Preparing the way: Fungal motors in microtubule organization

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Running title: Motors involved in microtubule organization

Abbreviations: MT, microtubule; CLIP, cytoplasmic linker protein; +TIP, plus-end tracking protein

Manuscript length: 3353 words; Abstract: 104 words; References: 66

Abstract

Fungal growth, development, and pathogenicity require hyphal tip growth. This process is supported by polar exocytosis at the expanding growth region. It is assumed that molecular motors transport growth supplies along the fibrous elements of the cytoskeleton, such as microtubules, to the hyphal apex. Recent advances in live cell imaging of fungi revealed additional roles of motors in organizing their own tracks. This can be achieved by (1) modifying microtubule dynamics directly, (2) targeting stability-determining factors to microtubule plus ends, and (3) transport and arrangement of already-assembled microtubules. In this review I discuss these unexpected roles of motors in organization of the microtubule cytoskeleton.

Molecular motors in fungi

Fungi are a large and important group of microorganisms that have enormous impact on the ecosystem and on human health, agriculture and industry (for an overview see introduction in [1]). A hallmark of fungi is the ability to expand by polarized growth [2], and transport processes along the cytoskeleton are thought to play important parts in fungal growth [3,4]. The eukaryotic cytoskeleton consists of two types of fibrous biopolymers: the filamentous actin assemblies (F-actin or microfilaments) and the microtubules (MTs). MTs are hollow cylinders composed of tubulin dimers that undergo phases of elongation and shrinkage (Box 1). They are nucleated by MT organizing centers, from where the growing plus ends of the MTs emanate and extend into the 3-D space of the cell. In addition, complex MT structures, such as the mitotic spindle [5] and the polarized MT array in specialized cells, e.g., neurons (summarized in Ref. [6]), are organized by molecular motors. These mechanoenzymes hydrolyze ATP for the transport of their cargo along the F-actin or the MT cytoskeleton (Fig. 1). The kinesin motors "walk" towards the MT plus end, whereas the large dynein complex motors move in the opposite direction. Motors have a broad spectrum of cargo, ranging from membranous organelles and vesicles to RNA and protein complexes, which explains why the motors play central parts in the organization and functioning of eukaryotic cells.

F-actin and associated myosin motors are long known to be essential for fungal growth and pathogenicity (see Ref. [7,8]). By contrast, the role of MTs in fungal growth has long been a

matter of debate and is currently under intense investigation. Two recent studies clearly showed that intact MTs are required for fast and extended hyphal growth of *Aspergillus nidulans* [9] and the plant pathogen *Ustilago maydis* [8]. In the dimorphic fungus *U. maydis*, MTs orient their plus ends towards the growing hyphal apex and in yeast-like cells, as shown by *in vivo* observation of a plus-end-binding EB1-like protein [10-12]. Thus, plus-end-directed kinesins most likely support hyphal tip growth . Published genomic sequences indicate that fungi contain a limited set of molecular motors: only 4 types of myosins, about 10–12 kinesins (Fig. 2), and 1 dynein. In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, many kinesins, and dynein, function in nuclear migration and chromosome segregation, whereas class V myosins are active in exocytosis and vacuole inheritance in interphase [13 14]. In the filamentous fungus *A. nidulans*, by contrast, the kinesin-7 KIPA focuses MTs at the growing tip [15], thereby supporting growth directionality during hyphal tip growth. However, in *U. maydis*, hyphal growth does not involve kinesin-7, but rather requires kinesin-1 and kinesin-3, which function in concert with myosin V [11]. Both kinesin motor types are absent in *S. cerevisiae*, but are found in higher eukaryotes and filamentous fungi (Fig. 2), where they are thought to support tip-directed traffic of vesicles and organelles, such as early endosomes [12] and mitochondria [16]. The data taken together indicate that only a small subset of MT motors maintain polarized hyphal growth by supporting membrane traffic along MTs (kinesin-1, kinesin-3, kinesin-7), whereas the majority of kinesins are involved in nuclear migration, mitosis or meiosis (kinesin-5, kinesin-7, kinesin-8, kinesin-14) and karyogamy (Kinesin-14), which often are linked to an effect on MT dynamics (see later). However, this functional classification was recently challenged by the finding that kinesin-14 in *S. pombe* mediates sliding of interphase MTs along each other [17] thereby helping to polarize the MT cytoskeleton. Furthermore, the organelle transporter kinesin-1 in *U. maydis* cross-links MTs *in vivo* [18], which in concert with dynein-dependent MT transport [19] might organize the interphase MT array (see later). Thus it is time to reconsider the simple concept of motors being involved in defined processes, such as membrane traffic. Here I review recent evidence for important functions of molecular motors in organizing and dynamics of the MT cytoskeleton. I suggest that motors exert their effect on MTs by three fundamentally different mechanisms: (1) direct modification of MT dynamics, (2) targeting factors to MT plus-ends, and (3) transport assembled MTs.

