-kDa dynein intermediate chain required to assemble the I1 inner arm complex. *Mol. Biol. Cell*, *9*(12), 3351–3365.

Phillips, D. M. (1969). Exceptions to the prevailing pattern of tubules (9 + 9 + 2) in the sperm flagella of certain insect species. *J. Cell Biol., 40*(1), 28–43.

Piao, T., Luo, M., Wang, L., Guo, Y., Li, D., Li, P., et al. (2009). A microtubule depolymerizing kinesin functions during both flagellar disassembly and flagellar assembly in Chlamydomonas. *Proc. Natl. Acad. Sci. USA, 106*(12), 4713–4718.

Pickett-Heaps, J. (1976). Cell division in eucaryotic algae. *Bioscience, 26*(7), 445–450.

Piperno, G., and Mead, K. (1997). Transport of a novel complex in the cytoplasmic matrix of Chlamydomonas flagella. *Proc. Natl. Acad. Sci. USA, 94*(9), 4457–4462.

Piperno, G., Siuda, E., Henderson, S., Segil, M., Vaananen, H., and Sassaroli, M. (1998). Distinct mutants of retrograde intraflagellar transport (IFT) share similar morphological and molecular defects. *J. Cell Biol, 143*(6), 1591–1601.

Porter, M. E., Bower, R., Knott, J. A., Byrd, P., and Dentler, W. (1999). Cytoplasmic dynein heavy chain 1b is required for flagellar assembly in Chlamydomonas. *Mol. Biol. Cell, 10*(3), 693–712.

Portman, N., and Gull, K. (2010). The paraflagellar rod of kinetoplastid parasites: from structure to components and function. *Int. J. Parasitol., 40*(2), 135–148.

Preuss, M. L., Kovar, D. R., Lee, YR. J., Staiger, C. J., Delmer, D. P., and Liu, B. (2004). A plant-specific kinesin binds to actin microfilaments and interacts with cortical microtubules in cotton fibers. *Plant Physiol., 136*(4), 3945-3955.

Prigge, M. J. and Bezanilla, M. (2010). Evolutionary crossroads in developmental biology: Physcomitrella patens. *Development, 137*(21), 3535–3543.

Pryer, K. M., Schuettpelz, E., Wolf, P. G., Schneider, H., Smith, A. R., & Cranfill, R. (2004). Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. *Am. J. Bot., 91*(10), 1582–1598.

Qin, H., Rosenbaum, J. L., and Barr, M. M. (2001). An autosomal recessive polycystic kidney disease gene homolog is involved in intraflagellar transport in *C. elegans* ciliated sensory neurons. *Curr. Biol., 11*(6), 457–461.

Quintyne, N. J., Reing, J. E., Hoffelder, D. R., Gollin, S. M., and Saunders, W. S. (2005). Spindle multipolarity is prevented by centrosomal clustering. *Science, 307*(5706), 127-129.

Rasmussen, C. G., Humphries, J. A., and Smith, L. G. (2011). Determination of symmetric and asymmetric division planes in plant cells. *Ann. Rev. Plant Biol., 62*, 387-409.

Raven, P. H., Evert, R. F., and Eichhorm, S. E. (1999) Biology of Plants. Ed 6. W.H. Freeman: New York.

Reddy, A. S. N. and Day, I. S. (2001). Kinesins in the Arabidopsis genome: A comparative analysis among eukaryotes. *BMC Genomics, 2*, 2.

Reddy, A. S. N. and Day, I. S. (2011). Microtubule Motor Proteins in the Eukaryotic Green Lineage: Functions and Regulation. In B. Liu (Ed.), The Plant Cytoskeleton (pp. 119–141). New York, NY: Springer New York.

Richardson, D. N., Simmons, M. P., and Reddy, A. S. N. (2006). Comprehensive comparitive analysis of kinesins in photosynthetic eukaryotes. *BMC Genomics, 7*, 18.

Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics, 26*(1), 139–140.

