Review TRENDS in Microbiology Vol.15 No.1

Preparing the way: fungal motors in microtubule organization

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Fungal growth, development and pathogenicity require hyphal tip growth, which 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 for motors in organizing their own tracks. These unexpected roles of the molecular motors are modifying microtubule dynamics directly, targeting stability-determining factors to microtubule plus ends, and transporting and arranging already-assembled microtubules.

Molecular motors in fungi

Fungi are a large and important group of microorganisms that have an enormous impact on the ecosystem and on human health, agriculture and industry (for an overview, see introduction in Ref. [1]). A hallmark of fungi is their ability to expand by polarized growth [2], and transport processes along the cytoskeleton are thought to have important roles in this process [3,4]. The fungal 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 three-dimensional 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 (Figure 1). The kinesin motors 'walk' towards the MT plus end, whereas the large dynein 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 have a central role in the organization and functioning of eukaryotic cells.

F-actin and associated myosin motors have long been known to be essential for fungal growth [7]. By contrast, the role of MTs in fungal growth has been a matter of debate and is currently under intense investigation. Two recent studies showed that intact MTs are required for the fast and extended hyphal growth of *Aspergillus nidulans* [8] and the plant pathogen *Ustilago maydis* [9]. In the dimorphic fungus *U. maydis,* live-cell imaging of a plus-end-binding EB1-like protein revealed that MT tips reach to the cell poles and the apex in yeast-like and hyphal cells, respectively, suggesting that MTs take part in polarized growth in both stages [10–12]. Consequently, plus-end-directed kinesins probably support hyphal tip growth. Published genomic sequences indicate that fungi contain a limited set of molecular motors: only four types of myosins, ~10–12 kinesins (Figure 2), and one 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 (Figure 2), where they are thought to support the 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 and kinesin-7), whereas the majority of kinesins are involved in nuclear migration, mitosis or meiosis (kinesin-5, kinesin-7, kinesin-8 and kinesin-14) and karyogamy (kinesin-14), which are often 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 the sliding of interphase MTs along each other [17], thereby helping to polarize the MT cytoskeleton. Furthermore, the organelle transporter kinesin-1 in *U. maydis* crosslinks MTs *in vivo* [18], which, in concert with dynein-dependent MT transport [19], might organize the interphase MT array (see later).

It is therefore 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 the organization and dynamics of the MT cytoskeleton. I suggest that motors exert their effect on MTs by three fundamentally different mechanisms: (i) directly modifying MT dynamics; (ii) targeting factors to MT plus ends; and (iii) transporting assembled MTs.

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Available online 28 November 2006.

 0966-842X/$ – see front matter doi:10.1016/j.tim.2006.11.007

Box 1. The dynamic instability of microtubules

Microtubules (MTs) are essential elements 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 the 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 (Figure I). Subsequent depolymerization at the plus end leads to a rapid shortening of the MT, which either results in their complete disappearance or in a switch back to MT elongation, termed rescue (Figure 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 four parameters: elongation rate, shortening rate, catastrophe frequency and rescue frequency; modifying the parameters enables the cell to control the stability and turnover of tubulin polymers, which in turn affects the intracellular transport processes along the MTs. Green fluorescent protein fused to tubulin enabled observation of MTs in living fungal cells [59,62,64,67]. 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 homologs that stabilize MTs by reducing all four parameters of dynamic instability in *Saccharomyces cerevisiae* (Bik1p [49]), or by suppressing catastrophes in *Schizosaccharomyces 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, MTs in Δ*clipA* mutants are less dynamic, a phenotype that was also reported in *Ustilago maydis* [12]. Surprisingly, minus-end-directed dynein motors 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 *U. maydis* [46,51,64]. Thus, the control of MT dynamics is a complex and important process that is not yet fully understood.

