The cellular roles of molecular motors in fungi

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Motors are molecular machines that move their cargo along F-actin or microtubules. Fungal representatives of myosin, kinesin and dynein motors support many cellular processes including polar growth, cell division and mitosis. Recent progress in understanding their cellular roles has revealed common principles. However, it has become obvious that fungi have also developed diverse strategies to cope with long-distance organelle transport.

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Organelle transport, chromosome segregation and cytokinesis have fascinated biologists for decades. Over the past 20 years, intensive research on the molecular basis of motility has identified the mechanochemical enzymes responsible for intracellular movements along the cytoskeleton. These molecular motors are grouped into three families: microtubule-based kinesins and dyneins, and F-actin-associated myosins. Motors are large protein complexes commonly found in eukaryotic cells, and it has been speculated that they might have prokaryotic counterparts, such as MukB from *Escherichia coli*. MukB was recently shown to bind to bacterial FtsZ, a possible tubulin homologue, as well as to vertebrate microtubules[1,2].

Most motors comprise two chains known as heavy chains that dimerize and form globular motor 'heads', containing the enzymatic activity and a binding site for their cytoskeletal 'tracks' (Fig. 1). They hydrolyze ATP to move their 'cargo' unidirectionally along microtubules or F-actin. The direction of transport along microtubules depends on the polymerization activity of these dynamic polymers. With some exceptions, kinesins move towards the 'plus' end (high polymerization rates), whereas dynein is 'minus'-end (low polymerization rate) directed. Alternatively, some motors modify the dynamic properties of microtubules by altering polymerization activity at the end of the polymer (Box 1; Fig. 2).

Based on sequence similarity in the motor region, several classes of myosins, kinesins and dyneins have been identified in systems ranging from vertebrates to fungi. Although fungi and higher eukaryotes have motor classes in common, to date only a few classes have been identified in fungi. Genome sequencing of *Saccharomyces cerevisiae* has revealed genes encoding five myosins, six kinesins and one dynein[3], whereas in some vertebrate systems the identification of many kinesins, myosins and dyneins could raise the number of different motors to 50 per cell. It is thus not surprising that, in fungi, a single motor participates in multiple cellular processes. Moreover, some motors appear to have adapted new functions, as indicated by Smy1p, a kinesin-like protein from *S. cerevisiae*. This putative kinesin was identified as a multicopy suppressor of myosin[4]. However, as it neither hydrolyzes ATP nor functions along microtubules[5], it is unlikely that Smy1p functions as a kinesin motor, and its cellular role remains to be clarified.

Motor-mediated intracellular transport of organelles along cytoskeletal tracks is of central importance for the spatial organization, growth, and cell division of yeasts and filamentous fungi[3]. Additionally, endocytic membrane traffic adds to intracellular vesicle transport in fungi[3,67] (Fig. 3). At present, fungal motors are known to be involved in (1) secretion and endocytosis, (2) cell divison, (3) organelle positioning and inheritance, (4) mitosis and, unexpectedly, (5) genetic recombination and RNA transport. Although this review will deal with fungi as a homogenous group, it is important to note that some aspects of intracellular transport are extremely diverse. For example, in *S. cerevisiae*, transport of mitochondria, secretory vesicles and vacuoles is based mainly on F-actin and its associated myosin motors[3], whereas in the fission yeast *Schizosaccharomyces pombe*, microtubules are involved in many aspects of intracellular membrane traffic[6,7]. It is the aim of this short review to reflect this diversity as well as to outline some general principles of motor function in yeasts and filamentous fungi. Owing to space limitations, recent progress on the regulation of fungal motors will not be discussed.

Fungal motors in cytokinesis, exocytosis and endocytosis

Cell separation during cytokinesis requires conventional (class II) myosins. Initially identified in skeletal muscle, class II myosins are found in 'lower' eukaryotes such as *S. cerevisiae* and *S. pombe*[8–11]. In these cells, class II myosin assembles into a ring-like structure at the cell cleavage plane[9,12], where it interacts with F-actin and supports cytokinesis.