Rogers, G. C., Rogers, S. L., Schwimmer, T. A., Ems-McClung, S. C., Walczak, C. E., Vale, R. D., et al. (2004). Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. *Nature, 427(*6972), 364–370.

Rompolas, P., Pedersen, L. B., Patel-King, R. S., and King, S. M. (2007). Chlamydomonas FAP133 is a dynein intermediate chain associated with the retrograde intraflagellar transport motor. *J. Cell Sci., 120*(Pt 20), 3653–3665.

Rosenbaum, J. L., and Witman, G. B. (2002). Intraflagellar transport. *Nat. Rev. Mol. Cell Biol., 3*(11), 813–825.

Ross, J., and Robison, W. G. Jr. (1969). Unusual microtubule patterns and three-dimensional movement of mealybug sperm and sperm bundles. *J. Cell Biol., 40*(2), 426–445.

Sakai, T., Honing, H. V. D., Nishioka, M., Uehara, Y., Takahashi, M., Fujisawa, N., et al. (2008). Armadillo repeat-containing kinesins and a NIMA-related kinase are required for epidermal-cell morphogenesis in Arabidopsis. *Plant J., 53*(1), 157–171.

Sale, W. S. (1986). The axonemal axis and Ca2+-induced asymmetry of active microtubule sliding in sea urchin sperm tails. *J. Cell Biol., 102*(6), 2042–2052.

Sale, W. S. and Satir, P. (1977). Direction of active sliding of microtubules in Tetrahymena cilia. *Proc. Natl. Acad. Sci. USA, 74*(5), 2045–2049.

Sarpal, R., Todi, S. V., Sivan-Loukianova, E., Shirolikar, S., Subramanian, N., Raff, E. C., et al. (2003). *Drosophila* KAP interacts with the kinesin II motor subunit

KLP64D to assemble chordotonal sensory cilia, but not sperm tails. *Curr. Biol., 13*(19), 1687–1696.

Sasabe, M, and Machida, Y. (2012). Regulation of organization and function of microtubules by the mitogen-activated protein kinase cascade during plant cytokinesis. *Cytoskeleton (Hoboken), 69*(11), 913-918.

Satir, P. (1968) Studies on cilia. 3. Further studies on the cilium tip and a "sliding filament" model of ciliary motility. J. *Cell Biol., 39*(1), 77–94.

Satir, P. and Christensen, S. T. (2007). Overview of structure and function of mammalian cilia. *Annu. Rev. Physiol., 69*(1), 377–400.

Sawitzky, H. and Grolig, F. (1995). Phragmoplast of the green alga Spirogyra is functionally distinct from the higher plant phragmoplast. *J. Cell Biol.*, *130*(6), 1359–1371.

Schaar, B. T., Chan, G. K., Maddox, P., Salmon, E. D., and Yen, T. J. (1997). CENP-E function at kinetochores is essential for chromosome alignment. *J. Cell Biol., 139*(6), 1373–1382.

Schafer, J. C., Haycraft, C. J., Thomas, J. H., Yoder, B. K., and Swoboda, P. (2003). XBX-1 encodes a dynein light intermediate chain required for retrograde intraflagellar transport and cilia assembly in *Caenorhabditis elegans*. *Mol. Biol. Cell*, *14*(5), 2057–2070.

Schallus, T., Jaechk, C., Feher, K., Palma, A. S., Liu, Y., Simpson, J. C., et al. (2008). Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. *Mol. Biol. Cell, 19*(8), 3404-3414

Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Wei, F., Pasternak, S., et al. (2009). The B73 maize genome: complexity, diversity, and dynamics. *Science, 326*(5956), 1112–1115.

Schneider, N., Ludwig, H., and Nick, P. (2015). Suppression of tubulin detyrosination by parthenolide recruits the plant-specific kinesin KCH to cortical microtubules. *J. Exp. Bot., 66*(7), 2001–2011.