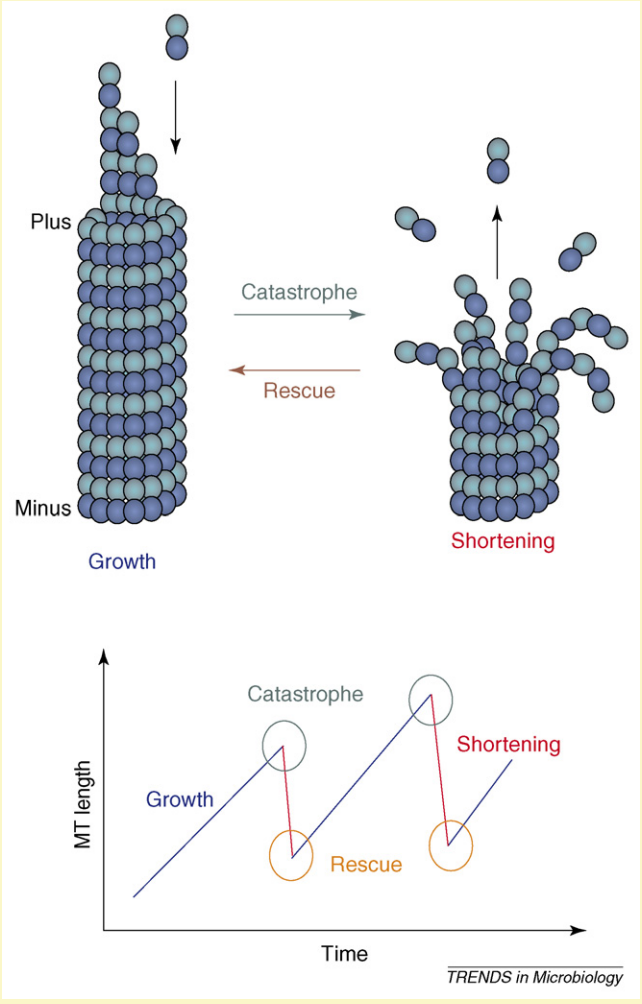

Figure I. The dynamic instability of MTs. MTs are biopolymers that consist of tubulin dimers. During MT growth, dimers assemble into protofilaments at the plus ends. MTs usually switch between phases of growth and rapid shrinkage, a behavior known as dynamic instability. The transition from growth to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

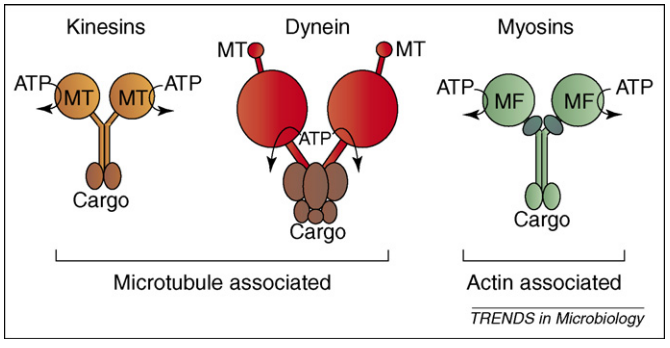

Figure 1. The organization of molecular motors. The known motors can be classified into three major types: the MT-associated kinesins and dyneins and the actin-associated myosins. In most cases, motors consist of a homodimer of heavy chains (light colors) and a variable number of associate light chains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

Kinesins modify MT dynamics

Mitotic roles of kinesin-14 and kinesin-8

MTs are dynamic polymers of αβ-tubulin heterodimers that grow and shrink by the 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 behaviour, and thereby the length and organization of MTs, is controlled by microtubule-associated proteins and plus-end tracking proteins (+TIPs) that 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 turnover [25]. The first indications for such a role for 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]. Deleting both

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

	Homolog	Organism	Motor	Type	Function at MT tip	Cell stage	Refs
Yeasts							
Dyn1p	Dynein	*S. cerevisiae*	Kip2p[a]	Kinesin-7	Nuclear migration; modifies MT dynamics	Mitosis	[50,64]
Bik1p	CLIP-170	*S. cerevisiae*	Kip2p	Kinesin-7	Anchors Pac1p (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[b] KINA[b]	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]

