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Review

A complete inventory of fungal kinesins in representative filamentous ascomycetes

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Abstract

Complete inventories of kinesins from three pathogenic filamentous ascomycetes, *Botryotinia fuckeliana*, *Cochliobolus heterostrophus*, and *Gibberella moniliformis*, are described. These protein sequences were compared with those of the filamentous saprophyte, *Neurospora crassa* and the two yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Data mining and phylogenetic analysis of the motor domain yielded a constant set of 10 kinesins in the filamentous fungal species, compared with a smaller set in *S. cerevisiae* and *S. pombe*. The filamentous fungal kinesins fell into nine subfamilies when compared with well-characterized kinesins from other eukaryotes. A few putative kinesins (one in *B. fuckeliana* and two in *C. heterostrophus*) could not be defined as functional, due to unorthodox organization and lack of experimental data. The broad representation of filamentous fungal kinesins across most of the known subfamilies and the ease of gene manipulation make fungi ideal models for functional and evolutionary investigation of these proteins.
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1. Introduction

The first description of fast axonal transport in longfin squid *Loligo pealei* (Brady et al., 1982) set the stage for the identification (Brady, 1985), purification, and eventual demonstration of a new cellular motor associated with microtubules, now known as kinesin (Vale et al., 1985). Proteins with 30–40% identity in a previously undescribed domain consisting of approximately 350 amino acids were isolated subsequently from two fungi, *Saccharomyces cerevisiae* and *Aspergillus nidulans* (Enos and Morris, 1990; Meluh and Rose, 1990); this exposed the kinesins as a new superfamily, found in all eukaryotes. The conserved domain, named the motor domain, is the defining characteristic of all kinesins. Over the last decade, these proteins have been demonstrated to be integral components of basic cellular functions ranging from chromosome movement and spindle elongation (Heck, 1999; Sharp et al., 2000) to transport of mRNA, organelles, and protein complexes (Brendza et al., 2000; Hirokawa, 1998). Historically, the squid protein and its orthologs in other organisms have been referred to as kinesin or kinesin heavy chain (KHC) with related proteins designated as kinesin related proteins (KRPs). However, the term kinesin is now commonly used to describe all members of the superfamily (Goldstein and Philp, 1999; Vale and Fletterick, 1997) while "conventional kinesin" is used to refer to the subfamily containing the KHC.

The kinesin motor domain contains a globular catalytic core that acts as the force-generating part of the protein. Specific motifs in the motor domain are responsible for ATP-hydrolysis and promote microtubule binding (Yang et al., 1990). This and other domains in conventional kinesin are depicted in Fig. 1. The combination of these domains allows kinesins to move cargo along microtubule "tracks" in the cell. Microtubules, consisting of long chains of alternating α and β tubulin proteins, are polar structures with fast and slow polymerizing ends (referred to as plus and minus ends,

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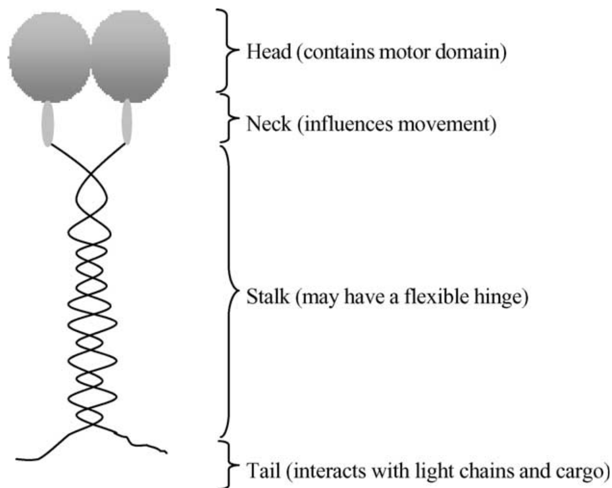

Fig. 1. Diagrammatic representation of the structure of a dimeric conventional kinesin, consisting of two heavy chains with distinct domains.

respectively). A given kinesin moves unidirectionally towards one or the other of these poles. Most kinesins contain either N-terminal or centrally located motor domains (respectively, known as N- and I-type motors) that move towards the plus end, while proteins with C-terminal domains (C-type) move toward minus ends (Hirokawa, 1998). A small number of kinesins function as a single heavy chain (Okada and Hirokawa, 1999), but most function as homo- (Bloom et al., 1988) or hetero-oligomers, (Cole, 1999a). N- and C-type motors have highly variable α-helical coiled-coil domains, called stalks. In some kinesins an additional globular domain is present at one end, known as the tail. Tail interactions are thought to be important for targeting of specific cargo, as reviewed in Manning and Snyder (2000), and have been shown also to play an important role in regulation of motor domain activity (Seiler et al., 2000; Stock et al., 1999). Another region, the neck, situated between the motor domain and the stalk is conserved only within certain subfamilies of kinesins and is known to participate in regulation of kinesin function. It contains approximately 40 amino acids that influence direction of movement and motility (Case et al., 2000; Endow and Waligora, 1998).

Since the first kinesin was discovered, additional experimentation with model fungi such as *S. cerevisiae*, *A. nidulans*, and more recently *Schizosaccharomyces pombe* and *Neurospora crassa* has yielded a wealth of data on structure, function, and interactions with other proteins. A number of reviews on various aspects of motor protein function that include discussions of fungal kinesins are available (Fischer, 1999; Steinberg, 1998, 2000).

Complete kinesin inventories are readily accessible for the two ascomycetous yeasts (*S. cerevisiae* and *S. pombe*). These data, combined with the proprietary filamentous fungal genome data described below, positioned us to address a number of questions. Do the kinesin profiles of filamentous ascomycetes differ from those of the yeasts? Are differences in growth and survival strategies reflected? Do fungal kinesin profiles differ fundamentally from those of other eukaryotes? This review provides a framework, supported by phylogenetic inference and available experimental data, for illuminating relationships and functions. Due to the limited data sets available, most of the discussion will focus on comparisons among ascomycete kinesins. However the body of knowledge about kinesin function in other fungal groups, such as basidiomycetes, is increasing and will be referred to where appropriate.

2. Kinesin phylogenies

Dayhoff (1976) suggested that all proteins could be grouped in families. Increased availability of genome sequence has enabled comprehensive gene taxonomies, which help us distinguish between genes sharing common ancestry (orthologs) and genes arising via duplications in single species (paralogs). Like organismal taxonomies, these gene classifications can be divided into hierarchies such as superfamilies, families, and subfamilies (Henikoff et al., 1997). Phylogenomic grouping of proteins (Eisen, 1998) has uncovered novel organism-specific proteins and allowed functional prediction of uncharacterized proteins (Eisen and Wu, 2002).

The characterization of specific subfamily members from a spectrum of eukaryotes has shown that phylogenetic analysis of large groups of kinesins based on motor domain alignments can successfully group kinesins of similar function (Goodson et al., 1994; Hirokawa, 1998; Kim and Endow, 2000; Lawrence et al., 2002; Moore and Endow, 1996). Additional areas of the primary amino acid sequence, such as the unique sequences of the neck region (Vale and Fletterick, 1997) and binding sites in the tail have also provided clues to protein function. Initially, five clearly defined subfamilies were delineated by Goodson et al. (1994) but subsequent analyses with expanded data sets yielded up to ten subfamilies plus a number of ungrouped "orphans" (Hirokawa, 1998; Kim and Endow, 2000). More recent phylogenetic comparisons include a study of human kinesins, grouping them into 14 subfamilies by means of maximum parsimony (Miki et al., 2001) and a comparison by means of maximum likelihood that delineated 11 subfamilies from a spectrum of eukaryotic kinesins, placing some, previously classified as orphans, into known subfamilies (Lawrence et al., 2002). Traditionally, kinesin subfamilies have been named after their founding members (e.g., Kim and Endow, 2000) but there are proposals for a more systematic labeling system (Hirokawa, 1998; Miki et al., 2001). Despite this,

the naming system used by The Kinesin Home Page (www.proweb.org/kinesin/) remains in recent phylogenetic reports (Kim and Endow, 2000; Lawrence et al., 2002; Siddiqui, 2002) and will be followed here. Most of the above-mentioned protein classifications largely agree, but certain differences in the groupings of the variable "orphan" proteins persist. This, coupled with addition of new data, renders our view of the kinesin family tree subject to continuous adjustment, as discussed below.

Data mining of genome sequences continues to add novel members to the kinesin superfamily, which already contains more than 400 members (pfam.wustl.edu/). In organisms for which the complete gene set is known, the numbers range from 72 for *Arabidopsis thaliana* (Reddy and Day, 2001), 45 for human (Miki et al., 2001), and 33 for *Caenorhabditis elegans* (Siddiqui, 2002); pfam.wustl.edu/) to only 6 for *S. cerevisiae* (Hildebrandt and Hoyt, 2000). A cartoon reflecting the phylogeny of a selection of representative organisms and their kinesin inventories is shown in Fig. 2.

The only complete profiles of fungal kinesins described so far are from two yeasts. *S. pombe*, belonging to the subphylum Taphrinomycotina, has nine proteins that fall into six subfamilies (Su and Yanagida, 1997); *S. cerevisiae*, belonging to the subphylum Saccharomycotina, has six proteins that fall into four subfamilies (Hildebrandt and Hoyt, 2000). Although much less is known about filamentous fungi, kinesins have been isolated from a number of ascomycetes, such as the eurotiomycete, *A. nidulans* (Enos and Morris, 1990; O'Connell and Morris, 1993), the sordariomycetes *N. crassa* (Steinberg and Schliwa, 1995), and *Nectria haematococca* (Wu et al., 1998) and *Thermomyces lanuginosus* (a mitosporic ascomycete of uncertain taxonomic grouping) (Sakowicz et al., 1999), as well as from the basidiomycete *Ustilago maydis* (Lehmler et al., 1997) and the zygomycete *Syncephalastrum racemosum* (Steinberg, 1997). Table 1 is a summary of known ascomycete kinesins and their functions.