Scholey, J. M. (2013). Kinesin-2: a family of heterotrimeric and homodimeric motors with diverse intracellular transport functions. *Annu. Rev. Cell Dev. Biol., 29*(1), 443–469.

Schroer, T. A., Steuer, E. R., and Sheetz, M. P. (1989). Cytoplasmic dynein is a minus end-directed motor for membranous organelles. *Cell, 56*(6), 937–946.

Setou, M., Nakagawa, T., Seog, D. H., and Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science, 288*(5472), 1796–1802.

Shah, A. S., Farmen, S. L., Moninger, T. O., Businga, T. R., Andrews, M. P., Bugge, K., et al. (2008). Loss of Bardet-Biedl syndrome proteins alters the morphology and function of motile cilia in airway epithelia. Proc. Natl. Acad. Sci. USA, 105(9), 3380–3385.

Sharp, D. J., Brown, H. M., Kwon, M., Rogers, G. C., Holland, G., and Scholey, J. M. (2000). Functional coordination of three mitotic motors in *Drosophila* embroys. *Mol. Biol. Cell, 11*(1), 241-253.

Sharp, D. J., Rogers, G. D., and Scholey, J. M. (2000). Roles of motor proteins in building microtubule-basd structures: a basic principle of cellular design. *Biochim. Biphys. Acta., 1496*(1), 128-141.

Sharp, L. W. (1914). Spermatogenesis in Marsilia. *Bot. Gaz.*, 419-431.

Shen, Z., Collatos, A. R., Bibeau, J. P., Furt, F., and Vidali, L. (2012). Phylogenetic analysis of the Kinesin superfamily from Physcomitrella. *Front. Plant Sci., 3*, 230.

Shen, Z., Liu, Y.-C., Bibeau, J. P., Lemoi, K. P., Tüzel, E., and Vidali, L. (2015). The kinesin-like proteins, KAC1/2, regulate actin dynamics underlying chloroplast light-avoidance in Physcomitrella patens. *J. Integr. Plant Biol., 57*(1), 106–119.

Signor, D., Wedaman, K. P., Rose, L. S., and Scholey, J. M. (1999). Two heteromeric kinesin complexes in chemosensory neurons and sensory cilia of *Caenorhabditis elegans*. *Mol. Biol. Cell, 10*(2), 345–360.

Silflow, C. D., and Lefebvre, P. A. (2001). Assembly and motility of eukaryotic cilia and flagella. Lessons from Chlamydomonas reinhardtii. *Plant Physiol., 127*(4), 1500–1507.

Smith, E. F. (2002). Regulation of flagellar dynein by the axonemal central apparatus. *Cell Motil. Cytoskeleton, 52*(1), 33–42.

Smith, E. F. and Lefebvre, P. A. (1997). The role of central apparatus components in flagellar motility and microtubule assembly. *Cell Motil. Cytoskeleton, 38*(1), 1–8.

Smith, E. F. and Sale, W. S. (1992). Structural and functional reconstitution of inner dynein arms in Chlamydomonas flagellar axonemes. *J. Cell Biol.*, *117*(3), 573–581.

Smith, E. F., and Yang, P. (2004). The radial spokes and central apparatus: mechano-chemical transducers that regulate flagellar motility. *Cell Motil. Cytoskeleton, 57*(1), 8–17.

Snow, J. J., Ou, G., Gunnarson, A. L., Walker, M. R. S., Zhou, H. M., Brust-Mascher, I., and Scholey, J. M. (2004). Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. *Nat. Cell Biol., 6*(11), 1109–1113.

Soyano, T., Nishihama, R., Morikiyo, K., Ishikawa, M., and Machida, Y. (2003). NQK1/NtMEK1 is a MAPKK that acts in the NPK1 MAPKKK-mediated MAPK cascade and is required for plant cytokinesis. *Genes Dev, 17*(8), 1055-1067.

Sparkes, I. A. (2010). Motoring around the plant cell: insights from plant myosins. *Biochem. Soc. Trans., 38*(3), 833–838.