[a]Dyn1p localization depends on Kip2p, but a direct role for Kip2p in dynein transport is not shown.
[b]At 42 °C, but not at 32 °C, the deletion of both motors significantly reduces plus-end localization of CLIPA.

kip3 and *kar3* results in 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, by its depolymerization activity, supports nuclear migration shortly before karyogamy [28]. The destabilizing activity of fungal kinesin-8 might be of importance in pre-anaphase 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*. Deleting the genes encoding the kinesin-14 members results in longer anaphase spindles [31]. Kinesin-8 motors in *S. pombe* (Klp5 and Klp6; Figure 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 delayed in mitotic progression [35] and kinesin-14 mutants of these fungi show similar defects in spindle architecture [36,37].

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

How fungal kinesin-14 and kinesin-8 motors exert their destabilizing activity on MTs is an important question. 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-homolog 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* is a depolymerase that directly disassembles MTs [40,41]. However, Kip3p probably reaches the MT plus end by its own motor activity, and the combination of translocation activity and depolymerization activity clearly distinguishes fungal kinesin-8 from its animal counterpart kinesin-13.

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) [21]. In fungi, homologs 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 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 for kinesin motors in targeting some +TIPs to the MT ends in fungi (Table 1).

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

Genetic experiments in *S. cerevisiae* have demonstrated that deleting Kip2p (kinesin-7) decreases the number of cytoplasmic MTs, whereas overexpression results in much longer MTs [26]. How this control over MT dynamics is achieved has long been elusive. Important insights into this question have come from a study by Carvalho *et al.* [50], which convincingly demonstrated that Kip2p delivers the CLIP-170-like homolog Bik1p to the plus ends, where this +TIP participates in stabilizing MTs [49]. Bik1p also serves as an anchor for the putative dynein activator Pac1p, a member of the Lis1-like proteins, 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