3. A fungal kinesin phylogeny

Recently obtained fungal genome sequences have enabled the identification of complete sets of kinesins for a number of filamentous fungi. In an effort to gain more insight into functional and structural diversity of these proteins in fungi, we have used motor domain conserved motifs to query the Torrey Mesa Research Institute/Syngenta sequences of *Cochliobolus heterostrophus*, *Gibberella moniliformis*, and *Botryotinina fuckeliana*. These ascomycete fungi are all plant pathogens, but are taxonomically diverse (Fig. 2). We also included a data set from the genome sequence of the saprophyte *N. crassa*. In addition to kinesins from these databases, a selection of previously characterized proteins from fungi and other eukaryotes, available in GenBank, was used for comparison.

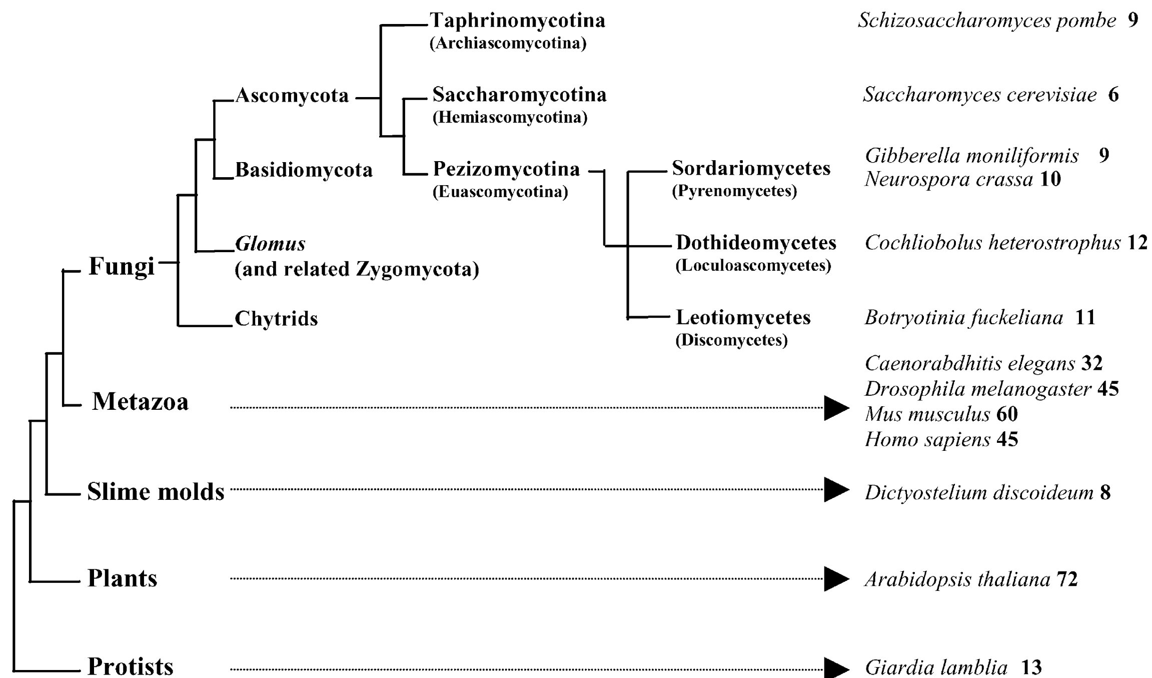

Fig. 2. A selection of kinesin inventories. Total number of kinesins found in each species is shown next to a consensus phylogeny of representative organisms (based on Eriksson, 2002; Sipiczki, 2000). Synonyms and former names of taxonomic groups applying to the fungal species in question are shown in brackets.

Table 1
Summary of phenotypes assigned to previously characterized ascomycetous kinesins

Gene and subfamily	Species	Function	Selected references
BimC			
BimC	*A. nidulans*	Spindle pole separation, mitotic spindle formation	Enos and Morris (1990)
Cin8p	*S. cerevisiae*	Mitotic spindle formation, mitotic chromosome segregation, mitotic anaphase B	Gheber et al. (1999) and Hoyt et al. (1993)
Kip1p	*S. cerevisiae*	Microtubule nucleation, mitotic anaphase B	Hoyt et al. (1992) and Saunders et al. (1997)
Cut7p	*S. pombe*	Mitotic spindle formation and elongation	Drummond and Hagan (1998)
KHC			
KinA	*A. nidulans*	Nuclear positioning, destabilizing microtubules	Requena et al. (2001)
KIN1	*N. haematococca*	Apical transport of secretory vesicles and mitochondria	Wu et al. (1998)
NKIN	*N. crassa*	Vesicle movement, nuclear distribution in hyphae	Seiler et al. (1997) and Seiler et al. (1999)
Klp3p	*S. pombe*	Organelle organization	Brazer et al. (2000)
Kar3p			
KlpA	*A. nidulans*	Counterbalancing spindle mitotic spindle motor forces of BimC, establishment of spindle bipolarity	O'Connell and Morris (1993)
KRP1	*N. haematococca*	Produces an inwardly directed force that opposes the astral pulling force	Aist (2002)
Kar3p	*S. cerevisiae*	Mitosis, meiosis, spindle positioning in the absence of dynein, MT bundling	Cottingham et al. (1999) and Cottingham and Hoyt (1997)
Pkl1	*S. pombe*	Mitotic spindle function	Troxell et al. (2001)
Klp2p	*S. pombe*	Mitotic spindle function, promoting spindle disassembly	Troxell et al. (2001)
Kip2p			
Tea2p	*S. pombe*	Polar vegetative growth	Browning et al. (2000)
Kip2p	*S. cerevisiae*	Nuclear migration, counteracting Kip3 and Dyn1	Miller et al. (1998)
Kip3p			
Klp5p	*S. pombe*	MT disassembly and meiosis	West et al. (2001)
Klp6p	*S. pombe*	MT disassembly and meiosis	West et al. (2001)
Kip3p	*S. cerevisiae*	Nuclear migration towards the budding neck, spindle positioning in the absence of dynein	Cottingham and Hoyt (1997), DeZwaan et al. (1997) and Miller et al. (1998)

A tblastn search of the *C. heterostrophus* sequence database yielded 10 predicted proteins with similarity to kinesin motor domain motifs (see http://www.proweb.org/kinesin//KinesinAlign.html). All conserved MT and ATP binding motifs were present. The presence of a Walker A type ATP-binding motif (AYGX TGXGKX), followed by conserved motifs consisting of SSRSH and LAGSE, or similar amino acids, as well as a YXXXXXDLL motif were taken as sound criteria for identifying kinesin motors (Miki et al., 2001). A search of the genome assemblies of *B. fuckeliana* and *G. moniliformis* using these criteria yielded additional candidates. We then conducted a more specific search with single motifs, which yielded one *B. fuckeliana* and two *C. heterostrophus* additional open reading frames with similarity to kinesin motor domains. Although these latter proteins did not contain clear ATP- or YXXXXXDLL-MT binding motifs, all other motifs were present. No unambiguous homologs could be found in sequence databases, but the motor domain structure was similar enough to allow their alignment with our data set of kinesins that carried a complete set of motifs and they were included as possible basal groups in our phylogenetic assessment. This gave a total of 12 kinesin or kinesin-like proteins in *C. heterostrophus* and 11 and 9 proteins in *B. fuckeliana* and *G. moniliformis*, respectively. This number is in line with the 10 proteins identified in *N. crassa* (see http://www-genome.wi.mit.edu/annotation/fungi/neurospora/geneindex.html). Table 2 lists the open reading frames analyzed in this study. A total phylogenetic analysis with representative fungal and eukaryotic kinesins is shown in Fig. 3. In order to limit our data set we analyzed the previously unknown predicted *C. heterostrophus* kinesins together with a representative selection of proteins from other eukaryotes used in previous analyses (Kim and Endow, 2000; Lawrence et al., 2002; Miki et al., 2001). A similar analysis with only fungal representatives that included all of the uncharacterized putative proteins obtained from the genomes in this study is shown in Fig. 4. The proteins predicted from *C. heterostrophus* are boxed for reference (Figs. 3 and 4). Finally, a domain analysis comparison of the putative kinesins obtained from the three representative ascomycete genomes is shown in Fig. 5.