St Johnston, D. (2005). Moving messages: the intracellular localization of mRNAs. *Nat. Rev. Mol. Cell Biol., 6*(5), 363–375.

Stauber, T., Simpson, J. C., Pepperkok, R., and Vernos, I. (2006). A role for kinesin-2 in COPI-dependent recycling between the ER and the Golgi complex. *Curr. Biol., 16*(22), 2245–2251.

Strompen, G., El Kasmi, F., Richter, S., Lukowitz, W., Assaad, F. F., Jurgens, G., and Mayer, U. (2002). The Arabidopsis HINKEL gene encodes a kinesin-related protein involved in cytokinesis and is expressed in a cell cycle-dependent manner. *Curr. Biol., 12*(2), 153-155.

Summers, K. E. and Gibbons, I. R. (1971). Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. *Proc. Natl. Acad. Sci. USA, 68*(12), 3092–3096.

Takada, S. and Kamiya, R. (1994). Functional reconstitution of Chlamydomonas outer dynein arms from alpha-beta and gamma subunits: requirement of a third factor. *J. Cell Biol., 126*(3), 737–745.

Takahashi, Y., Soyano, T., Kosetsu, K., Sasabe, M., and Machida, Y. (2010). HINKEL kinesin, ANP MAPKKKs and MKK6/ANQ MAPKK, which phosphorylates and activates MPK4 MAPK, constitute a pathway that is required for cytokinesis in Arabidopsis thaliana. *Plant Cell Physiol., 51*(10), 1766–76.

Takeda, S., Yamazaki, H., Seog, D. H., Kanai, Y., Terada, S., and Hirokawa, N. (2000). Kinesin superfamily protein 3 (KIF3) motor transports fodrin-associating vesicles important for neurite building. *J. Cell Biol., 148*(6), 1255–1265.

Tamura, K., Nakatani, K., Mitsui, H., Ohashi, Y., and Takahashi, H. (1999). Characterization of katD, a kinesin-like protein gene specifically expressed in floral tissues of Arabidopsis thaliana. *Gene, 230*(1), 23-32.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol., 30*(12), 2725–2729.

Taschner, M., Bhogaraju, S., Vetter, M., Morawetz, M., and Lorentzen, E. (2011). Biochemical mapping of interactions within the intraflagellar transport (IFT) B core complex: IFT52 binds directly to four other IFT-B subunits. *J. Biol. Chem*, *286*(30), 26344–26352.

Tian, J., Han, L., Feng, Z., Wang, G., Liu, W., Ma, Y., Yu, K., and Kong, Z. (2015). Orchestration of microtuble and the actin cytoskeleton in trichome cell shape determination by a plant-unique kinesin. *Elife, 19*(4), 1711.

Tokai-Nishizumi, N., Ohsugi, M., Suzuki, E., and Yamamoto, T. (2005). The Chromokinesin Kid Is Required for Maintenance of Proper Metaphase Spindle Size. *Mol. Biol. Cell, 16*(11), 5455–5463.

Tokai, N., Fujimoto-Nishiyama, A., Toyoshima, Y., Yonemura, S., Tsukita, S., Inoue, J., and Yamamota, T. (1996). Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. *EMBO J., 15*(3), 457–467.

Tomei, E. J. and Wolniak, S. M. (2016). Transcriptome analysis reveals a diverse family of kinesins essential for spermatogenesis in the fern Marsilea. *Cytoskeleton (Hoboken)*,

Trapnell, C., Pachter, L., and Salzberg, S. L. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics, 25*(9), 1105-1111.

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., Van Baren, M. J., et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol., 28*(5), 511- 515.

Tsai, C. W. and Wolniak, S. M. (2001). Cell cycle arrest allows centrin translation but not basal body formation during spermiogenesis in Marsilea. *J. Cell Sci., 114*(23), 4256–4272.

Tsai, C. W., Van der Weele, C. M., and Wolniak, S. M. (2004). Differential segregation and modification of mRNA during spermiogenesis in *Marsilea vestita*. *Dev. Biol., 269*(2), 319-330.