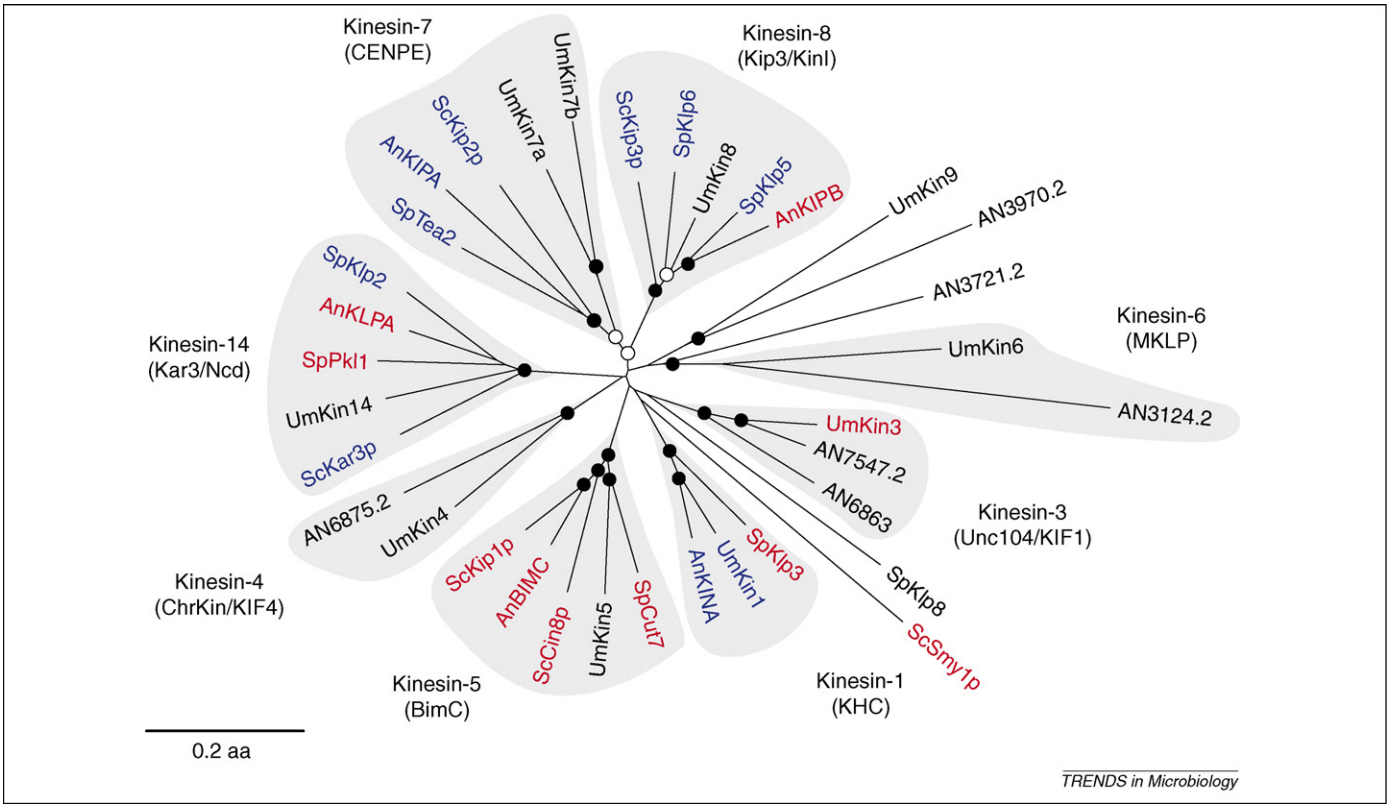

Figure 2. The biological role of fungal kinesins. Whereas the yeast fungi *Saccharomyces cerevisiae* (Sc) and *Schizosaccharomyces pombe* (Sp) contain six and eight kinesins, respectively, filamentous fungi such as *Aspergillus nidulans* (An or AN) and the dimorphic fungus *Ustilago maydis* (Um) encode 10–11 kinesin motors belonging to eight subfamilies. There is experimental evidence for the cellular role of many motors (names shown in red). Members of the subfamily kinesin-8, kinesin-14 and kinesin-5 are involved in mitosis, whereas members of 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 described previously [7,9]. Bootstrap values are indicated as 60–80% (open circles) and >80% (closed circles). Alternative names for kinesin subfamilies are given in brackets.

absence of dynein, MT dynamics are deregulated [46,51]. Despite the 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 required for dynein and dynactin targeting to plus ends, suggesting that this motor takes the dynein complex to the MT tip [12,52] (Figure 3). Consequently, *A. nidulans* with a mutation in *kinA*, the gene encoding kinesin-1 (Figure 2), shows defects in nuclear migration and has more stable MTs [53]. By contrast, CLIP-170 homologs seem to be targeted to the plus end of MTs by a motor-independent treadmilling mechanism [45], although deleting kinesin-7 affects plus-end targeting at a higher temperature, as does deleting kinesin-1 [45]. Furthermore, dynein localization at plus ends is independent of the CLIP-170 protein [12,45] (Figure 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*. The polarity of the cylindrical fission yeast cell depends on MTs because 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 Tea2, a kinesin-7 (Figure 2) that takes Tea1 and the CLIP-170-like Tip1p to MT plus ends [22,56]. Tip1 and the EB1 homolog Mal3 promote the continuous growth of the MT until it reaches the cell end [43], where it undergoes catastrophe, that is, 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.

The role of kinesin-7 in filamentous fungi is elusive

Whereas the studies in the yeasts *S. cerevisiae* and *S. pombe* indicate a general role of kinesin-7 motors in targeting +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; Figure 2) has a less noticeable role in targeting the CLIP-170 homolog CLIPA, which is only evident in Δ*kipA* mutants at a 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 the proper organization of the hyphal growth machinery. However, such a role for kinesin-7 in hyphal growth might not be common among fungi. The basidiomycete *U. maydis* contains two kinesin-7 members (Figure 2), but neither Δ*kin7a* or Δ*kin7b* single mutants