1. Kinesins modify MT dynamics

1.1 Mitotic roles of kinesin-14 and kinesin-8

MTs are dynamic polymers of αβ-tubulin heterodimers that grow and shrink by assembly and disassembly of subunits at their plus end (Box 1). MTs stochastically switch between phases of elongation and shortening, a behavior known as dynamic instability [20]. This behavior and thereby the length and organization of MTs is controlled by microtubule-associated proteins and plus-end tracking proteins (+TIPs), which bind specifically to MT plus ends [21]. In addition, some kinesins also accumulate at MT plus ends [15,22-24], which suggests that motors participate in the regulation of MT dynamics and turn-over [25]. The first indications for such a role of kinesins were found in the yeasts *S. cerevisiae* and *S. pombe.* Out of six kinesins in *S. cerevisiae*, Kar3p (kinesin-14), Kip3p (kinesin-8), and Kip2p (kinesin-7) are implicated in regulating MT stability [26]. Deletion of both *kip3* and *kar3* leads to longer and more stable MTs, and this phenotype is partially rescued by the destabilizing drug benomyl, suggesting that Kar3p and Kip3p function as MT-destabilizing factors in *S. cerevisiae* (see [27]; summarized in Ref. [26]). Indeed, it was recently shown that Kar3p supports nuclear migration shortly before karyogamy by its depolymerization activity [28]. The destabilizing activity of fungal Kinesin-8 might be of importance in preanaphase spindle positioning [29] and in anaphase A, where Kip3p localizes to kinetochores and mediates chromosome segregation [30]. A similar situation occurs in the fission yeast *S. pombe.* Deletion of the genes encoding the kinesin-14 members results in longer anaphase spindles [31]. Kinesin-8 motors in *S. pombe* (Klp5, Klp6; Fig. 2) are thought to destabilize MTs at chromosome kinetochores [32,33]. However, both fission yeast kinesin-8 motors also localize along interphase MTs, and *klp* deletion mutants contain much longer interphase MTs [34]. The destabilizing Klp5 and Klp6 proteins might therefore participate in organizing the MT array in growing *S. pombe* cells. At least the mitotic role of kinesin-14 and kinesin-8 is conserved between yeasts and filamentous fungi, because null mutants in *kipB*, the kinesin-8 in *A. nidulans* are are delayed in mitotic progression [35] and kinesin-14 mutants of these fungi show similar defects in spindle architecture (see [36,37]).

1.2 Kinesin-14 and Kinesin-8 directly destabilize MT plus-ends

It is an important question how fungal kinesin-14 and kinesin-8 motors exert their destabilizing activity on MTs. It was recently shown that *S. cerevisiae* Kar3p dimerizes with the non-motor protein Cik1, which helps to target Kar3p to the MT plus-end [24]. There, Kar3p uses its ATP-dependent motor activity to destabilize the MT end by removing subunits while moving towards the minus-end [24]. Fungal kinesin-8 proteins show weak sequence similarity with KinI kinesins (kinesin-13) from vertebrates [38]. Like Klp5 and Klp6, vertebrate KinI motors localize to kinetochores of chromosomes, where they directly modify the conformation of MT plus ends to foster depolymerization (summarized in Ref. [39]). This activity is counteracted by XMAP215-like MT-associated proteins, a functional cooperation that also occurs in *S. cerevisiae,* involving Kip3p and the XMAP215-homologue Stu2 which, counteract to regulate MT length in anaphase [38]. Thus, it seems likely that fungal kinesin-8 motors are functionally related to KinI motors. Indeed, two recent reports provide compelling evidence that Kip3p from *S. cerevisiae* are depolymerases that directly disassembles MTs [40,41]. However, Kip3p most likely reaches the MT plus-end by its own motor activity, and the combination of translocation activity and depolymerization activity clearly distinguishes fungal kinesin-8 form its animal counterpart kinesin-13.