Table 2
GenBank Accession Nos. of known kinesin heavy chain proteins

Organism and protein[a]	Accession No.
A. nidulans (*An*)	
BimC	AAA33298
KlpA	CAA45887
A. thaliana (*At*)	
F8K7.17	AAD41428
KATA	S34830
KCBP	AAC37475
PAK	AAG08965
B. fuckeliana (*Bf*)	
Klp1p	AY230415
Klp2p	AY230416
Klp3p	AY230417
Klp4p	AY230418
Klp5p	AY230419
Klp6p	AY230420
Klp7p	AY230421
Klp8p	AY230422
Klp9p	AY230423
Klp10p	AY230424
ORFM39L	AY230425
C. elegans (*Ce*)	
UNC-116	P34540
KLP-3	CAA85331
OSM-3	BAA07612
UNC-104	AAA03517
VAB-8	CAD36504
ZEN-4	AAM97993
C. heterostrophus (*Ch*)	
Klp1p	AY230426
Klp2p	AY230427
Klp3p	AY230428
Klp4p	AY230429
Klp5p	AY230430
Klp6p	AY230431
Klp7p	AY230432
Klp8p	AY230433
Klp9p	AY230434
Klp10p	AY230435
ORFM3NI	AY230436
ORFM3KO	AY230437
Cricetulus griseus (*Cg*)	
CHO1	CAA58558
MCAK	U11790
Chlamydomonas reinhardtii (*Cr*)	
KLP1	P46870
Dictyostelium discoideum (*Dd*)	
K7	AAB07748
Drosophila melanogaster (*Dm*)	
KHC	AAA28652
KLP38B	CAA67928
KLP67A	AE003552
KLP68D	AAA69929
KLP98A	NP_524532
NCD	CAA36998
NOD	AAA28653
G. moniliformis (*Gm*)	
Klp1	AY230438
Klp2	AY230439
Klp4	AY230440
Klp5	AY230441
Klp6	AY230442
Klp7	AY230443
Klp8	AY230444
Klp9	AY230445
Klp10	AY230446
Gallus gallus (*Gg*)	
CHRKIN	AAC59666
Homo sapiens (*Hs*)	
ATSV	CAA62346
CENP-E	CAA78727
HKSP	AAA86132
GAKIN	Q9NQT8
KID	BAA33019
MKLP1	CAA47628
Loligo pealei (*Lp*)	
KHC	AAA29990
Mus musculus (*Mm*)	
KHCX	AAB53940
KIF1A	BAA06221
KIF1B	BAA04503
KIF2	BAA02165
KIF3A	BAA02166
KIF4	BAA02167
KIF16A	BAA22385
KIFC1	BAA19676
KIFC2	BAA19677
KLP174	CAA70845
N. crassa (*Nc*)[b]	
NKIN	AAB52961
KLP2	3.236 (15)
KLP3	3.37 (2)
KLP4	3.136 (7)
KLP5	3.356 (25)
KLP6	3.285 (18)
KLP7	CAC28851
	3.201 (11)
KLP8	3.389 (30)
KLP9	T49451
	3.391 (31)
KLP10	3.292 (19)
N. haematococca (*Nh*)	
KIN1	AAB47851
KRP1	AAC99460
KRP2	AAC99461
Nicotiana tabacum (*Nt*)	
KRP125	BAA23159
TCK1	AAC49393
S. cerevisiae (*Sc*)	
Cin8p	AAA34495
Kar3p	AAA34715
Kip1p	CAA78019
Kip2p	CAA78021

Table 2 (*continued*)

Organism and protein[a]	Accession No.
Kip3p	CAA96933
Smy1p	AAA35056
S. pombe (*Sp*)	
Cut7p	CAA40738
Pkl1p	AAB88235
Klp2p	CAB65811
Klp3p	AAF14525
Tea2p	CAA22353
Klp5p	BAB69885
Klp6p	BAB69886
Klp8p	CAB59694
SPBC15D4	CAA20476
Strongylocentrotus purpuratus (*Spu*)	
KRP85	AAA16098
KRP95	AAA87393
S. racemosum (*Sr*)	
KIN1	CAA12647
Solanum tuberosum (*St*)	
KCBP	AAB37756
Ustilago maydis (*Um*)	
KIN1	AAB63336
KIN2	AAB63337
KIN3	AAL87137
Xenopus laevis (*Xl*)	
CTK2	AAB40402
EG52	CAA50695
KCM1	AAC59743
KCM2	AAC59744
KLP1	CAA57539
KLP2	CAA63826
KLP3	CAA08879

[a] Motor domains and open reading frame predictions of kinesins discovered in this study have been deposited in GenBank. Newly predicted fungal kinesins (this study) were named Klp (for kinesin-like protein) plus a numerical designation following conventions established for known *S. cerevisiae* and *S. pombe* proteins and others. Open reading frame designations were used for three proteins that do not have clearly defined motor domains.

[b] To facilitate comparison with orthologs in the filamentous fungi, *Neurospora* proteins are designated by contig numbers with the scaffold given in brackets (http://www-genome.wi.mit.edu/annotation/fungi/neurospora/). To our knowledge, these names have not been sanctioned in any other publication. In two cases, contig numbers are listed as well a GenBank accession number.

The large-scale analysis (Fig. 3) revealed that filamentous fungi encode proteins belonging to nine known subfamilies. Two subfamilies, KRP85/95 and MCAK, clearly do not have any known fungal members. In the KRP85/95 subfamily, KRP85 and KRP95 are separate but closely related motor domain containing proteins isolated from sea urchin (Cole et al., 1993). Several additional members of this subfamily have since been identified in organisms ranging from *Chlamydomonas* to human. A number of proteins in this group form heterotrimeric complexes containing the two proteins with a motor domain and a third non-motor protein analogous to kinesin light chains. This protein complex, known as kinesin II, is a plus end directed, heteromeric protein with a range of functions. These functions include participation in a conserved intraflagellar transport system designed to assemble and maintain ciliary and flagellar organelles and a role in axonal transport in mammalian neuron cells (Cole, 1999b; Marszalek and Goldstein, 2000). Given the lack of related cellular structures in fungi, their absence is not surprising.

The founding member of MCAK, or mitochondrial centromere-associated kinesin, was determined to be associated with the centromeres during mitosis in Chinese hamster (Wordeman and Mitchison, 1995). MCAK and related motors are I-type motors with the motor domain centrally located in the protein. Two other proteins, the kinesin CENP-E and a complex containing another MT motor, dynein, are also targeted to the mammalian centromeres but both of these proteins are released from the kinetochore during early phases of mitosis (Brown et al., 1996; Echeverri et al., 1996; Schaar et al., 1997). In contrast to this, MCAK is located at the kinetochores throughout telophase (Wordeman and Mitchison, 1995) and has roles in chromosome movement at the initiation of anaphase and in microtubule destabilization (Maney et al., 1998, 2001). A number of key differences in the spindles of fungi and mammals could account for the absence of a fungal MCAK. For example, the amount of DNA in fungal centromeres is much lower than in mammalian centromeres and fungal kinetochores are attached to single MTs instead of up to 50 MTs as in mammalian cells (Maney et al., 1998). Additionally, absence of a fungal metaphase plate may result in MCAK homologs being unnecessary in fungi.

It should be noted that MCAK fell out as a separate group in a number of recent phylogenetic studies in which parsimony was used for analysis (Kim and Endow, 2000; Miki et al., 2001), while MCAK grouped closely with the Kip3p subfamily in other studies in which maximum likelihood and distance methods were used (Iwabe and Miyata, 2002; Lawrence et al., 2002). Our analysis also shows high similarity between the Kip3 and MCAK subfamilies (Fig. 3). Furthermore, some workers refer to the *S. pombe* Klp5 and Klp6 proteins as members of the MCAK subfamily (Garcia et al., 2002). Although the members of MCAK and Kip3p appear to have closely related functions, such as microtubule depolymerization, the placement of their motor domains is different (central in MCAK and N-terminal in Kip3p). Therefore, we will continue to describe them as separate entities untill more data and phylogenetic analysis prove otherwise.

In our Bayesian analysis, a kinesin group with homologs in *S. pombe* and the filamentous species has been placed with members of the MKLP1 subfamily. However, parsimony analysis did not find enough support to group these proteins together and both clades were placed

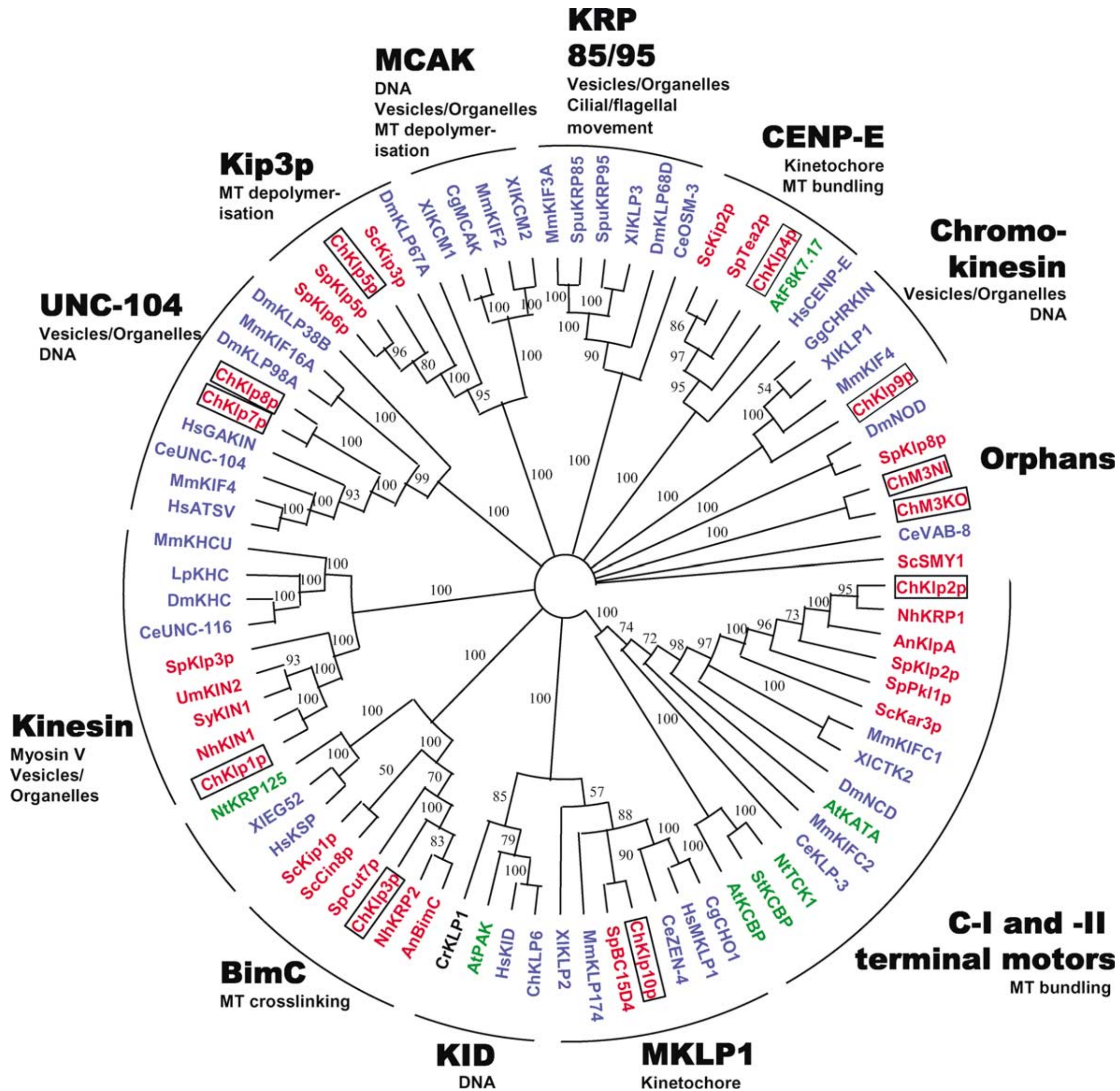

Fig. 3. A 50% Majority Rule tree, with midpoint rooting, obtained from 10,000 maximum likelihood trees, selected from 1,000,000 generations, inferred by Bayesian logic using a Jones amino acid substitution model in the program MrBayes (Huelsenbeck and Ronquist, 2001). Protein sequences were aligned with T-Coffee (Notredame et al., 2000) and deposited at TreeBASE (alignments can be obtained from the corresponding author.). The first 500 trees were discarded. The percentage of trees containing specific branches is shown. (For an explanation of abbreviations used, see Table 2.) Kinesins of metazoans are shown in blue, from plants in green, protists in black, and fungi in red. *C. heterostrophus* proteins are boxed.

