Cytoskeleton and motor proteins in filamentous fungi

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In filamentous fungi, the actin cytoskeleton is required for polarity establishment and maintenance at hyphal tips and for formation of a contractile ring at sites of septation. Recently, formins have been identified as Arp (actin-related protein) 2/3-independent nucleators of actin polymerization, and filamentous fungi contain a single formin that localizes to both sites. Work on cytoplasmic dynein and members of the kinesin and myosin families of motors has continued to reveal new information regarding the function and regulation of motors as well as demonstrate the importance of microtubules in the long-distance transport of vesicles/organelles in the filamentous fungi.

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Current Opinion in Microbiology 2003, **6**:628–633

This review comes from a themed issue on
Growth and development
Edited by Claudio Scazzocchio and Jeff Errington

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2003.10.009

Abbreviations

Arp	actin-related protein
KINA	kinesin of *Aspergillus nidulans*
nud	nuclear distribution
SPB	spindle pole body

Introduction

Filamentous fungi grow in a highly polarized fashion to form extremely elongated hyphae. How cytoskeletal elements are organized to support hyphal growth and organelle distribution in hyphal compartments is a question being addressed in various fungal systems. Our current understanding of cytoskeletal organization during polarized cell growth has benefited greatly from studies on the budding and the fission yeasts [1]. However, the growth pattern of filamentous fungi differs substantially from those of budding or fission yeasts, and current studies indicate interesting and important differences in cytoskeletal organization and function. In this review, we discuss recent studies using filamentous fungi that focus on the actin and microtubule cytoskeletons and the motor proteins. Although intermediate filaments and septins have also been studied in filamentous fungi, information concerning these cytoskeletal elements has been presented elsewhere [2••,3•].

Cytoskeleton

Actin

Actin microfilaments are required in fungi for organelle movement, growth polarity establishment/maintenance and septation (i.e. cytokinesis). In filamentous fungi, filamentous actin is organized as patches that localize to actively growing or emerging hyphal tips and at sites of septation. The distinctive actin cables observed in yeast are not observed in the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa*; however, actin filaments are detected in the cytoplasm and as contractile rings at sites of septation. The dimorphic basidiomycete fungus *Ustilago maydis* is similar to yeast in that the actin cytoskeleton consists of both cables and patches that orientate toward the bud site and patches [4•].

Formins are actin nucleation factors in eukaryotic cells. It has been found in yeasts that formins are required for actin cable assembly and maintenance independently of the Arp2/3 complex [1]. Interestingly, whereas *Saccharomyces cerevisiae* encodes two distinct formins and *Schizosaccharomyces pombe* encodes three formins, *A. nidulans* and *N. crassa* encode a single formin. The *A. nidulans* formin, SEPA, localizes to both septation sites and hyphal tips, suggesting that filamentous fungi use site-specific regulatory mechanisms to control formin-mediated actin polymerization [5••]. Recently, a large-scale screen for morphogenesis mutants was conducted using *N. crassa* and, as expected, some of the mutants define genes involved in regulation of the actin cytoskeleton [6••]. The Rho-type GTPases (Rho1-4 and CDC42) that regulate the actin cytoskeleton and other aspects of polarized growth has been studied in *A. gossypii* and other filamentous fungi, and most of these studies have been covered in a previous review [7].

Microtubules

The microtubule cytoskeleton is essential for spindle assembly and function, and in many eukaryotes, is also required for transport of various organelles/cargoes and the maintenance of growth polarity. Interestingly, there is species-specific variation in organelle transport mechanisms as mitochondria travel along actin tracks in budding yeast and some filamentous fungi, but in *N. crassa*, their movement is dependent on microtubules [8•].

In both the yeasts and filamentous fungi, a nuclear-membrane-embedded structure known as the spindle pole body (SPB) acts as the microtubule-organizing

centre. SPBs contain γ-tubulin; a specialized universal tubulin isoform in eukaryotic cells that was first discovered in *A. nidulans* and is required for nucleation of microtubule polymerization. Mutational analysis of *A. nidulans* γ-tubulin suggests that it also carries out functions essential to mitosis and the organization of cytoplasmic microtubules [9]. In *A. nidulans* and *N. crassa*, it appears that all microtubule nucleation occurs at nuclear-associated SPBs. However, in *U. maydis*, microtubule nucleation occurs at both nuclear and non-nuclear organizing centers and is regulated in a cell-cycle-dependent manner, indicating that there is significant flexibility in the ability of fungi to spatially regulate the formation of microtubules [10••].