2. Kinesins affect interphase MT organization by targeting factors to MT plus ends

Dynamic instability is tightly regulated by proteins that specifically locate at plus ends (Box 1, Ref. [21]). In fungi, homologues of EB1 [10,42,43]and CLIP-170 [12,44,45] and components of the dynein-transport machinery [12,46-48] are located at MT plus ends. There is good evidence that all of these +TIPs modify the dynamic behavior of MTs [44,46,49]. To exert their effect on the dynamic instability of MTs, +TIPs have to reach the plus end of MTs. Recent evidence confirms a crucial role of kinesin motors in the targeting of some +TIPs to the MT ends in fungi (Table 1).

2.1 Kinesin-7 targets plus-end binding proteins in budding and fission yeast

Genetic experiments in *S. cerevisiae* have demonstrated that deletion of Kip2p (kinesin-7) decreases the number of cytoplasmic MTs, whereas overexpression leads to much longer

MTs [26]. How this control over MT dynamics is achieved has long been elusive. Important insights into this question has come from a study by Carvalho et al. (2004) that convincingly demonstrated that Kip2p delivers the CLIP-170-like homologue Bik1p to the plus ends [50], where this +TIP participates in stabilizing MTs [49]. Bik1p also serves as an anchor for the putative dynein activator Pac1p (=Lis1), which in turn anchors the dynein complex to MT plus ends in *S. cerevisiae* [47,48]. The mechanism by which dynein reaches the plus end is not known, but it was speculated that Kip7p is involved in dynein targeting [50]. In filamentous fungi, dynein also locates to MT plus ends [12,46], and in the absence of dynein, MT dynamics is deregulated [46,51]. Despite these similarities between *S. cerevisiae* and filamentous fungi, the molecular mechanism of targeting the dynein machinery to MT plus ends might by fundamentally different. In *A. nidulans* and *U. maydis* kinesin-1 (=conventional kinesin) is used for direct transport of dynein and dynactin to the plus ends [12,52] (Fig. 3), and consequently *A. nidulans* mutated in *kinA*, the gene encoding kinesin-1 (Fig. 2), shows defects in nuclear migration and has more stable MTs [53]. By contrast, good indication exists that CLIP-170 homologues are targeted to the plus end of MTs by a motor-independent treadmilling mechanism [45], although deletion of Kinesin-7, but also Kinesin-1, affects plus end targeting at higher temperature [45]. Furthermore, dynein localization at plus ends is independent of the CLIP-170 protein [12,45] (Fig. 3), which further emphasizes the differences between yeast and filamentous fungi. The reason for these variations is not known, but it is tempting to speculate that the different cell architecture of elongated hyphae versus yeast cells might favor an active transport process of the large dynactin-dynein complex.

Similar to budding yeast, kinesin-7 motors participate in targeting +TIPs in the fission yeast *S. pombe*. Polarity of the cylindrical fission yeast cell depends on MTs, as cells that contain less and short MTs start branching [54]. This occurs because of the crucial role of MTs in the delivery of polarity factors, such as Tea1p, to the cell poles of fission yeast cells [55]. A key factor in this process is Tea2p, a kinesin-7 (Fig. 2) that takes Tea1 and the CLIP-170-like Tip1p to MT plus ends [22,56]. Tip1 and the EB1 homologue Mal3 promote continuous growth of the MT until it reaches the cell end [43], where it undergoes catastrophe, i.e., switches to shortening of the MTs [44]. This coincides with the off-loading of Tea1 to the cell cortex; Tea1 subsequently recruits additional polarity factors and helps to orient the myosin-actin based secretion apparatus.