Tsao, C.-C., and Gorovsky, M. A. (2008). Tetrahymena IFT122A is not essential for cilia assembly but plays a role in returning IFT proteins from the ciliary tip to the cell body. *J. Cell Sci., 121*(4), 428–436.

Tuskan, G. A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., et al. (2006). The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). *Science, 313*(5793), 1596–1604.

Umezu, N., Umeki, N., Mitsul, T., Kondo, K., and Maruta, S. (2011). Characterization of a novel rice kinesin O12 with a calponin homology domain. J. *Biochem., 149*(1), 91-101.

Vale, R. D., Spudich, J. A., and Griffis, E. R. (2009). Dynamics of myosin, microtubules, and Kinesin-6 at the cortex during cytokinesis in *Drosophila* S2 cells. *J. Cell Biol., 186*(5), 727–738.

van Dam, T. J. P., Townsend, M. J., Turk, M., Schlessinger, A., Sali, A., Field, M. C., and Huynen, M. A. (2013). Evolution of modular intraflagellar transport from a coatomer-like progenitor. *Proc. Natl. Acad. Sci. USA, 110*(17), 6943–6948.

Van der Weele, C. M., Tsai, C. W., and Wolniak, S. M. (2007). Mago Nashi is essential for spermatogenesis in Marsilea. *Mol. Biol. Cell, 18*(10), 3711-3722.

Vanstraelen, M., Inze, D., and Geelen, G. (2006). Mitosis-specific kinesins in Arabidopsis. *Trends Plant Sci., 11*(4), 167-175

Varga, V., Helenius, J., Tanaka, K., Hyman, A.A., Tanaka, T.U., and Howard, J. (2006). Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nat. Cell Biol., 8*(9), 957–962.

Varga, V., Leduc, C., Bormuth, V., Diez, S., and Howard, J. (2009). Kinesin-8 motors act cooperatively to mediate length-dependent microtubule depolymerization. Cell, 138(6), 1174–1183.

Varma, D., Monzo, P., Stehman, S. A., and Vallee, R. B. (2008). Direct role of dynein motor in stable kinetochore-microtubule attachment, oriengation, and alignment. *J. Cell Biol., 182*(6), 1045-1054.

Vashishtha, M., Walther, Z., and Hall, J. L. (1996). The kinesin-homologous protein encoded by the Chlamydomonas FLA10 gene is associated with basal bodies and centrioles. *J. Cell Sci., 109*(3), 541–549.

Vasudevan, K. K., Jiang, Y.-Y., Lechtreck, K. F., Kushida, Y., Alford, L. M., Sale, W. S., et al. (2015). Kinesin-13 regulates the quantity and quality of tubulin inside cilia. *Mol. Biol. Cell, 26*(3), 478–494.

Verhey, K. J. and Hammond, J. W. (2009). Traffic control: regulation of kinesin motors. *Nat. Rev. Mol. Cell Biol., 10*(11), 765–777.

Villalba, A., Coll, O., and Gebauer, F. (2011). Cytoplasmic polyadenylation and translational control. *Curr. Opin. Genet. Dev., 21*(4), 452–457.

Walczak, C. E., Mitchison, T. J., and Desai, A. (1996). XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell, 84*(1), 37–47.

Walter, W. J., Machens, I., Fereshteh, R., and Diez, S. (2015). The non-processive rice kinesin-14 OsKCH1 transports actin filaments along microtubules with two distinct velocities. *Nat. Plants, 1*, 15511.

Walther, Z., Vashishtha, M., and Hall, J. L. (1994). The Chlamydomonas FLA10 gene encodes a novel kinesin-homologous protein. *J. Cell Biol., 126*(1), 175–188.

Wang, L., Piao, T., Cao, M., Qin, T., Huang, L., Deng, H., et al. (2013). Flagellar regeneration requires cytoplasmic microtubule depolymerization and kinesin-13. *J. Cell Sci., 126*(6), 1531–1540.