In most fungi, microtubules are found as part of intranuclear spindles and as tracks within the cytoplasm. Astral microtubules can be seen emanating from the poles of elongated mitotic spindles. In *A. nidulans*, where mitosis does not require the breakdown of the nuclear envelope, tubulins are found to enter the nucleus before mitotic spindle formation, and leave the nucleus during M to G1 transition, suggesting that regulation of the intranuclear level of tubulins and other proteins may be important for mitotic onset in fungi with intranuclear mitosis [11••].

Motor proteins

Cytoplasmic dynein

Cytoplasmic dynein, a multi-subunit complex, is a minus-end-directed microtubule motor. In filamentous fungi, loss of cytoplasmic dynein function causes a nuclear distribution defect [12]. Although the exact mechanism(s) controlling dynein-mediated nuclear positioning remain unclear, evidence suggests that the dynamic status of microtubules is important for nuclear positioning in filamentous fungi. Less dynamic or longer microtubules have been observed in dynein mutants [13,14,15••]. This may be at least partially responsible for the nuclear migration defect as the microtubule-destabilizing drug benomyl can partially suppress the nuclear migration defect in *A. nidulans* and completely suppress the defect in *Ashbya gossypii* [16,17]. In *Nectria haematococca*, dynein is also important for anchoring interphase nuclei along the hyphae, for astral microtubule formation and for anaphase B spindle elongation [18,19].

Filamentous fungi also use dynein for retrograde transport of vesicles and organelles [20]. In *U. maydis*, dynein is important for endoplasmic reticulum (ER) organization and for endosome positioning [21•,22••]. *N. crassa* dynein mutants show defects in the organization and stability of the Spitzenkörper, an aggregation of apical vesicles that has been implicated in supporting hyphal growth [23•]. In *A. nidulans*, dynein loss-of-function also causes abnormal positioning of septa [24].

Many mutants in the cytoplasmic dynein pathway have been isolated as *nud* (nuclear distribution) mutants in *A. nidulans* and as *ropy* mutants in *N. crassa* [12]. Cloning of the *nud* and *ropy* genes in *A. nidulans* and *N. crassa* identified many components of the cytoplasmic dynein complex and the dynactin complex, a complex that is involved in dynein–cargo interaction and motor activity (Table 1). Genes encoding dynein regulators that were not identified initially as components of the dynein and

Table 1

Motor proteins in filamentous fungi.

Family/class	Possible functions
Proteins in the cytoplasmic dynein pathway [12,51–54]	
1. Components of the cytoplasmic dynein complex	
Heavy chain	Nuclear positioning
Intermediate chain	Mitosis, retrograde, vesicle transport
Light intermediate chain	
Light chain, LC8	
Light chain, roadblock/LC7	
Light chain, Tctex-1	
2. Components of the dynactin complex	
p150Glued	Regulation of dynein–cargo interactions, dynein motor activity and processivity
p62	
Arp11	
P50/dynamitin	
Arp1	
p27	
p25	
p24	
3. LIS1 and its interacting proteins	
LIS1	Regulation of dynein activity
NUDE/RO11	
NUDC	
Kinesins [32••]	
Conventional kinesin/KHC	Vesicle/organelle transport, nuclear positioning
Unc104/KIF1 (a long and a short version)	Vesicle/organelle transport
Chromokinesin/KIF4	Vesicle/organelle transport, DNA binding
BimC	Spindle assembly
C-terminal motor	Spindle assembly
Kip2/CENP-E	Microtubule stabilizing, kinetochore binding
Kip3	Microtubule dynamics
KID	Chromosome movement to the metaphase plate
MKLP1	Spindle midzone organization and cytokinesis
Myosins [45•,46,47,48••,49•]	
Class I	Actin organization, endocytosis
Class II	Actin organization, cytokinesis
Class V	Vesicle/organelle transport
Chitin-synthase-myosin fusion protein	Polarized cell wall synthesis

dynactin complexes have also been isolated, including NUDF/LIS1, NUDE/RO11 and NUDC (Table 1) [12]. A recent analysis of the annotated *N. crassa* genome indicates that the dynein/dynactin complexes of filamentous fungi are more similar to that of mammals than they are to that of yeasts (S Seiler, M Plamann, unpublished data).