2.2 The role of Kinesin-7 in filamentous fungi elusive

Whereas these studies in the yeasts *S. cerevisiae* and *S. pombe* argue for a general role of kinesin-7 motors in targeting of +TIPs to fungal MT ends, recent studies in the filamentous fungi *A. nidulans* and *U. maydis* challenge this conclusion. In contrast to the situation in both yeasts, the kinesin-7 in *A. nidulans* (KIPA, Fig. 2) has a less noticeable role in targeting of the CLIP-170 homologue CLIPA, which is only evident in Δ*kipA* mutants at higher temperature [45]. Instead, *A. nidulans* KIPA accumulates at MT plus ends in the growing hyphal tip, where it is required to focus MTs at the hyphal apex [15]. Because Δ*kipA* mutants lose growth direction, this influence on MTs is thought to be a prerequisite for proper organization of the hyphal growth machinery. However, such a role of kinesin-7 in hyphal growth might not be common among fungi. The basidiomycete *U. maydis* contains two kinesin-7 members (Fig. 7), but neither Δ*kin7a* or Δ*kin7b* single mutants nor Δ*kin7a*Δ*kin7b* double mutants show defects in hyphal growth or cell polarity [11]. Whether this discrepancy is because of novel functions of kinesin-7 in basidiomycetes, or whether there are functional redundancies with other, yet uncharacterized kinesins is presently not known.

3. Motors organize the cytoskeleton by transporting assembled MTs

3.1 Kinesin-14 supports sliding of MTs along each other

In contrast to other kinesins, kinesin-14 motors are minus-end-directed motors that have important roles in the mitotic spindle of all fungi. In addition to its destabilizing activity (see earlier), fungal kinesin-14 is able to bundle MTs [57], which is mediated by an additional MT binding site in their tail domain. Owing to these unique features, kinesin-14 is able to cross-bridge and actively organize them by sliding MTs along each other. This activity is of particular importance during early mitosis, when kinesin-14 is thought to establish the bipolar spindle by sliding MTs along each other [57]. However, a recent report on Klp2 in *S. pombe* suggests that kinesin-14 might also organizes the MT array in interphase [17]. In the fission yeasts, MTs are predominantly nucleated at the central nucleus, where they form short regions

of overlapping MTs that extend the plus ends towards the cell poles [58-60]. However, MTs can also be nucleated at cytoplasmic sites [61]. In a set of elegant experiments, Carazo-Sales et al. (2005) demonstrated that MTs in *S. pombe* slide along each other to focus near the cell center [17]. This MT transport is minus-end directed, which suggests that dynein or one of the two kinesin-14 motors (Pkl1 and Klp2) mediate this motility. During interphase, Pkl1 is located in the nucleus but concentrates in the spindle [37], pointing to a function in mitosis. By contrast, Klp2 is found along interphase MTs [31]; this motor is therefore a good candidate for the observed MT sliding in growing fission yeast cells. Indeed, mutant analysis revealed that Klp2 is responsible for MT transport [17]. This activity focuses the minus ends of MTs at the cell center, thereby polarizing the MT cytoskeleton, which is a prerequisite for polarized growth of *S. pombe* (see above).