Wang, X., and Schwarz, T. L. (2009). The mechanism of Ca2+-dependent regulation of kinesin-mediated mitochondrial motility. *Cell, 136*(1), 163–174.

Wang, Z., Fan, Z.-C., Williamson, S. M., and Qin, H. (2009). Intraflagellar transport (IFT) protein IFT25 is a phosphoprotein component of IFT complex B and physically interacts with IFT27 in Chlamydomonas. *PloS One,* 4(5), e5384.

Wargo, M. J., McPeek, M. A., and Smith, E. F. (2004). Analysis of microtubule sliding patterns in Chlamydomonas flagellar axonemes reveals dynein activity on specific doublet microtubules. *J. Cell Sci., 117*(12), 2533–2544.

Warner, F. D. and Satir, P. (1974). The structural basis of ciliary bend formation. Radial spoke positional changes accompanying microtubule sliding. *J. Cell Biol., 63(*1), 35–63.

Weaver, L. N., Ems-McClung, S. C., Stout, J. R., LeBlanc, C., Shaw, S. L., Gardner, M. K., and Walczak, C. E. (2011). Kif18A uses a microtubule binding site in the tail for plus-end localization and spindle length regulation. *Curr. Biol., 21*(17), 1500–1506.

Wedaman, K. P., Meyer, D. W., Rashid, D. J., Cole, D. G., and Scholey, J. M. (1996). Sequence and submolecular localization of the 115-kD accessory subunit of the heterotrimeric kinesin-II (KRP85/95) complex. *J. Cell Biol., 132*(3), 371–380.

Wei, L., Zhang, W., Liu, Z., and Li, Y. (2009). AtKinesin-13a is located on Golgi-associated vesicle and involved in vesicle formation/budding in Arabidopsis root-cap peripheral cells. *BMC Plant Biol. 9*, 138.

Wei, Q., Zhang, Y., Li, Y., Zhang, Q., Ling, K., and Hu, J. (2012). The BBSome controls IFT assembly and turnaround in cilia. *Nat. Cell Biol.,* 14(9), 950–957.

Wickstead, B. and Gull, K. (2006). A "holistic" kinesin phylogeny reveals new kinesin families and predicts protein functions. *Mol. Biol. Cell, 17*(4), 1734–1743.

Wickstead, B. and Gull, K. (2007). Dyneins across eukaryotes: a comparative genomic analysis. *Traffic, 8*(12), 1708-1721.

Wickstead, B., Carrington, J. T., Gluenz, E., and Gull, K. (2010a). The expanded Kinesin-13 repertoire of trypanosomes contains only one mitotic Kinesin indicating multiple extra-nuclear roles. *PloS One, 5*(11), e15020.

Wickstead, B., Gull, K., and Richards, T. A. (2010b). Patterns of kinesin evolution reveal a complex ancestral eukaryote with a multifunctional cytoskeleton. *BMC Evol. Biol., 10,* 110.

Wilkerson, C. G., King, S. M., Koutoulis, A., Pazour, G. J., and Witman, G. B. (1995). The 78,000 M(r) intermediate chain of Chlamydomonas outer arm dynein isa WD-repeat protein required for arm assembly. *J. Cell Biol., 129*(1), 169–178.

Wilkes, D. E., Bennardo, N., Chan, C. W. C., Chang, Y.-L., Corpuz, E. O., DuMond, J., et al. (2009). Identification and characterization of dynein genes in Tetrahymena. *Methods Cell Biol., 92*, 11–30.

Wilkes, D. E., Rajagopalan, V., Chan, C. W. C., Kniazeva, E., Wiedeman, A. E., and Asai, D. J. (2007). Dynein light chain family in Tetrahymena thermophila. *Cell Motil. Cytoskeleton, 64*(2), 82–96.