basally in the tree. It must be emphasized therefore that the placement of these proteins is tenuous, and that they may constitute a unique fungal clade. In other eukaryotes, MKLP1 subfamily are plus end directed motors whose functions include MT crosslinking and midzone positioning during cytokinesis (Chen et al., 2002; Kobayashi et al., 1998; Matuliene and Kuriyama, 2002).

4. Subfamilies: N-terminal motors

4.1. Conventional kinesin

This group contains the first reported kinesin and consists of N-terminal plus end directed motors. Conventional kinesin is known to be involved in transport of a wide variety of organelles in eukaryotes, including the filamentous fungi (Lehmler et al., 1997; Seiler et al., 1997; Steinberg et al., 1998; Wu et al., 1998) and *S. pombe* (Brazer et al., 2000). Differences between profiles of the two yeasts are notable. The conventional kinesin heavy chain protein is absent in *S. cerevisiae* but present in *S. pombe*. Thus, uniquely in budding yeast, kinesins play important roles in mitotic spindle formation and chromosome movement, but not in organelle movement. Organelle movement and related functions are the function of myosins, actin associated motors (Jacobs et al., 1988; Simon and Pon, 1996). Fission yeast, on the other hand, has well-developed cytoplasmic microtubule arrays (Hagan, 1998) and its microtubule-associated

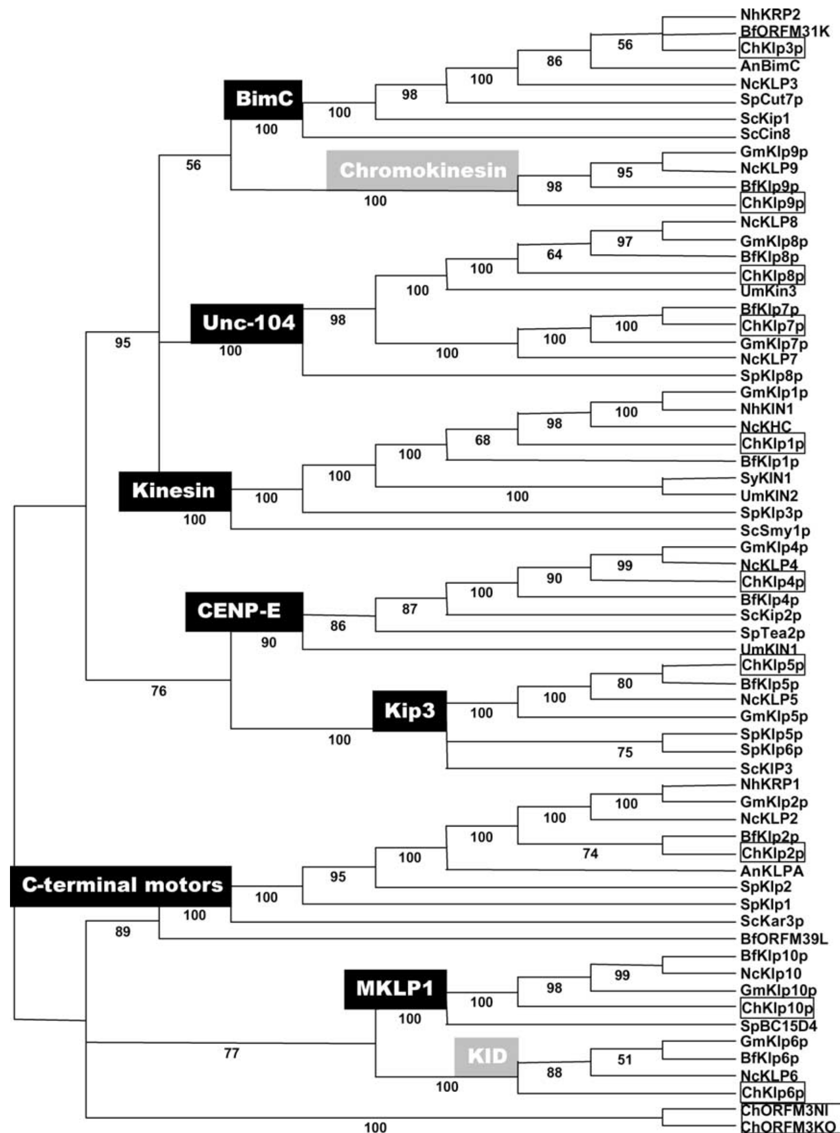

Fig. 4. A 50% Majority Rule tree, with midpoint rooting, obtained from 10,000 maximum likelihood trees, selected from 1,000,000 generations, inferred by Bayesian logic using the Jones amino acid substitution model in the program MrBayes (Huelsenbeck and Ronquist, 2001). The first 500 trees were discarded. Protein sequences were aligned with T-Coffee (Notredame et al., 2000) and deposited at TreeBASE (alignments can be obtained from the corresponding author.). Numbers refer to the percentage of trees containing a specific branch. (For explanation of abbreviations used, see Table 2.) Protein subfamilies are listed on the nodes and colored as follows: *Black*—found in yeasts, filamentous ascomycetes, and other organisms; *Grey*—present in filamentous ascomycetes and other eukaryotes, but not in the yeasts surveyed for this study. *C. heterostrophus* proteins are boxed.

motors appear to play roles comparable to those in filamentous fungi and other eukaryotes.

Although deletions of kinesin genes in a number of fungal species all result in hyphal deformation and slower growth, additional phenotypes vary. In *N. crassa*, nuclear distribution is impaired, while in *U. maydis* and *N. haematococca* alterations occur in vacuole formation and mitochondrial distribution at the hyphal tip (Steinberg et al., 1998; Suelmann and Fischer, 2000; Wu et al., 1998). In *A. nidulans* kinesin deletion mutants, polar growth and nuclear positioning were affected, while movement of vacuoles and mitochondria were not (Requena et al., 2001). The finding that kinesin function can influence MT stability may provide clues to differences among phenotypes in these species (Requena et al., 2001).

Some unique filamentous fungal features present in kinesin have also been noted. No light chains have been isolated in association with KHC or other fungal kinesins, suggesting differences in interactions at the tail region (Steinberg, 2000; Steinberg and Schliwa, 1995). Four- and fivefold higher speeds of movement than reported for animal counterparts has been observed for fungal kinesins isolated from *N. crassa* (Kirchner et al., 1999; Steinberg and Schliwa, 1996) and the zygomycete *S. racemosum* (Grummt et al., 1998a). Several distinctive