The cell wall determines the shape of the cell and is synthesized at polar growth sites during cell expansion. Directed intracellular transport enables the cell

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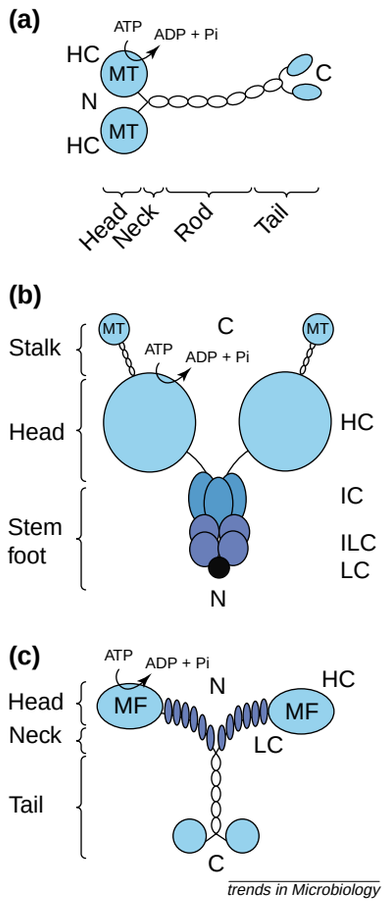

Fig. 1. Schematic drawing of **(a)** fungal kinesins, **(b)** dyneins and **(c)** myosins. The figures are based on electron microscopy studies on native proteins from animal sources, and biochemical data and sequence predictions for fungal motors. **(a)** Biochemical data indicate that conventional kinesins from fungi comprise a homodimer of two heavy chains (~105 kDa) that provides the enzymatic activity. Interestingly, no evidence for carboxy-terminal light chains that are typical for kinesins from animals has been found[28,33,64]. **(b)** The dynein complex contains two heavy chains of ~400 kDa, which probably bind associated polypeptides[65]. **(c)** The heavy chain (~180 kDa) of Myo2p, a class V myosin from *Saccharomyces cerevisiae* provides six IQ motifs (filled ovals), which are putative binding sites for a recently identified light chain[66]. Abbreviations: C, carboxyl terminus; HC, heavy chain; IC, intermediate chain; ILC, intermediate light chain; LC, light chain; MF, F-actin-binding site; MT, microtubule-binding site; N, amino terminus.

to construct and modify the cell wall and allows polar secretion of exoenzymes. These transport processes might involve up to 38 000 vesicles fusing with the fungal tip per minute, as estimated for fast-growing *Neurospora crassa* hyphae[13]. This process is supported by polar delivery of vesicles along F-actin and the microtubule cytoskeleton[14]. Some unconventional myosins (class V) as well as conventional kinesins participate in polar secretion. Additionally, class I myosins appear to support polar growth, but they are also involved in endocytosis.

The role of motors in exocytosis is best understood in *S. cerevisiae*, in which a class V myosin, Myo2p (Ref. 15), appears to support polar growth during budding. Genetic approaches, combined with cytological analysis, suggest that Myo2p binds secretory vesicles via its carboxy-terminal tail[16] and moves its cargo along F-actin filaments to sites of growth[17]. At present, Myo2p appears to be the main membrane-bound motor in yeast and, therefore, is likely to participate in the transport of many components. One such component is Chs3p (Ref. 18), a chitin synthase required for cell wall synthesis. Interestingly, some fungi have chitin synthases fused to their molecular motor. Examples include CsmA from *A. nidulans*[19] and Csm1 from the rice-blast fungus *Pyricularia oryzae*[20]. Mutational analysis has confirmed the cellular relevance of CsmA and indicated that this fusion protein requires both the chitin synthase domain and the myosin domain for correct cellular function[21].

Class I myosins, which have a role in actin organization and endocytosis, have been identified in fungi. These include Myo3p and Myo5p from *S. cerevisiae*[22,23]. Defects in endocytosis were also found in constitutively active mutants of another fungal myosin I, MYOA from *Aspergillus nidulans*[24,25]. However, deletion studies suggest that MYOA supports polar growth and secretion[24,26], indicating that this myosin is involved in both endo- and exocytosis in *A. nidulans*.