Wilkes, D. E., Watson, H. E., Mitchell, D. R., and Asai, D. J. (2008). Twenty-five dyneins in Tetrahymena: A re-examination of the multidynein hypothesis. *Cell Motil Cytoskeleton, 65*(4), 342–351.

Wirschell, M., Yang, C., Yang, P., Fox, L., Yanagisawa, H.-A., Kamiya, R., et al. (2009). IC97 is a novel intermediate chain of I1 dynein that interacts with tubulin and regulates interdoublet sliding. *Mol. Biol. Cell, 20*(13), 3044–3054.

Witman, G. B., Plummer, J., and Sander, G. (1978). Chlamydomonas flagellar mutants lacking radial spokes and central tubules. Structure, composition, and function of specific axonemal components. *J. Cell Biol., 76*(3), 729–747.

Woehlke, G., and Schliwa, M. (2000). Walking on two heads: the many talents of kinesin. *Nat. Rev. Mol. Cell Biol., 1*(1), 50–58.

Wojcik, E., Basto, R., Serr, M., Scaerou, F., Karess, R., and Hays, T. (2001). Kinetochore dynein: its dynamics and role in the transport of the Rough deal checkpoint protein. *Nat. Cell Biol., 3*(11), 1001-1007.

Wolniak, S. M. and Cande, W. Z. (1980). Physiological requirements for ciliary reactivation of bracken fern spermatozoids. *J. Cell Sci., 43*, 195-207.

Wolniak, S. M. and Swamy, R. N. (2004). Microfilaments and spermatid differentiation in Marsilea. MBC. 15(Suppl. S): 90A–91A

Wolniak, S. M., Boothby, T. C., and van der Weele, C. M. (2015). Posttranscriptional control over rapid development and ciliogenesis in Marsilea. *Methods Cell Biol., 127*, 403-444.

Wolniak, S. M., van der Weele, C. M., Deeb, F., Boothby, T., and Klink, V. P. (2011). Extremes in rapid cellular morphogenesis: post-transcriptional regulation of spermatogenesis in *Marsilea vestita*. *Protoplasma, 248*(3), 457-473.

Wood, K. W., Sakowicz, R., Goldstein, L. S., and Cleveland, D. W. (1997). CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. *Cell, 91*(3), 357–366.

Wu, G. and Chen, P.-L. (2008). Structural requirements of chromokinesin Kif4A for its proper function in mitosis. *Biochem. Biophys. Res. Commun., 372*(3), 454–458.

Xu, T., Qu, Z., Yang, X., Qin, X., Xiong, J., Wang, Y., et al. (2009). A cotton kinesin GhKCH2 interacts with both microtubules and microfilaments. *Biochem. J., 421*(2), 171-180.

Yagi, T. (2009). Bioinformatic approaches to dynein heavy chain classification. *Methods Cell Biol, 92*, 1–9.

Yamamoto, R., Yanagisawa, H.-A., Yagi, T., and Kamiya, R. (2006). A novel subunit of axonemal dynein conserved among lower and higher eukaryotes. *FEBS Lett., 580*(27), 6357–6360.

Yamamoto, R., Yanagisawa, H.-A., Yagi, T., and Kamiya, R. (2008). Novel 44-kilodalton subunit of axonemal Dynein conserved from chlamydomonas to mammals. *Eukaryotic Cell, 7*(1), 154–161.

Yamaoka, S., and Leaver, C. J. (2008). EMB2473/MIRO1, an Arabidopsis Miro GTPase, is required for embryogenesis and influences mitochondrial morphology in pollen. *Plant Cell, 20*(3), 589–601.

Yamaoka, S., Nakajima, M., Fujimoto, M., and Tsutsumi, N. (2011). MIRO1 influences the morphology and intracellular distribution of mitochondria during embryonic cell division in Arabidopsis. *Plant Cell Rep., 30*(2), 239–244.

Yang, G., Gao, P., Zhang, H., Huang, S., and Zheng, Z.-L. (2007). A mutation in MRH2 kinesin enhances the root hair tip growth defect caused by constitutively activated ROP2 small GTPase in Arabidopsis. *PloS One, 2*(10), e1074.