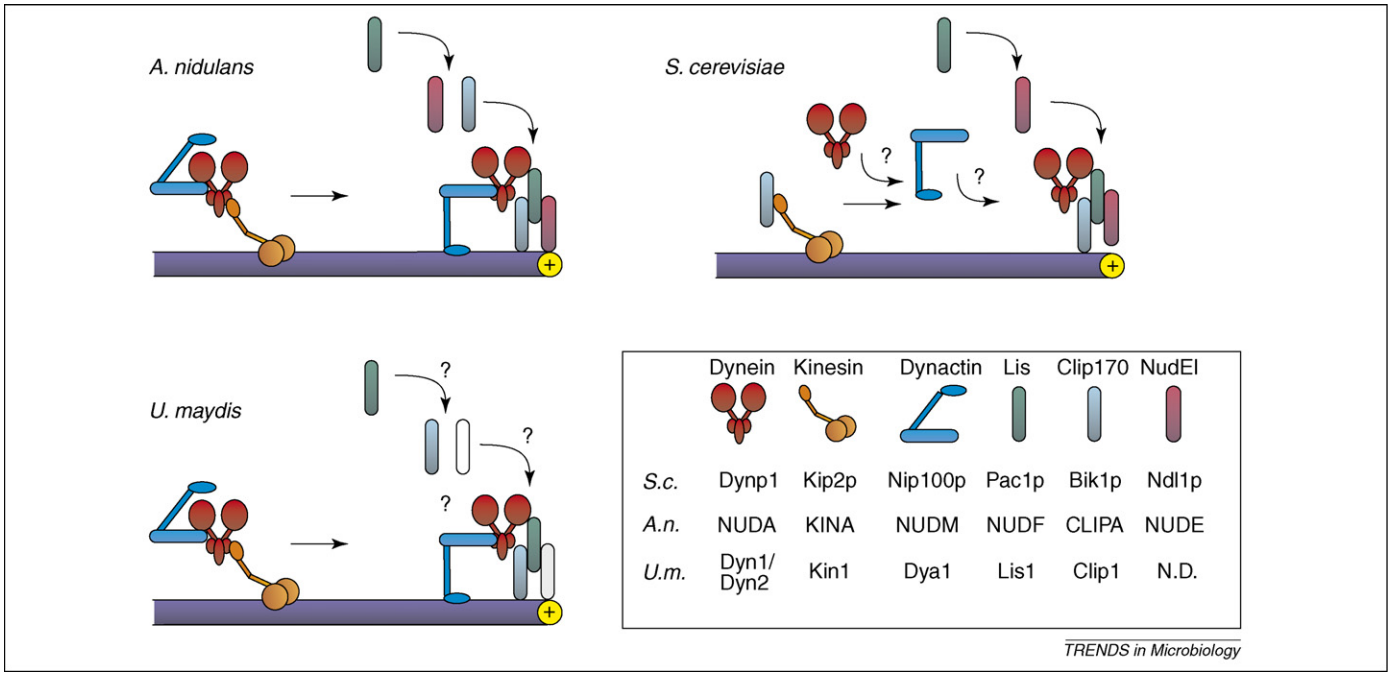

Figure 3. A summary of the current understanding of motor-based, plus-end targeting of the dynein transport machinery in fungi. *Saccharomyces cerevisiae* uses kinesin-7 (Kip2p) for plus-end targeting of the CLIP-170 homolog Bik1p, which binds to Pac1p. Pac1p subsequently anchors the dynein complex. By contrast, filamentous fungi utilize kinesin-1 for delivering dynein and dynactin to the MT plus end. Other compounds depend on each other for plus-end localization, including NUDF, which is recruited by CLIPA and NUDE [45], or Pac1p, which requires Ndl1p for plus-end localization. Note that Bik1 is crucial for targeting of dynein in *S. cerevisiae*, whereas in *Aspergillus nidulans*, CLIPA cooperates with NUDE in recruitment of NUDF and dynein [63]. Furthermore, in *S. cerevisiae*, deletion of dynactin increases dynein at plus ends [47,48], whereas it is released from MT tips in dynactin mutants in *A. nidulans* [52] and *Ustilago maydis* [12]. However, many open questions remain (indicated by ?), for example, whether Kip2p also participates in the delivery of components of the dynein complex to the MT tip [50]. The genome of *U. maydis* contains a NUDE homolog but nothing is known about its localization. Kip2p is a kinesin-7; KINA and Kin1 belong to the kinesin-1 motor family. Abbreviation: N.D., not determined.