The availability of the large number of mutants in the dynein pathway makes filamentous fungi good systems to use to study how cytoplasmic dynein is regulated *in vivo*. In *A. nidulans*, dynein, dynactin, NUDF/LIS1 and NUDE/RO11 all form comet-like structures at the plus-ends of microtubules, a site implicated in microtubule–cortex interaction and in dynein cargo loading [25••,26••]. A similar dynein/dynactin localization pattern has also been observed in *N. crassa* [27]. Interestingly, dynein comets in the *nudF* and *nudE/ro-11* loss-of-function mutants are more prominent relative to the wild-type, suggesting that these proteins may be required for activating dynein-mediated transport. It has not yet been determined whether cargo binding is a prerequisite for dynein motor activation. The dynactin complex is important for dynein–membrane interaction, and the pointed-end proteins of the dynactin complex, such as Arp11, p62 and p25 in *N. crassa*, may be important in modulating the structure of the dynactin complex in some way to allow recycling of the motor from membranous cargoes [28]. The carboxyl terminus of the p150 dynactin is also involved in regulating cargo binding [29]. It has been shown in *N. crassa* that dynactin is important in regulating dynein ATPase activity via phosphorylation of putative dynein light chains [30]. Interestingly, the p25 null mutant (Δ*ro*-12) has a significantly lower dynein ATPase activity than the wild-type, but does not show a nuclear migration defect, suggesting that a higher ATPase activity may be needed for vesicle traffic rather than for nuclear migration [28]. Additional information regarding proteins in the dynein pathway has been obtained using genetic approaches. Interestingly, the *A. nidulans* 8 kDa dynein light chain is only essential for dynein function at high temperatures [15••]. Overproduction of NUDF inhibits the growth of all the tested mutants of *apsA*, which encodes a cortical protein required for nuclear migration during asexual spore development [26••,31].

Kinesins

In filamentous fungi, members of the kinesin superfamily of microtubule-associated motors are not only involved in spindle formation and function, but are also important for long-distance transport of organelles and vesicles. Analysis of fungal genomes indicates that there are at least 10 distinct kinesins in filamentous fungi (Table 1), and several of these motors are not found in yeasts [32••]. Conventional kinesin has been defined as the founding member of the kinesin superfamily, and the conventional kinesin of filamentous fungi shows sequence similarity to, and has the same domain organization as, conventional kinesins from higher eukaryotes. The *N. crassa* conventional kinesin (Nkin or NcKin) was the first isolated and has been the most extensively studied [33]. Although most fungal conventional kinesins are involved in polarized growth and secretion, some of them are also involved in vacuole organization and mitochondria transport [34–37]. The conventional kinesin of *A. nidulans* (KINA) is partially required for nuclear positioning [38]. Its mutant phenotype is suppressed by conditions that destabilize microtubules, suggesting that KINA is also involved in regulating microtubule dynamics [38]. Interestingly, the localization of dynein and dynactin at plus-ends of microtubules is significantly diminished in a *kinA* deletion mutant, suggesting that KINA may transport dynein/dynactin to the plus ends of microtubules [25••].