3.2 Dynein transports assembled MTs along the cortex thereby polarizing the MT array

The site where MTs are nucleated usually determines the orientation of the MT array in the cell. In the corn smut fungus *Ustilago maydis*, MTs are nucleated at γ-tubulin-containing nucleation sites at the neck constriction between mother and daughter cell and consequently extend their plus ends towards the ends of the budding cell [10]. Surprisingly, assembled MTs move rapidly within the cell; therefore, this motility is likely based on motor activity [62]. It is well-known that in animal neurons, dynein powers MT motility, which organizes the unipolar MT array in the axon [6]. Indeed, a recent report demonstrates that cytoplasmic dynein has similar roles in *U. maydis* [19]. Dynein concentrates on the leading end of moving MTs, from where it gets "off-loaded" to anchorage sites and powers cortical sliding of the MTs. A similar "off-loading" mechanism was initially described in the yeast *S. cerevisiae*, where dynein mediates cortical MT sliding to pull the mitotic nucleus into the bud [47,48]. Localization studies with EB1 fused to red fluorescent protein demonstrated that moving MT structures often have two plus ends and a central spot of γ-tubulin [19]. This suggests that bipolar nucleation sites are transported in *U. maydis*. In dynein mutants both MT motility and polarization of the MT array is lost, which is most consistent with the idea that motor-dependent transport of MTs focuses cytoplasmic MT nucleation sites at the neck region. Furthermore, MT polarization is abolished when MTs are experimentally disrupted, which indicates that nucleation sites are anchored at

the neck by MT–MT interactions. This is most likely mediated by the cross-bridging activity of a yet unknown MT-associated protein. Surprisingly, kinesin-1 in *U. maydis* has such a cross-linking activity [18]. This is surprising because kinesin-1 in *U. maydis* and other fungi is implied in membrane traffic (summarized in Ref. [4]). Taken together, these results clearly demonstrate that in *U. maydis* molecular motors actively organize the MT array by transport of assembled MTs.

Concluding remarks and future perspectives

In particular, work on the model yeast *S. cerevisiae* demonstrated that MT motors support mitosis, whereas polarized growth involves myosins and F-actin. However, recent advances in fungal cell biological research changed this view. It now is evident that MTs and associated motors are crucial for polarized growth of the fission yeast *S. pombe*, and for hyphal growth of *A. nidulans* and *U. maydis*. In the filamentous fungi, MT motors support hyphal growth by transporting membranous cargo along MTs. In addition, recent work has shown that kinesins and dynein also actively participate in organizing their MT tracks in fungal cells. However, we are just beginning to understand the molecular mechanisms by which motors, and in particular kinesins, regulate MT stability and turnover. The emerging picture suggests that kinesin-7 and kinesin-1 motors participate in transport of regulatory compounds to MT plus ends, thereby affecting MT dynamics and organization (Fig. 4 a; *S. pombe* is depicted as a model for other fungi). Furthermore, kinesin-14 and kinesin-8 motors might directly affect the stability of MTs in mitosis and interphase (Fig. 4b), which in analogy to results from animal cell systems is achieved by direct modification of the plus-end conformation of the tubulin polymers. Finally, minus-end-directed motors (kinesin-14 and dynein) are able to transport assembled MTs to focus MT nucleation sites at certain regions of the cell, thereby polarizing the MT array (Fig. 4c). In the light of these results, it becomes evident that individual motors participate in numerous processes that are of key importance in organization and polarized growth of fungal cells. However, these results also raise new questions (Box 2). Among these is why yeast-like and filamentous fungi show significant differences in the use of motors. A good example is kinesin-1, which is not present in *S. cerevisiae*, but involved in hyphal growth, dynein targeting to plus-ends and MT organization in filamentous fungi. Whereas it is tempting

to speculate that these variations are a consequence of the different cellular dimensions and shaping of yeasts and filamentous fungi, solid evidence for such a causal relation remains to be provided. Thus some principles emerge, but much more work is needed in order to understand the functional repertoire of molecular motors in fungi. Moreover, almost nothing is known about the cargo specificity and regulation of motors. Considering the importance of motors in fungal cells, future research on these questions will not only provide insights into basic cell biological problems, but might also help to develop new strategies in fungicide development.

Acknowledgements

I wish to thank Petra Happel and Daniela Aßmann for technical support and Dr. Isabel Schuchardt for help with calculating the phylogenetic tree. Dr. Karen Brune is acknowledged for language corrections. I am grateful to Dr. Xin Xiang, her helpful comments greatly improved the manuscript. I apologize to those colleagues, whose work could not be cited due to space constrictions. Our work is supported by the Max-Planck Gesellschaft and the Deutsche Forschungsgemeinschaft.