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, as yet uncharacterized kinesins is presently not known.

Kinesin-14 motors organize the cytoskeleton by transporting assembled MTs

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, fungal kinesin-14 can bundle MTs [57], which is mediated by an additional MT-binding site in their tail domain. Owing to these unique features, kinesin-14 can cross-bridge and actively organize MTs by sliding them 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 organize 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.* [17] demonstrated that MTs in *S. pombe* slide along each other to focus near the cell center. 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 the polarized growth of *S. pombe*.

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 *U. 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; this motility is therefore probably 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

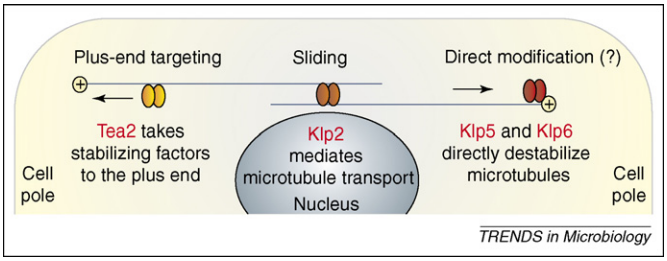

Figure 4. The mechanism by which motors organize MTs in *Schizosaccharomyces pombe*. Kinesin-7 motors (Tea2) participate in the transport of stabilizing factors to the plus end in *S. pombe*. The direct action of Klp5 and Klp6 (kinesin-8) on MT plus ends is speculative. However, kinesin-8 motors share a similar biological function and a weak but significant sequence similarity with the KinI motors, which are known to destabilize MTs by directly modifying the MT plus end. Kinesin-14 (Klp2) cross-links MTs and mediates sliding, which helps to polarize the MT array.

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 consistent with the idea that the 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. These interactions are probably mediated by the cross-bridging activity of an as yet unknown MT-associated protein. Kinesin-1 in *U. maydis* has such a crosslinking activity [18], which is surprising because kinesin-1 in *U. maydis* and other fungi is implied in membrane traffic (summarized in Ref. [4]). Taken together, these results demonstrate that in *U. maydis* molecular motors actively organize the MT array by transporting assembled MTs.

Concluding remarks and future perspectives

Early work on the model yeast *S. cerevisiae* had demonstrated that MT motors support mitosis, whereas polarized growth involved myosins and F-actin, suggesting that MTs are not involved in fungal growth. However, recent advances in fungal cell biological research changed this view. It now is evident that MTs and associated motors are crucial for the polarized growth of the fission yeast *S. pombe*, and for the 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 the transport of regulatory compounds to MT plus ends, thereby affecting MT dynamics and organization (Figure 4; *S. pombe* is shown as a model for other fungi but note that kinesin-1 is not involved in this species). Furthermore, kinesin-14 and kinesin-8 motors might directly affect the stability of MTs in mitosis and interphase (Figure 4), which in analogy to results from animal cell systems is achieved by directly modifying the plus-end conformation of the tubulin polymers. Finally, minus-end-directed motors (kinesin-14 and dynein) can transport assembled MTs to focus MT nucleation sites at certain regions of the cell, thereby polarizing the MT array (Figure 4). In the light of these results, it becomes evident that individual motors participate in numerous processes that are of key importance in the 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 is 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 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.

Box 2. Outstanding questions

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

Update

Two recent reports demonstrate that Klp2-based self-organization of the MT array in fission yeast happens even in the absence of nuclei and their associated microtubule-organizing centers [68,69], which further emphasizes the importance of motor activity in organizing MTs in fungi.

Acknowledgements

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

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