Fungal kinesins show interesting differences in composition, structure and properties relative to conventional kinesins of higher eukaryotes. For example, the fungal kinesins apparently lack light chains that are typically part of conventional kinesin of higher eukaryotes [33]. Fungal kinesins are also about four times faster in *in vitro* motility assays and show greater processivity when compared to human conventional kinesin [33,39•]. Structural analysis of the *N. crassa* fast kinesin revealed a nucleotide-binding pocket that is more open [40]. In addition, Nckin shows interaction with not only the β-tubulin but also the α-tubulin of microtubules [40]. These features may allow the fungal kinesin to have a higher ATP turn-over rate. Studies have also shown that the fungal kinesin has a special neck domain directly adjacent to the motor domain. The presence of the neck region together with its adjacent motor domain containing the head and the neck-linker regions is not sufficient for dimerization, which is different from the case in higher eukaryotes [41]. A conserved tyrosine in the neck domain may directly interact with the head domain to negatively regulate its ATPase activity [42••].

Besides conventional kinesins, the previously identified BIMC (blocked in mitosis C) and KLPA (kinesin-like protein A) kinesins have been found to play mitotic roles in filamentous fungi and many other organisms [43,44]. In *U. maydis*, endosome positioning depends on balanced forces between cytoplasmic dynein and Kin3, a kinesin that belongs to the Unc-104 class [22••].

Myosins

The genomes of *N. crassa* and *A. nidulans* encode one class I myosin (single-headed motor), one class II myosin (two-headed motor implicated in actin-filament sliding), one class V myosin (two-headed motor implicated in vesicle transport), and contain an interesting filamentous fungus-specific gene, *csmA*, encoding a myosin motor domain at the amino terminus and a chitin synthase domain at its carboxyl terminus (Table 1) [45•]. The class I myosin MYOA from *A. nidulans* localizes to hyphal tips, is essential

for initiating polarized growth, and is also involved in endocytosis [46]. Interestingly, a *myoA* mutant that contains only 1% of its normal actin-activated ATPase activity and has no detectable *in vitro* motility can support polarized growth, suggesting that MYOA's role may be primarily structural [47]. In the dimorphic pathogen *Candida albicans*, the class I myosin CaMyo5 is also required for hyphal formation, and the null mutant forms random buds [48••]. Interestingly, a CaMyo5 mutant with depolarized actin patches still undergoes hyphal growth, suggesting that the polarized distribution of actin patches is not essential for polarized growth [48••]. The class V myosin of *C. albicans* (CaMYO2) is not essential for viability; however, germ tube formation and nuclear distribution are affected in the deletion mutant [49•].

Conclusions

The genetic tractability of filamentous fungi has made them excellent systems to study the function and regulation of the cytoskeleton and motor proteins. The recent availability of fungal genomes has revealed that many components of the cytoskeleton, including the cytoplasmic dynein pathway and the kinesin superfamily, are more closely related to those of higher eukaryotes than to those of the yeasts. These observations support experimental evidence that indicate that, in filamentous fungi, microtubules support long-distance-transport functions, whereas actin microfilaments are required for localized targeting events. Future studies are needed to further define specific cargoes for each motor, and to address the interaction between the microtubule and the actin cytoskeleton for coordinated intracellular transport.

Update

Osmani and co-workers have recently published the characterization of a protein that interacts with the NIMA (never in mitosis A) kinase in *A. nidulans*, and this protein, named TINA (two-hybrid interactors of NIMA A), is involved in the control of astral microtubule formation during mitosis [50••].

Acknowledgements

We thank Stephan Seiler, Greg May, Bo Liu and Reinhard Fischer for helpful genome information on motor proteins. We thank the National Science Foundation (MCB-0235871 to MP and MCB-0093106 to XX), the University of Missouri Research Board (research grant 340975 to MP) and Uniformed Services University of the Health Sciences (intramural grant R071GO to XX) for support.

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The *A. nidulans* septin AspB localizes post-mitotically to the septation site before the cross-wall is visible, and this localization is actin-dependent. More interestingly, AspB is the only known branch site marker because it localizes premitotically as a ring at sites of branching and secondary germ-tube emergence. AspB is also important for conidiophore development.

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This work represents the first characterization of an intermediate filament gene in filamentous fungi. The *A. nidulans mbmB* gene product, a homologue of the *S. cerevisiae mdm*10 gene product, co-localizes with mitochondria. The deletion of *mdmB* affects mitochondrial morphology and distribution at a low temperature of 20 °C, but not at 37 °C.