Figure legends

Figure 1. Organization of molecular motors. The known motors can be classified into three major types: the MT-associated kinesins and dyneins and the actin-associated myosins. In most cases, motors consist of a homodimer of heavy chains (light colors) and a variable number of associate light chains that often have regulatory roles (dark colors). The heavy chain forms the globular motor domain, which binds microtubules (MT) or F-actin (microfilaments, MF). ATP cleavage leads to conformational changes in the two motor domains, which results in coordinated "walking" of the motor along the fibrous cytoskeleton. Note that myosin I and kinesin-3 motors are thought to be single-headed motors.

Figure 2. The biological role of fungal kinesins. Whereas the yeast fungi *S. cerevisiae* and *S. pombe* contain 6 and 8 kinesins, respectively, filamentous fungi such as *A. nidulans* and the dimorphic fungus *U. maydis* encode 10–11 kinesin motors belonging to 8 subfamilies. There is

experimental evidence for the cellular role of many motors (names shown in red). It turns out that members of subfamily kinesin-8, kinesin-14, and kinesin-5 are involved in mitosis, whereas kinesin-3 and kinesin-1 are predominantly organelle motors. However, members of almost all classes participate in the organization of microtubules (MTs; names shown in blue). Phylogenetic dendrograms were constructed as previously described [7,8]. Bootstrap values are indicated.

Figure 3. Summary of the current understanding of motor-based, plus-end targeting in fungi. *S. cerevisiae* uses kinesin-7 for plus-end targeting of the CLIP-170 homologue Bik1p, which binds Pac1 and subsequently the dynein complex. By contrast, filamentous fungi utilize kinesin-1 for delivery of dynein and dynactin to the MT plus end. Other compounds are depending on each other for plus-end localization. This includes NUDF, which is recruited by CLIPA and NUDE [45] or Pac1p that requires NudEp for plus-end localization [63]. However, many open questions remain to be answered (indicated by ?), e.g. whether Kip2p also participates in the delivery of components of the dynein complex to the MT tip [50]. Note that the genome of *U. maydis* contains a NUDE homologue, but nothing is known about its localization.

Figure 4. Mechanism by which motors organize MTs in *S. pombe*. Note that a direct action of Klp5 and Klp6 (kinesin-8) on MT plus ends is speculative. However, kinesin-8 motors share similar biological function and weak but significant sequence similarity with the KinI motors, which are known to destabilize MTs by direct modification of the MT plus end.

Figure I in Box 1. Dynamic instability of MTs. MTs are biopolymers that consist of tubulin dimers. During MT growth dimers assemble into protofilaments at the plus-ends. MTs usually switch between phases of growth and rapid shrinkage, a behavior known as dynamic instability. The transition from growth to disassembly is known as catastrophe, which can result in the disappearance of MTs or leads to a rescue, followed by another round of elongation. Note that dynamic instability is an intrinsic feature of the MT, which is modified by associated proteins in order to regulate MT length and dynamics in the living cell.

Table 1. Motor-based plus-end targeting of proteins

Cargo	Homologue	Organism	Motor	Type	Function at MT tip	Cell stage	Reference
Yeasts							
Dyn1p	dynein	*S. cerevisiae*	Kip2p*	Kinesin-7	Nuclear migration; modifies MT dynamics	Mitosis	[50,64]
Bik1p	CLIP-170	*S. cerevisiae*	Kip2p	Kinesin-7	Anchors Pac1 (=Lis1) and dynein; modifies MT dynamics	Mitosis	[49,50]
Tip1	CLIP-170	*S. pombe*	Tea2	Kinesin-7	Stabilizes MTs	Interphase	[22,44,56]
Tea1	-	*S. pombe*	Tea2	Kinesin-7	Marks cell ends	Interphase	[22,55]
Filamentous fungi							
NUDA	Dynein	*A. nidulans*	KINA	Kinesin-1	Retrograde traffic of organelles (?); nuclear migration; MT destabilization;	Interphase	[46,52,65]
NUDM	P150 *Glued* (Dynactin)	*A. nidulans*	KINA	Kinesin-1	Retrograde traffic of organelles (?); anchors dynein	Interphase	[52]
CLIPA	CLIP-170	*A. nidulans*	KIPA** KINA**	Kinesin-7 Kinesin-1	Promotes MT dynamics; anchors NUDE	Interphase	[45]
Dyn1	dynein	*U. maydis*	Kin1	Kinesin-1	Retrograde endosome traffic; nuclear migration; spindle elongation	Interphase	[12,51,66]
Dya1	P150 *Glued* (Dynactin)	*U. maydis*	Kin1	Kinesin-1	Anchors dynein; nuclear migration	Interphase	[12]