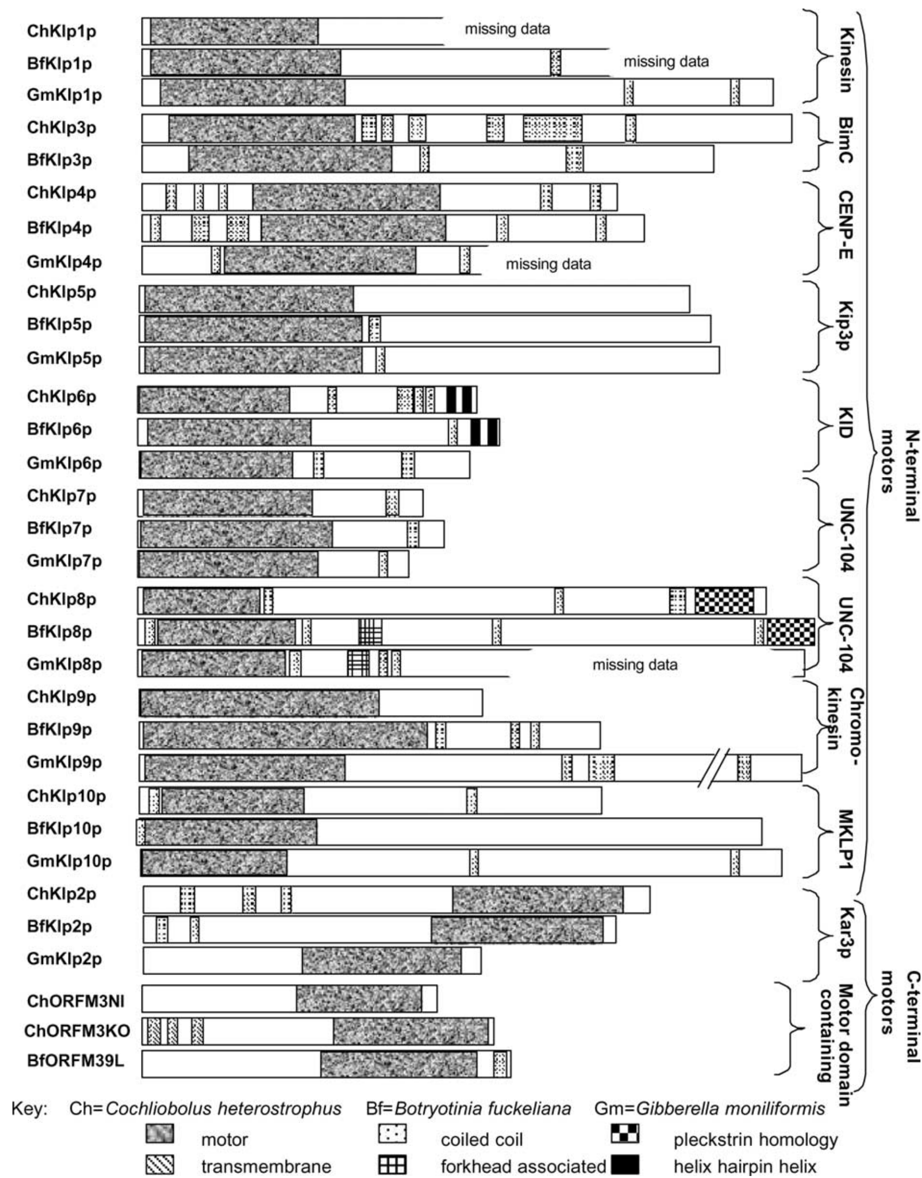

Fig. 5. SMART domain analysis (Letunic et al., 2002; Schultz et al., 1998) of representative ascomycete kinesins. Boxes represent proteins derived from open reading frames, in proportion to size. Open reading frames were annotated manually using comparisons among closely related species and known proteins where possible.

structural features associated with the fungal kinesins, such as a helix disrupting hinge in the neck domain may make this possible (Grummt et al., 1998b; Kallipolitou et al., 2001; Song et al., 2001). Taking all these differences into account, it was proposed that the fungal kinesins form a distinct and distantly related subgroup from the animal kinesins (Grummt et al., 1998a).

Previously, the budding yeast protein Smy1p was used as an outgroup in a number of phylogenetic studies of kinesins (Hirokawa, 1998; Kim and Endow, 2000). Smy1p interacts with a myosin-V and has not been found to bind microtubules (Lillie and Brown, 1998). In a recent maximum likelihood comparison of kinesin motor domains (Lawrence et al., 2002), it was inferred that Smy1p originated as a conventional kinesin. This assumption fits well with the budding yeast profile that demonstrates the absence of a conventional kinesin. Additionally, Smy1p shares its ability to interact with a myosin-V with another member of the conventional kinesin subfamily, the mouse protein KHCU (Beningo et al., 2000; Huang et al., 1999). The fungal phylogenetic comparison in Fig. 4 supports the above-mentioned placement of Smy1p with the conventional kinesins. However, a larger-scale analysis of kinesin motor domains including several orphan proteins (Fig. 3) could not group the Smy1 protein similarly, emphasizing the tenuousness of its phylogenetic placement based on sequence analysis only.

4.2. BimC

The founding member of this subfamily, BimC, was first described in *A. nidulans*, and is involved in cell division through mitotic spindle formation (Enos and Morris, 1990). This protein is a homotetramer important for bipolar spindle formation; it slides anti-parallel MTs by forming cross bridges between them and produces an outwardly directed force in the spindle (Kashina et al., 1996). Members of this subfamily have been found in all eukaryotes examined. A number of studies have shown that BimC and its homologs are essential in *A. nidulans* (Enos and Morris, 1990), *S. pombe* (Hagan and Yanagida, 1990), and *Drosophila* (Heck et al., 1993). Budding yeast has two functionally redundant proteins, Cin8p and Kip1p, playing essential roles in separation of spindle poles during mitosis (mutants of single genes are viable, but double mutants are not) (Hoyt et al., 1992; Roof et al., 1992). Like the *Aspergillus* protein, they function antagonistically with members of the Kar3p subfamily (O'Connell and Morris, 1993; Saunders et al., 1997; Saunders and Hoyt, 1992). In the fungal phylogenetic comparison (Fig. 4), all fungal species have homologs, except for *G. moniliformis*. Because of the importance of BimC to cell function and since it has already been found in at least one additional *Gibberella* species (data not shown) it seems likely that this absence can best be explained by a gap in the *G. moniliformis* genome assembly.

4.3. CENP-E

This subfamily is named after human CENP-E, which was first identified as a centromeric protein, functioning in chromosome segregation (Yen et al., 1991). Members of this subfamily are in an orphan group in the phylogenetic analysis by Kim and Endow (2000) but form a separate group in phylogenetic treatments by Miki et al. (2001) and Lawrence et al. (2002). CENP-E has been proposed to tether chromosomes to dynamic microtubule ends (Wood et al., 1997) and is important in the spindle checkpoint at metaphase (reviewed in Musacchio and Hardwick, 2002). *S. cerevisiae* Kip2p, the CENP-E homolog, stabilizes microtubules and functions in nuclear migration (Miller et al., 1998) but has not been detected in the nucleus (Hildebrandt and Hoyt, 2000). *S. pombe* Tea2p has been shown to be necessary for polarized vegetative growth; it localizes other proteins, such as Tea1p to MT ends and promotes MT growth (Behrens and Nurse, 2002; Browning et al., 2000; Niccoli and Nurse, 2002). In the basidiomycete *U. maydis,* a Kin1 deletion had no detectable phenotype (Lehmler et al., 1997). Members of this subfamily were found in all fungal species included in this study. Their role as fungal MT stabilizers is well supported in budding and fission yeast, however it is unclear whether they share additional functions with CENP-E, such as interaction with the kinetochore. The domain placements in Fig. 5 indicate differences in the fungal motor domain. It is placed internally in Klp4p, while in the CENP-E motor domain it is N-terminal (not shown). These filamentous ascomycete placements also agree with those in *S. pombe* and *S. cerevisiae*. Additional work is required, however, to confirm our annotations; thus we tentatively list these as N-type proteins here.

4.4. Kip3p

The budding yeast Kip3 protein is involved in spindle positioning and nuclear migration during mitosis (Cottingham and Hoyt, 1997; DeZwaan et al., 1997). *S. pombe* has undergone a gene duplication for this group of kinesins. The two gene products, Klp5p and Klp6p, are required for meiosis in fission yeast, play a role in MT disassembly, and are required for chromosome segregation in mitosis (West et al., 2002; West et al., 2001). They have been localized to the spindle midzone and kinetochores of mitotic spindles (Garcia et al., 2002). Although these proteins are not essential for vegetative growth, either separately or together, the presence of both is essential for meiosis (West et al., 2002; West et al., 2001). These proteins share several possible functions with the members of the MCAK subfamily (discussed previously). Separate proteins from human and fly that group with both subfamilies have already been shown in phylogenetic analyses (Iwabe and Miyata, 2002; Miki et al., 2001). Functional data for members of the Kip3p subfamily are still limited in higher eukaryotes, but the *Drosophila* ortholog, KLP67A, has been implicated in mitochondrial movement (Pereira et al., 1997). Like the CENP-E subfamily, the members of this subfamily likely conduct a conserved and important function in all fungal species.

4.5. UNC-104

In this analysis, two different fungal orthologous groups were clustered together with the human and *C. elegans* UNC-104 representatives (Figs. 3 and 4). According to a SMART analysis (Letunic et al., 2002; Schultz et al., 1998; Fig. 5), proteins in the fungal Klp8p clade carry a number of known motifs in the tail region. These include a motif with similarity to the pleckstrin homology domain. This domain has previously been reported in UNC-104 related kinesins in *C. elegans* where it has been proposed to bind lipids and lipid rafts in order to dock onto membrane cargo (Klopfenstein et al., 2002). Another domain, the forkhead associated domain, proposed to be involved in signaling and protein-protein interactions of kinesins (Westerholm-Parvinen et al., 2000) was predicted in this set of proteins. In

the higher eukaryotes, UNC-104-like motors are involved in transport of organelles such as mitochondria (Nangaku et al., 1994) or Golgi intermediates (Dorner et al., 1998). Recently, a fungal UNC-104 kinesin, Kin3, was isolated from the basidiomycete *U. maydis* and shown to be involved in binding endosomes, moving them in concert with dynein (Wedlich-Soldner et al., 2002).

In Figs. 3 and 4, a second clade (Klp7p) containing orthologous fungal proteins consisting of smaller predicted open reading frames is shown. The predicted fungal Klp7p representative has fewer than half the number of amino acids found in the Klp8 predicted protein (for *C. heterostrophus,* 614 versus 1653). No functional data are available to back up the assumed placement of these two clades, but in a parsimony analysis of the same alignment (data not shown) the two were congruent. However, the different domain predictions for the two groups of proteins seen in Fig. 5 indicate that the Klp7p clade constitutes a unique fungal subgroup of "truncated" UNC-104-like proteins that may constitute a new subfamily.

4.6. KID

A clade of predicted fungal proteins (Klp6p) groups with KID, a human chromosome binding protein (Fig. 3). This protein has a highly diverged motor domain and has been placed consistently among orphan kinesin groups in previous phylogenetic analyses (Hirokawa, 1998; Kim and Endow, 2000). More recent analyses have grouped KID in a new subfamily together with NOD from *Drosophila* and others (Miki et al., 2001) or placed it (together with NOD) among chromokinesins (Lawrence et al., 2002). NOD is a chromosome-associated protein with ATPase activity but no demonstrable microtubule gliding activity (Matthies et al., 2001). Placement with chromokinesins seems logical from a functional perspective, because, in humans, KID proteins are involved in moving chromosomes towards the metaphase plate (Tokai et al., 1996). No support for this placement is seen in Figs. 3 and 4, although a close relationship with centromere binding proteins in the Kip3p and MKLP1 subfamilies is inferred.