Yang, P. and Sale, W. S. (1998). The Mr 140,000 intermediate chain of Chlamydomonas flagellar inner arm dynein is a WD-repeat protein implicated in dynein arm anchoring. *Mol. Biol. Cell, 9*(12), 3335–3349.

Yang, P., Diener, D. R., Rosenbaum, J. L., and Sale, W. S. (2001). Localization of calmodulin and dynein light chain LC8 in flagellar radial spokes. *J. Cell Biol., 153*(6), 1315–1326.

Yang, P., Diener, D. R., Yang, C., Kohno, T., Pazour, G. J., Dienes, J. M., et al. (2006). Radial spoke proteins of Chlamydomonas flagella. *J. Cell Sci., 119*(6), 1165–1174.

Yang, X.-Y., Chen, Z.-W., Xu, T., Qu, Z., Pan, X.-D., Qin, X.-H., et al. (2011). Arabidopsis kinesin KP1 specifically interacts with VDAC3, a mitochondrial protein, and regulates respiration during seed germination at low temperature. *Plant Cell, 23*(3), 1093–1106.

Yao, X., Abrieu, A., Zheng, Y., Sullivan, K. F., and Cleveland, D. W. (2000). CENP-E forms a link between attachment of spindle microtubules to kinetochores and the mitotic checkpoint. *Nat. Cell Biol., 2*(8), 484–491.

Yardimci, H., van Duffelen, M., Mao, Y., Rosenfeld, S. S., and Selvin, P. R. (2008). The mitotic kinesin CENP-E is a processive transport motor. *Proc. Natl. Acad. Sci. USA, 105*(16), 6016–6021.

Yen, H.-J., Tayeh, M. K., Mullins, R. F., Stone, E. M., Sheffield, V. C., and Slusarski, D. C. (2006). Bardet-Biedl syndrome genes are important in retrograde intracellular trafficking and Kupffer's vesicle cilia function. *Hum. Mol. Genet., 15*(5), 667–677.

Yen, T. J., Compton, D. A., Wise, D., Zinkowski, R. P., Brinkley, B. R., Earnshaw, W. C., and Cleveland, D. W. (1991). CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. *EMBO J., 10*(5), 1245–1254.

Yokoyama, R., OToole, E., Ghosh, S., and Mitchell, D. R. (2004). Regulation of flagellar dynein activity by a central pair kinesin. *Proc. Natl. Acad. Sci. USA, 101*(50), 17398–17403.

Yu, J., Hu, S., Wang, J., Wong, G. K.-S., Li, S., Liu, B., et al. (2002). A draft sequence of the rice genome (Oryza sativa L. ssp. indica). *Science, 296*(5565), 79–92.

Zhang, H. and Dawe, R. K. (2011). Mechanisms of plant spindle formation. *Chromosome Res., 19*(3), 335-344

Zhang, M., Zhang, B., Qian, Q., Yu, Y., Li, R., Zhang, J., et al. (2010). Brittle Culm 12, a dual-targeting kinesin-4 protein, controls cell-cycle progression and wall properties in rice. *Plant J., 63*(2), 312–328.

Zhong, R., Burk, D. H., Morrison, W. H., and Ye, Z.-H. (2002). A kinesin-like protein is essential for oriented deposition of cellulose microfibrils and cell wall strength. *Plant Cell, 14*(12), 3101–3117.

Zhu, C. and Dixit, R. (2011). Single molecule analysis of the Arabidopsis FRA1 kinesin shows that it is a functional motor protein with unusually high processivity. *Mol. Plant, 4*(5), 879–885.

Zhu, C., Ganguly, A., Baskin, T. I., McClosky, D. D., Anderson, C. T., Foster, C., et al. (2015). The fragile Fiber1 kinesin contributes to cortical microtubule-mediated trafficking of cell wall components. *Plant Physiol., 167*(3), 780–792.