* Dyn1p localization depends on Kip2p, but a direct role of Kip2p in dynein transport is not shown;

** at 42°C, but not at 32°C, deletion of both motors significantly reduce plus end localization of CLIPA.

Box 1. Dynamic instability of microtubules

Microtubules (MTs) are essential compounds of the eukaryotic cytoskeleton. In mitosis, MTs form the mitotic spindle apparatus that mediates chromosome segregation. In interphase, they form long tracks that reach into the growth region. The MTs are used by molecular motors in long-distance transport of membranous organelles, vesicles, RNA, and protein complexes. Many cellular functions of MTs are based on their dynamic behavior[20]. MTs are formed by an assembly of α- and β-tubulin dimers at a nucleation site and elongate by addition of dimers at the fast-growing plus end. Purified tubulin dimers continuously assemble and MTs elongate until they stochastically switch to shrinkage, a transition termed catastrophe (Fig. I). Subsequent depolymerization at the plus end leads to rapid shortening of the MT, which either results in complete disappearance or in a switch back to MT elongation, termed rescue (Fig. I). This dynamic behavior or dynamic instability was initially described for MTs *in vitro* (summarized in Ref. [20]*)*. Theoretically, the length of the MTs is determined by these four parameters — elongation rate, shortening rate, catastrophe- and rescue frequency — and modification of each parameter allows the cell to control stability and turn-over of tubulin polymers, which in turn affects the intracellular transport processes along the MTs. Green fluorescent protein fused to tubulin allowed the observation of MTs in living fungal cells [59,62,64]. These studies confirmed that MTs undergo dynamic instability in living fungal cells, and it is now evident that proteins that specifically bind to the plus end of MTs regulate this behavior. Among these proteins are the CLIP-170 homologues that stabilize MTs by reducing all 4 parameters of dynamic instability in *Saccharomyces cerevisiae* (Bik1p [49]), or suppressing catastrophes in *S. pombe* (Tip1 [44]). In contrast to fission yeast, in *Aspergillus nidulans* CLIP-170 (CLIPA) promotes MT growth by doubling the rescue frequency [45]. In addition, Δ*clipA* mutants are dynamic [45], an effect that was also reported for Δ*clip1* mutants in *U. maydis* [12]. Surprisingly, minus-end-directed dynein motors and associated regulators, such as Lis1 and dynactin, are also concentrated at MT plus ends in fungi [12,46-48], where they apparently promote MT dynamics by affecting catastrophe and rescue rates in *S. cerevisiae, A. nidulans,* and *Ustilago maydis* [46,51,64]. Thus, control of MT dynamics is a complex and important process that is not yet fully understood.

Box 2. Outstanding questions

1. What is the exact molecular mechanism by which fungal kinesin-8 destabilizes MTs?
2. What regulates the assembly of +TIPs at the plus end of MTs?
3. How does the cell obtain spatial information in order to organize cytoplasmic microtubule organizing centers?
4. What is the role of uncharacterized orphan kinesins in filamentous fungi (see Fig. 2)?
5. What is the role of kinesin-7 in filamentous fungi?
6. How can a single motor participate in various cellular functions?