4.7. Chromokinesin/KIF4

Chromokinesin was initially isolated from chicken and found to be associated with the chromosome arms, functioning in the movement of DNA during mitosis (Wang and Adler, 1995). In a frog homolog, Xklp1, putative zinc finger domains were found in the tail and the protein was localized to the nucleus during anaphase and the chromosomes during mitosis (Vernos et al., 1995). The role of this protein in mitosis was confirmed when Xklp1 disrupted embryos were shown to have defects in cell division after fertilization, seen as unstable spindles and a failure of chromosomes to aggregate at the metaphase plate (Vernos et al., 1995). A human counterpart, KIF4A, is expressed mainly in juvenile neurons where it takes part in vesicle transport (Peretti et al., 2000). In our study, only filamentous fungi were found to have these proteins; none of the yeasts had any homolog. A recent phylogenetic study by Iwabe and Miyata (2002) grouped a protein from *Giardia lamblia* together with the *N. crassa* orthologs of this group and separate from the other eukaryote homologs (Fig. 4), emphasizing the ancient origin of these proteins and the uniqueness of the fungal subgroup.

5. C-terminal motors

5.1. Kar3p

The first member of this subfamily, Kar3p, was identified in *S. cerevisiae* and has been shown to move in the opposite direction to N-terminal motors (Meluh and Rose, 1990; Middleton and Carbon, 1994). In filamentous fungi, a similar function—generation of an inwardly directed force in the spindle—was found for KlpA in *Aspergillus* and KRP1 in *N. haematococca* (Aist, 2002). So far, uniquely in budding yeast, Kar3p has been shown to interact with another non-motor protein, Cik1p, to form a heteromeric complex (Page et al., 1994). These proteins function in spindle assembly, but act antagonistically to another kinesin and member of the CENP-E group of kinesins, Kip2p (Huyett et al., 1998). A second subfamily, named C-terminal II, containing poorly characterized human, worm, and plant genes is shown in some analyses (Lawrence et al., 2002; Miki et al., 2001). Most of the human genes in this group (which contains no fungal genes) are involved in dendritic transport of vesicles (Miki et al., 2001). However, a number of other analyses did not separate the C-terminal motors into two groups (Goodson et al., 1994; Iwabe and Miyata, 2002; Kim and Endow, 2000) and its status as a separate subfamily requires further analysis.

6. Kinesin motor domain-containing proteins and orphans

In the current study, three putative filamentous fungal proteins were detected with divergent motor domain sequences. Another divergent protein, Klp8p, previously denoted in *S. pombe*, was placed differently in our two analyses (Figs. 3 and 4). It grouped with the *Drosophila* protein NOD in the large-scale analysis (Fig. 3) which, as previously mentioned, binds microtubules without gliding activity (Matthies et al., 2001), but was placed with members of the UNC-104 clade in the fungi-only

comparison (Fig. 4). The fungi-only analysis grouped two of the putative filamentous proteins from *C. heterostrophus*, ORFM3NI and ORFM3KO, basally in both trees (Figs. 3 and 4). The third protein from *B. fuckeliana*, ORFM39L, was placed in the group of C-terminal motors (Fig. 4). This placement is supported by the C-terminal domain motifs of ORFM39L seen in Fig. 5. Additionally, one of the predicted proteins, *C. heterostrophus* ORFM3KO, has transmembrane domains (Fig. 5). Since the motor domain cannot be separated into modules (Kikkawa et al., 2000) and retain motor function, it seems unlikely from the structure of these predicted domains that they will function as traditional kinesins. Other proteins with similar degenerate motor domain structures (containing only single motifs) and unknown functions have been noted already from the genomes of *Drosophila* and human, (Miki et al., 2001). In all cases more biological data are needed to elucidate their function.

7. Conclusions

It is evident that the founding members of most kinesin subfamilies were present before fungi, metazoans, and plants diverged. The majority of the kinesin groups are conserved across the filamentous ascomycetes examined in this study and have corresponding distinct functions. This ancient subfamily origin was substantiated when a number of kinesin cDNAs were sequenced from *G. lamblia* (Iwabe and Miyata, 2002) and data compared with those of known kinesins, including a number of proteins from basally located metazoans. *Giardia* forms part of the Diplomonadida, proposed to be one of the earliest branching eukaryotic groups (Baldauf et al., 2000). A phylogenetic analysis that compared amino-acid sequences inferred from these *Giardia* kinesins revealed strong similarities that assisted in placing these proteins with the subfamilies containing MCAK/KIF2, BimC, Unc104/KIF1, KRP85/95, and C-terminal motors (Iwabe and Miyata, 2002). No representatives with similarity to MKLP1, Kip3p, and chromokinesin/KIF1 were found, indicating either more recent origins for these subfamilies or a loss in *Giardia*.

A comparison of kinesin inventories of mouse and human showed that the profiles of both organisms were highly similar (Miki et al., 2001). Akin to this, our comparison of kinesin inventories of filamentous ascomycetes has identified a constant set of 10 kinesins. Although some species-specific proteins with degenerate motor domains were found in *Botrytis* and *Cochliobolus,* no kinesin-related function can be inferred from their motor domains. It appears that a rigidly defined limit to the number of kinesins exists in the filamentous fungi and that these proteins have diverged following gene duplication events. In the two yeasts, some kinesins have been lost; extant differences in their profiles have been clarified by studies showing variation in their interactions with the actin and MT cytoskeletons. When the filamentous fungal kinesin profiles are compared with those of the two yeasts, there are notable differences. For example, proteins such as Klp6p and Klp9p are absent from the yeasts. These components are most likely involved in transport of subcellular components through multicellular, often fast growing, hyphae. In the comparison of the filamentous fungal kinesins to characterized proteins from other eukaryotes, a number of fungal groups (Klp4p, Klp7p, Klp9p, and Klp10p) were placed within subfamilies, but still appear unique, buttressed either by differences in domain placement or low support for their placement in other phylogenetic analyses.

In the higher eukaryotes, extensive gene duplication has given rise to multiple members in specific kinesin subfamilies, especially those used in transport in brain tissue. Alternative splicing has also expanded this group; members have related and sometimes overlapping, but still distinct, functions. Recent plant genome data has catapulted plants to the forefront in terms of total numbers of kinesins (Fig. 2; Reddy and Day, 2001). Several of the previously described subfamilies are present in plants, but it seems very likely that novel subfamilies will be described in the future. Currently, however, very little is known about function of plant kinesins, from the perspective of a complete profile. This underscores the fact that, despite the recent explosion of structural knowledge of kinesins, much remains to be understood about kinesin evolution and function. The fungi, with relatively "simple" kinesin inventories, and ease of functional analysis, are premier model systems for investigating these questions.

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References

Aist, J.R., 2002. Mitosis and motor proteins in the filamentous ascomycete, *Nectria haematococca*, and some related fungi. Int. Rev. Cytol. 212, 239–263.

Baldauf, S.L., Roger, A.J., Wenk-Siefert, I., Doolittle, W.F., 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290, 972–977.

Behrens, R., Nurse, P., 2002. Roles of fission yeast tea1p in the localization of polarity factors and in organizing the microtubular cytoskeleton. J. Cell Biol. 157, 783–793.

Beningo, K.A., Lillie, S.H., Brown, S.S., 2000. The yeast kinesin-related protein Smy1p exerts its effects on the class V myosin Myo2p via a physical interaction. Mol. Biol. Cell 11, 691–702.

Bloom, G.S., Wagner, M.C., Pfister, K.K., Brady, S.T., 1988. Native structure and physical properties of bovine brain kinesin and identification of the ATP-binding subunit polypeptide. Biochemistry 27, 3409–3416.

Brady, S.T., 1985. A novel brain ATPase with properties expected for the fast axonal transport motor. Nature 317, 73–75.

Brady, S.T., Lasek, R.J., Allen, R.D., 1982. Fast axonal transport in extruded axoplasm from squid giant axon. Science 218, 1129–1131.

Brazer, S.C., Williams, H.P., Chappell, T.G., Cande, W.Z., 2000. A fission yeast kinesin affects Golgi membrane recycling. Yeast 16, 149–166.

Brendza, R.P., Serbus, L.R., Duffy, J.B., Saxton, W.M., 2000. A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. Science 289, 2120–2122.

Brown, K.D., Wood, K.W., Cleveland, D.W., 1996. The kinesin-like protein CENP-E is kinetochore-associated throughout poleward chromosome segregation during anaphase-A. J. Cell Sci. 109, 961–969.

Browning, H., Hayles, J., Mata, J., Aveline, L., Nurse, P., McIntosh, J.R., 2000. Tea2p is a kinesin-like protein required to generate polarized growth in fission yeast. J. Cell Biol. 151, 15–28.

Case, R.B., Rice, S., Hart, C.L., Ly, B., Vale, R.D., 2000. Role of the kinesin neck linker and catalytic core in microtubule-based motility. Curr. Biol. 10, 157–160.

Chen, M.C., Zhou, Y., Detrich III, H.W., 2002. Zebrafish mitotic kinesin-like protein 1 (Mklp1) functions in embryonic cytokinesis. Physiol. Genomics 8, 51–66.

Cole, D.G., 1999a. Kinesin-II, the heterodimeric kinesin. Cell. Mol. Life Sci. 56, 217–226.

Cole, D.G., 1999b. Kinesin-II, coming and going. J. Cell Biol. 147, 463–466.

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

Cottingham, F.R., Gheber, L., Miller, D.L., Hoyt, M.A., 1999. Novel roles for *Saccharomyces cerevisiae* mitotic spindle motors. J. Cell Biol. 147, 335–350.

Cottingham, F.R., Hoyt, M.A., 1997. Mitotic spindle positioning in *Saccharomyces cerevisiae* is accomplished by antagonistically acting microtubule motor proteins. J. Cell Biol. 138, 1041–1053.

Dayhoff, M.O., 1976. The origin and evolution of protein superfamilies. Fed. Proc. 35, 2132–2138.