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During budding in *U. maydis*, actin patches and cables polarize to the presumptive bud site and the bud tip, and an actin ring forms at the neck region. Analysis of the microtubule cytoskeleton suggests that astral microtubules may be involved in the early stages of spindle orientation and nuclear migration into the bud.

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The *A. nidulans* multidomain formin SEPA is required for the formation of actin rings at septation sites. The SEPA–GFP fusion protein co-localizes with actin at both hyphal tips and septation sites, and the amino-terminal half of SEPA is sufficient for localization. The localization at both sites is dynamic. At septation sites, SEPA forms a ring that constricts as the septum is deposited. Actin filaments are required to maintain the proper localization pattern of SEPA.

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In *U. maydis*, microtubules are organized in a cell-cycle-dependent fashion. During G1 and S phase, multiple cytoplasmic microtubule nucleating centers are present. At the early budding G2 phase, a polar microtubule-organizing centre at the bud neck is active. The SPB is inactive in G2 phase, but continuously recruits gamma-tubulin and becomes active in mitosis. Depletion or overexpression of gamma-tubulin affects microtubule number in interphase cells.

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In *A. nidulans*, tubulin is substantially excluded from interphase nuclei, but is present in mitotic nuclei. Observation of a GFP-tubulin fusion indicates that tubulin levels are low in interphase nuclei and there is a rapid movement of tubulin into the nucleoplasm seconds before spindle formation begins. This may be important for mitotic onset in fungi that have closed mitosis.

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In *A. nidulans*, the highly conserved 8 kDa dynein light chain (NUDG) is only required for dynein function under certain conditions. Although the heavy chain motor is required for normal patterns of growth, nuclear migration, conidiation and septation at all tested temperatures, *nudG* deletion affects these patterns only at a high temperature. In addition, *nudG* deletion affects the localization of dynein to the microtubule plus ends near the hyphal tip and to the septa only at a high temperature.

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Cytoplasmic dynein and an UNC-104/KIF1-like kinesin, Kin3, counteract on the endosomes to arrange them in *U. maydis*. During early bud formation, dynein is important for endosome movement towards the growth region where a microtubule-organizing site is located. The Kin3 motor localizes to endosomes and is important for endosome transport towards the distal pole of medium-sized bud where the growing ends (most likely the plus ends) of microtubules are located. Deletion of *kin3* abolishes endosome clustering at the distal cell pole and at septa, and causes defects in budding patterns and septation.

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In a *N. crassa ro-1* dynein heavy-chain mutant, cytoplasmic regions are not as organized as in wild-type cells, and the motility and/or positioning of vesicles, mitochondria, and nuclei are defective. The microtubule cytoskeleton is also severely disrupted. The hyphal tip region of the dynein mutant contains a Spitzenkörper with a defined central core but with reduced numbers of apical vesicles.

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Dynein and dynactin's plus-end localization is significantly diminished in a deletion mutant of KINA, a conventional kinesin in *A. nidulans*. Interestingly, NUDF/LIS1's plus-end localization is not dramatically affected in the *kinA* mutant. Moreover, deletion of NUDF increases the plus-end accumulation of cytoplasmic dynein and dynactin. One hypothesis is that cytoplasmic dynein and dynactin are transported by KINA to the microtubule plus end where cytoplasmic dynein is activated by NUDF/LIS1 to gain the full motor activity needed for its departure from the plus end towards the minus end.

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New information is presented regarding the function of NUDF/LIS1 and its interacting protein, NUDE, in the cytoplasmic dynein pathway. Similar to GFP-labeled NUDF and dynein, GFP–NUDE forms comet-like structures. Interestingly, the non-functional carboxy-terminal NUDE, but not the functional amino terminus, is sufficient for this localization pattern. Overexpression of NUDF completely suppresses the phenotype of a NUDE deletion, suggesting that the function of NUDF is more directly involved in dynein function and the function of NUDE is secondary to that of NUDF. NUDF may be involved in dynein–cortex interaction because NUDF overexpression significantly inhibits the growth of the mutants of the *apsA* gene that encodes a cortical protein homologous to the yeast NUM1 protein interacting with dynein.

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