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Figure 1.
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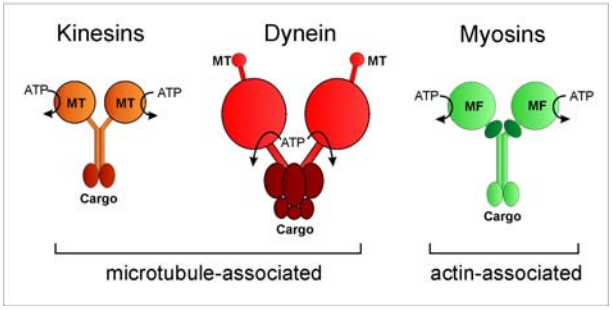

Figure 2
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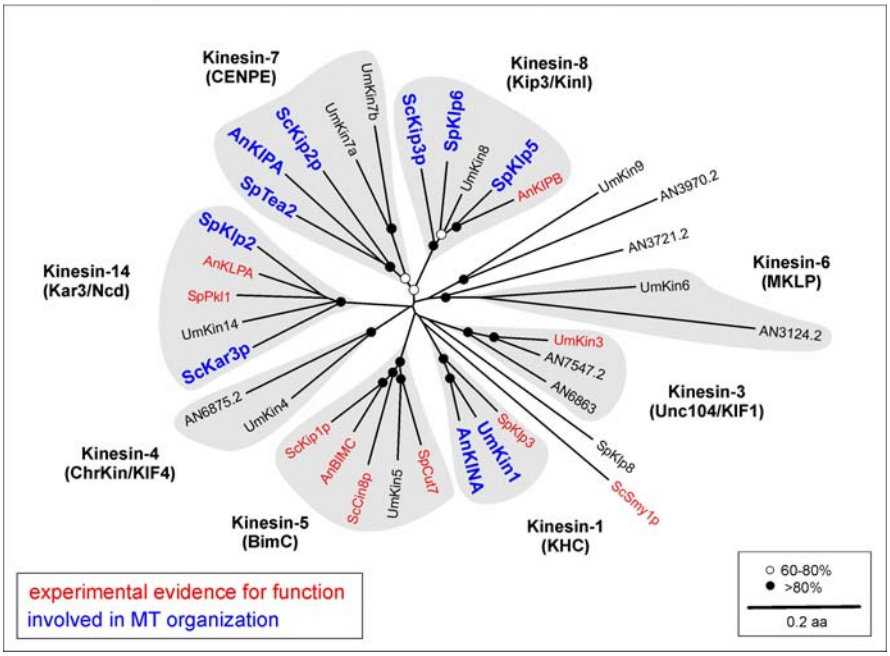

Figure 3
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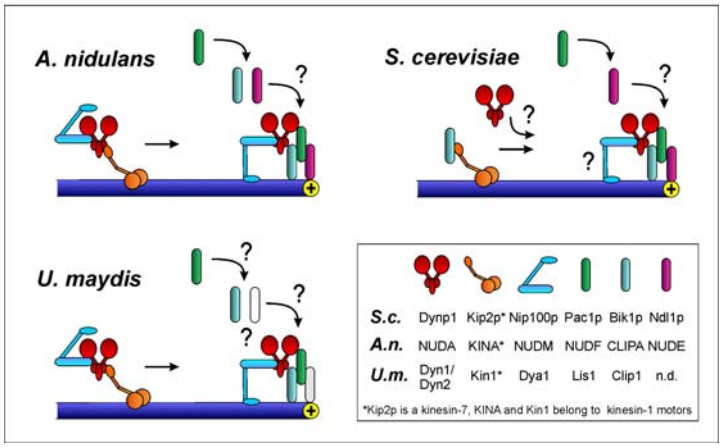

Figure 4
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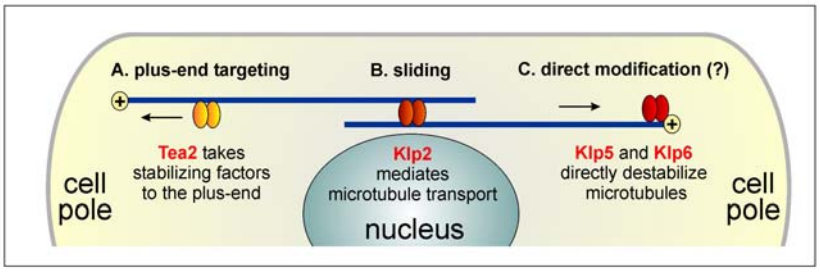

Fig I. Box1
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