DeZwaan, T.M., Ellingson, E., Pellman, D., Roof, D.M., 1997. Kinesin-related KIP3 of *Saccharomyces cerevisiae* is required for a distinct step in nuclear migration. J. Cell Biol. 138, 1023–1040.

Dorner, C., Ciossek, T., Muller, S., Moller, P.H., Ullrich, A., Lammers, R., 1998. Characterization of KIF1C, a new kinesin-like protein involved in vesicle transport from the Golgi apparatus to the endoplasmic reticulum. J. Biol. Chem. 273, 20267–20275.

Drummond, D.R., Hagan, I.M., 1998. Mutations in the bimC box of Cut7 indicate divergence of regulation within the bimC family of kinesin related proteins. J. Cell Sci. 111, 853–865.

Echeverri, C.J., Paschal, B.M., Vaughan, K.T., Vallee, R.B., 1996. Molecular characterization of the 50-kDa subunit of dynactin reveals function for the complex in chromosome alignment and spindle organization during mitosis. J. Cell Biol. 132, 617–633.

Eisen, J.A., 1998. Phylogenomics: improving functional predictions for uncharacterized genes by evolutionary analysis. Genome Res. 8, 163–167.

Eisen, J., Wu, M., 2002. Phylogenetic analysis and gene functional predictions: phylogenomics in action. Theor. Popul. Biol. 61, 481.

Endow, S.A., Waligora, K.W., 1998. Determinants of kinesin motor polarity. Science 281, 1200–1202.

Enos, A., Morris, N.R., 1990. Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *Aspergillus nidulans*. Cell 60, 1019–1027.

Eriksson, E.O., 2002. Outline of mycota. Myconet, www.umu.se/myconet/Myconet.html.

Fischer, R., 1999. Nuclear movement in filamentous fungi. FEMS Microbiol. Rev. 23, 39–68.

Garcia, M.A., Koonrugsa, N., Toda, T., 2002. Two kinesin-like Kin I family proteins in fission yeast regulate the establishment of metaphase and the onset of anaphase A. Curr. Biol. 12, 610–621.

Gheber, L., Kuo, S.C., Hoyt, M.A., 1999. Motile properties of the kinesin-related Cin8p spindle motor extracted from *Saccharomyces cerevisiae* cells. J. Biol. Chem. 274, 9564–9572.

Goldstein, L.S., Philp, A.V., 1999. The road less traveled: emerging principles of kinesin motor utilization. Annu. Rev. Cell. Dev. Biol. 15, 141–183.

Goodson, H.V., Kang, S.J., Endow, S.A., 1994. Molecular phylogeny of the kinesin family of microtubule motor proteins. J. Cell Sci. 107, 1875–1884.

Grummt, M., Pistor, S., Lottspeich, F., Schliwa, M., 1998a. Cloning and functional expression of a 'fast' fungal kinesin. FEBS Lett. 427, 79–84.

Grummt, M., Woehlke, G., Henningsen, U., Fuchs, S., Schleicher, M., Schliwa, M., 1998b. Importance of a flexible hinge near the motor domain in kinesin-driven motility. EMBO J. 17, 5536–5542.

Hagan, I., 1998. The fission yeast microtubule cytoskeleton. J. Cell Sci. 111, 1603–1612.

Hagan, I., Yanagida, M., 1990. Novel potential mitotic motor protein encoded by the fission yeast *cut7+* gene. Nature 347, 563–566.

Heck, M.M., 1999. Dr. Dolittle and the making of the mitotic spindle. BioEssays 21, 985–990.

Heck, M.M., Perreira, A., Pesavento, Y., Yannoni, A.C., 1993. The kinesin-like protein KLP61F is essential for mitosis in *Drosophila*. J. Cell Biol. 123, 665–679.

Henikoff, S., Greene, E.A., Pietrokovski, S., Bork, P., Attwood, T.K., Hood, L., 1997. Gene families: the taxonomy of protein paralogs and chimeras. Science 278, 609–614.

Hildebrandt, E.R., Hoyt, M.A., 2000. Mitotic motors in *Saccharomyces cerevisiae*. Biochem. Biophys. Acta. 1496, 99–116.

Hirokawa, N., 1998. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279, 519–526.

Hoyt, M.A., He, L., Loo, K.K., Saunders, W.S., 1992. Two *Saccharomyces cerevisiae* kinesin-related gene products required for mitotic spindle assembly. J. Cell Biol. 118, 109–120.

Hoyt, M.A., He, L., Totis, L., Saunders, W.S., 1993. Loss of function of *Saccharomyces cerevisiae* kinesin-related CIN8 and KIP1 is suppressed by KAR3 motor domain mutations. Genetics 135, 35–44.

Huang, J.D., Brady, S.T., Richards, B.W., Stenolen, D., Resau, J.H., Copeland, N.G., Jenkins, N.A., 1999. Direct interaction of microtubule- and actin-based transport motors. Nature 397, 267–270.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huyett, A., Kahana, J., Silver, P., Zeng, X., Saunders, W., 1998. The Kar3p and Kip2p motors function antagonistically at the spindle poles to influence cytoplasmic microtubule numbers. J. Cell Sci. 111, 295–301.

Iwabe, N., Miyata, T., 2002. Kinesin-related genes from diplomonad, sponge, amphioxus, and cyclostomes: divergence pattern of kinesin family and evolution of giardial membrane-bounded organella. Mol. Biol. Evol. 19, 1524–1533.

Jacobs, C.W., Adams, A.E., Szaniszlo, P.J., Pringle, J.R., 1988. Functions of microtubules in the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. 107, 1409–1426.

Kallipolitou, A., Deluca, D., Majdic, U., Lakamper, S., Cross, R., Meyhofer, E., Moroder, L., Schliwa, M., Woehlke, G., 2001.

Unusual properties of the fungal conventional kinesin neck domain from *Neurospora crassa*. EMBO J. 20, 6226–6235.

Kashina, A.S., Baskin, R.J., Cole, D.G., Wedaman, K.P., Saxton, W.M., Scholey, J.M., 1996. A bipolar kinesin. Nature 379, 270–272.

Kikkawa, M., Okada, Y., Hirokawa, N., 2000. A resolution model of the monomeric kinesin motor, KIF1A. Cell 100, 241–252.

Kim, A.J., Endow, S.A., 2000. A kinesin family tree. J. Cell Sci. 113, 3681–3682.

Kirchner, J., Woehlke, G., Schliwa, M., 1999. Universal and unique features of kinesin motors: insights from a comparison of fungal and animal conventional kinesins. Biol. Chem. 380, 915–921.

Klopfenstein, D.R., Tomishige, M., Stuurman, N., Vale, R.D., 2002. Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor. Cell 109, 347–358.

Kobayashi, N., Reiser, J., Kriz, W., Kuriyama, R., Mundel, P., 1998. Nonuniform microtubular polarity established by CHO1/MKLP1 motor protein is necessary for process formation of podocytes. J. Cell Biol. 143, 1961–1970.

Lawrence, C.J., Malmberg, R.I., Muzynski, M.G., Dawe, R.K., 2002. Maximum likelihood methods reveal conservation of function among closely related kinesin families. J. Mol. Evol. 54, 42–53.

Lehmler, C., Steinberg, G., Snetselaar, K.M., Schliwa, M., Kahmann, R., Bolker, M., 1997. Identification of a motor protein required for filamentous growth in *Ustilago maydis*. EMBO J. 16, 3464–3473.

Letunic, I., Goodstadt, L., Dickens, N.J., Doerks, T., Schultz, J., Mott, R., Ciccarelli, F., Copley, R.R., Ponting, C.P., Bork, P., 2002. Recent improvements to the SMART domain-based sequence annotation resource. Nucleic Acids Res. 30, 242–244.

Lillie, S.H., Brown, S.S., 1998. Smy1p, a kinesin-related protein that does not require microtubules. J. Cell Biol. 140, 873–883.

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

Maney, T., Wagenbach, M., Wordeman, L., 2001. Molecular dissection of the microtubule depolymerizing activity of mitotic centromere-associated kinesin. J. Biol. Chem. 276, 34753–34758.

Manning, B.D., Snyder, M., 2000. Drivers and passengers wanted! The role of kinesin-associated proteins. Tr. Cell Biol. 10, 281–289.

Marszalek, J.R., Goldstein, L.S., 2000. Understanding the functions of kinesin-II. Biochim. Biophys. Acta 1496, 142–150.

Matthies, H.J., Baskin, R.J., Hawley, R.S., 2001. Orphan kinesin NOD lacks motile properties but does possess a microtubule-stimulated ATPase activity. Mol. Biol. Cell 12, 4000–4012.

Matuliene, J., Kuriyama, R., 2002. Kinesin-like protein CHO1 is required for the formation of midbody matrix and the completion of cytokinesis in mammalian cells. Mol. Biol. Cell 13, 1832–1845.

Meluh, P.B., Rose, M.D., 1990. KAR3, a kinesin-related gene required for yeast nuclear fusion. Cell 6, 1029–1041.

Middleton, K., Carbon, J., 1994. KAR3-encoded kinesin is a minus-end-directed motor that functions with centromere binding proteins (CBF3) on an in vitro yeast kinetochore. Proc. Natl. Acad. Sci. USA 91, 7212–7216.

Miki, H., Setou, M., Kaneshiro, K., Hirokawa, N., 2001. All kinesin superfamily protein, KIF, genes in mouse and human. Proc. Natl. Acad. Sci. USA 98, 7004–7011.

Miller, R.K., Heller, K.K., Frisen, L., Wallack, D.L., Loayza, D., Gammie, A.E., Rose, M.D., 1998. The kinesin-related proteins, Kip2p and Kip3p, function differently in nuclear migration in yeast. Mol. Biol. Cell 9, 2051–2068.

Moore, J.D., Endow, S.A., 1996. Kinesin proteins: a phylum of motors for microtubule-based motility. BioEssays 18, 207–219.

Musacchio, A., Hardwick, K.G., 2002. The spindle checkpoint: structural insights into dynamic signalling. Nat. Rev. Mol. Cell Biol. 3, 731–741.

Nangaku, M., Sato-Yoshitake, R., Okada, Y., Noda, Y., Takemura, R., Yamazaki, H., Hirokawa, N., 1994. KIF1B, a novel microtubule plus end-directed monomeric motor protein for transport of mitochondria. Cell 79, 1209–1220.

Niccoli, T., Nurse, P., 2002. Different mechanisms of cell polarisation in vegetative and shmooing growth in fission yeast. J. Cell Sci. 115, 1651–1662.

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

O'Connell, M.J., Morris, N.R., 1993. Suppression of the bimC4 mitotic spindle defect by deletion of klpA, a gene encoding a KAR3-related kinesin-like protein in *Aspergillus nidulans*. J. Cell Biol. 120, 153–162.

Okada, Y., Hirokawa, N., 1999. A processive single-headed motor: kinesin superfamily protein KIF1A. Science 283, 1152–1157.

Page, B.D., Satterwhite, L.L., Rose, M.D., Snyder, M., 1994. Localization of the Kar3 kinesin heavy chain-related protein requires the Cik1 interacting protein. J. Cell Biol. 124, 507–519.

Pereira, A.J., Dalby, B., Stewart, R.J., Doxsey, S.J., Goldstein, L.S., 1997. Mitochondrial association of a plus end-directed microtubule motor expressed during mitosis in *Drosophila*. J. Cell Biol. 136, 1081–1090.

Peretti, D., Peris, L., Rosso, S., Quiroga, S., Caceres, A., 2000. Evidence for the involvement of KIF4 in the anterograde transport of L1-containing vesicles. J. Cell Biol. 149, 141–152.

Reddy, A.S., Day, I.S., 2001. Kinesins in the Arabidopsis genome: a comparative analysis among eukaryotes. BMC Genomics 2, 1–13.

Requena, N., Alberti-Segui, C., Winzenburg, E., Horn, C., Schliwa, M., Philippsen, P., Liese, R., Fischer, R., 2001. Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. Mol. Microbiol. 42, 121–132.

Roof, D.M., Meluh, P.B., Rose, M.D., 1992. Kinesin-related proteins required for assembly of the mitotic spindle. J. Cell Biol. 118, 95–108.

Sakowicz, R., Farlow, S., Goldstein, L.S., 1999. Cloning and expression of kinesins from the thermophilic fungus *Thermomyces lanuginosus*. Protein Sci. 8, 2705–2710.

Saunders, W., Lengyel, V., Hoyt, M.A., 1997. Mitotic spindle function in *Saccharomyces cerevisiae* requires a balance between different types of kinesin-related motors. Mol. Biol. Cell 8, 1025–1033.

Saunders, W.S., Hoyt, M.A., 1992. Kinesin-related proteins required for structural integrity of the mitotic spindle. Cell 70, 451–458.

Schaar, B.T., Chan, G.K., Maddox, P., Salmon, E.D., Yen, T.J., 1997. CENP-E function at kinetochores is essential for chromosome alignment. J. Cell Biol. 139, 1373–1382.

Schultz, J., Milpetz, F., Bork, P., Ponting, C.P., 1998. SMART, a simple modular architecture research tool: identification of signaling domains. Proc. Natl. Acad. Sci. USA 95, 5857–5864.

Seiler, S., Kirchner, J., Horn, C., Kallipolitou, A., Woehlke, G., Schliwa, M., 2000. Cargo binding and regulatory sites in the tail of fungal conventional kinesin. Nat. Cell Biol. 2, 333–338.

Seiler, S., Nargang, F.E., Steinberg, G., Schliwa, M., 1997. Kinesin is essential for cell morphogenesis and polarized secretion in *Neurospora crassa*. EMBO J. 16, 3025–3034.

Seiler, S., Plamann, M., Schliwa, M., 1999. Kinesin and dynein mutants provide novel insights into the roles of vesicle traffic during cell morphogenesis in Neurospora. Curr. Biol. 9, 779–785.

Sharp, D.J., Rogers, G.C., Scholey, J.M., 2000. Microtubule motors in mitosis. Nature 407, 41–47.

Siddiqui, S.S., 2002. Metazoan motor models: kinesin superfamily in *C. elegans*. Traffic 3, 20–28.

Simon, V.R., Pon, L.A., 1996. Actin-based organelle movement. Experientia 52, 1117–1122.

Sipiczki, M., 2000. Where does fission yeast sit on the tree of life? Genome Biol. 1, 1–4.

Song, Y.H., Marx, A., Muller, J., Woehlke, G., Schliwa, M., Krebs, A., Hoenger, A., Mandelkow, E., 2001. Structure of a fast kinesin:

implications for ATPase mechanism and interactions with microtubules. EMBO J. 20, 6213–6225.

Steinberg, G., 1997. A kinesin-like mechanoenzyme from the zygomycete *Syncephalastrum racemosum* shares biochemical similarities with conventional kinesin from *Neurospora crassa*. Eur. J. Cell Biol. 73, 124–131.

Steinberg, G., 1998. Organelle transport and molecular motors in fungi. Fungal. Genet. Biol. 24, 161–177.

Steinberg, G., 2000. The cellular roles of molecular motors in fungi. Tr. Microbiol. 8, 162–168.

Steinberg, G., Schliwa, M., 1995. The *Neurospora* organelle motor: a distant relative of conventional kinesin with unconventional properties. Mol. Biol. Cell 6, 1605–1618.

Steinberg, G., Schliwa, M., 1996. Characterization of the biophysical and motility properties of kinesin from the fungus *Neurospora crassa*. J. Biol. Chem. 271, 7516–7521.

Steinberg, G., Schliwa, M., Lehmler, C., Bolker, M., Kahmann, R., McIntosh, J.R., 1998. Kinesin from the plant pathogenic fungus *Ustilago maydis* is involved in vacuole formation and cytoplasmic migration. J. Cell Sci. 111, 2235–2246.

Stock, M.F., Guerrero, J., Cobb, B., Eggers, C.T., Huang, T.G., Li, X., Hackney, D.D., 1999. Formation of the compact confomer of kinesin requires a COOH-terminal heavy chain domain and inhibits microtubule-stimulated ATPase activity. J. Biol. Chem. 274, 11423–14617.

Su, S., Yanagida, M., 1997. Mitosis and cytokinesis in the fission yeast, *Schizosaccharomyces Pombe*. In: Pringle, J.R., Broach, J.R., Jones, E.W. (Eds.), The molecular and cellular biology of the yeast *Saccharomyces cerevisiae*, vol. 3. Cold Spring Harbor Laboratory Press, Plainview, New York, pp. 765–825.

Suelmann, R., Fischer, R., 2000. Mitochondrial movement and morphology depend on an intact actin cytoskeleton. Cell Motil. Cytoskeleton 45, 42–50.

Tokai, N., Fujimoto-Nishiyama, A., Toyoshima, Y., Yonemura, S., Tsukita, S., Inoue, J., Yamamota, T., 1996. Kid, a novel kinesin-like DNA binding protein, is localized to chromosomes and the mitotic spindle. EMBO J. 15, 457–467.

Troxell, C.L., Sweezy, M.A., West, R.R., Reed, K.D., Carson, B.D., Pidoux, A.L., Cande, W.Z., McIntosh, J.R., 2001. pkl1(+)and klp2(+): two kinesins of the Kar3 subfamily in fission yeast perform different functions in both mitosis and meiosis. Mol. Biol. Cell 12, 3476–3488.

Vale, R.D., Fletterick, R.J., 1997. The design plan of kinesin motors. Annu. Rev. Cell Dev. Biol. 13, 745–777.

Vale, R.D., Reese, T.S., Sheetz, M.P., 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 42, 39–50.

Vernos, I., Raats, J., Hirano, T., Heasman, J., Karsenti, E., Wylie, C., 1995. Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning. Cell 81, 117–127.

Wang, S.Z., Adler, R., 1995. Chromokinesin: a DNA-binding, kinesin-like nuclear protein. J. Cell Biol. 128, 761–768.

Wedlich-Soldner, R., Straube, A., Friedrich, M.W., Steinberg, G., 2002. A balance of KIF1A-like kinesin and dynein organizes early endosomes in the fungus *Ustilago maydis*. EMBO J. 21, 2946–2957.

West, R.R., Malmstrom, T., McIntosh, J.R., 2002. Kinesins klp5(+) and klp6(+) are required for normal chromosome movement in mitosis. J. Cell Sci. 115, 931–940.

West, R.R., Malmstrom, T., Troxell, C.L., McIntosh, J.R., 2001. Two related kinesins, klp5(+) and klp6(+), foster microtubule disassembly and are required for meiosis in fission yeast. Mol. Biol. Cell 12, 3919–3932.

Westerholm-Parvinen, A., Vernos, I., Serrano, L., 2000. Kinesin subfamily UNC104 contains a FHA domain: boundaries and physicochemical characterization. FEBS Lett. 486, 285–290.

Wood, K.W., Sakowicz, R., Goldstein, L.S., Cleveland, D.W., 1997. CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. Cell 91, 357–366.

Wordeman, L., Mitchison, T.J., 1995. Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. J. Cell Biol. 128, 95–104.

Wu, Q., Sandrock, T.M., Turgeon, B.G., Yoder, O.C., Wirsel, S.G., Aist, J.R., 1998. A fungal kinesin required for organelle motility, hyphal growth, and morphogenesis. Mol. Biol. Cell 9, 89–101.

Yang, J.T., Saxton, W.M., Stewart, R.J., Raff, E.C., Goldstein, L.S.B., 1990. Evidence that the head of kinesin is sufficient for force generation and motility in vitro. Science 249, 42–47.

Yen, T.J., Compton, D.A., Wise, D., Zinkowski, R.P., Brinkley, B.R., Earnshaw, W.C., Cleveland, D.W., 1991. CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. EMBO J. 10, 1245–1254.