Conventional kinesin purified from animal sources was the first identified member of the kinesin superfamily, and it is assumed that this motor is involved in a wide spectrum of membrane transport processes[27]. In fungi, microtubule motors of the conventional kinesin class have been identified in a zygomycete[28], a basidiomycete[29] and ascomycetes[30,31], in which they appear to participate in polar exocytosis[29,32]. However, disruption of kinesin in *Nectria haematococca*[31] leads to defects in mitochondrial positioning rather than impaired morphology. Another conventional kinesin, Kin2, was shown to be required for polar growth in the phytopathogenic fungus *Ustilago maydis*[29], but appears to also function in vacuole organization[33]; this argues for multiple functions of kinesin motors within fungi (see later).

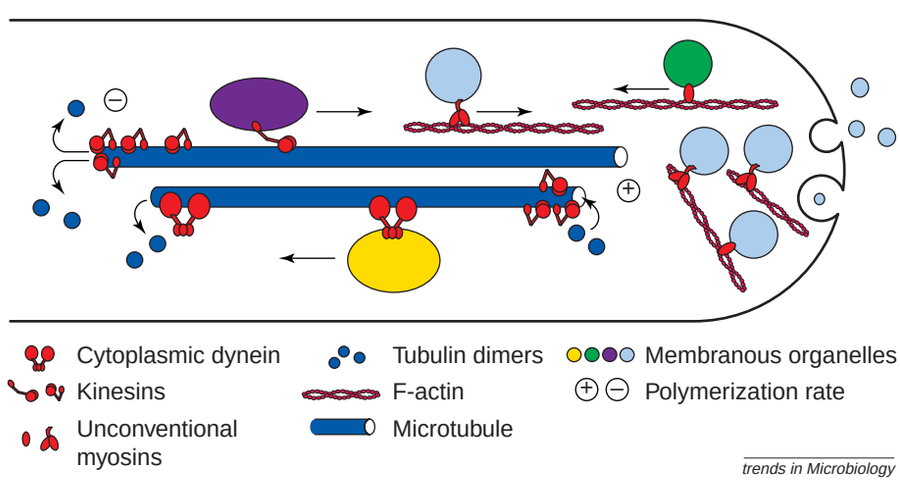

Fig 2. Hypothetical model of motor activity within a fungal cell. Kinesins and dyneins move in opposite directions along polar microtubules (+ marks the end with a high polymerization rate, − marks the end with a low polymerization rate), whereas myosins use F-actin, which is often concentrated at the tip region. In a classical model, motors translocate their 'cargo' (e.g. vesicles) along these filamentous tracks. However, it is now evident that at least some motors influence microtubule stability, presumably by modifying the microtubule ends (Box 1). Note that the described processes occur simultaneously within a growing cell.

Box 1. Motors and microtubule dynamics

Shortly after the first description of the amino acid sequence of conventional kinesin from *Drosophila melanogaster*, kinesin-like motors were identified in several fungi. Among these, Kar3p was found to be involved in *Saccharomyces cerevisiae* karyogamy. It was suggested that Kar3p supports karyogamy by crosslinking microtubules, a view which is consistent with a model of motors moving their cargo (in this case a microtubule) along cytoskeletal tracks. Indeed, *in vitro*, recombinant Kar3p translocates microtubules at rates of ~1–2 μm min^{-1}. However, in the presence of Kar3p, microtubules shorten while they are moving, suggesting that Kar3p also destabilizes microtubules[a]. Cellular defects owing to mutations in *kar3* could be rescued by treatment with benomyl[b], a cytoskeletal inhibitor that destabilizes fungal microtubules. This suggests that the destabilization activity of Kar3p could be biologically relevant; further studies have indicated that the mitotic function of Kar3p is mainly a result of microtubule destabilization at the spindle pole bodies[c,d]. Therefore, it is tempting to speculate that the activity of Kar3p in the kinetochore of chromosomes[e] is also the result of an effect on microtubule dynamics. Two other kinesin motors, Kip2p and Kip3p, are also involved in modifying microtubule stability during nuclear migration and mitosis in yeast[b,d,f], suggesting that an influence on cytoskeletal dynamics might be an essential aspect of their cellular role. Work from the group of R. Morris has indicated that this activity is not only restricted to yeast kinesins. In a screen for suppressors of nuclear migration mutants of *Aspergillis nidulans*, a tubulin mutation was found that leads to instable microtubules. Surprisingly, in tubulin and dynein double mutants, nuclei were able to move, and this effect could also be achieved by low concentrations of benomyl[g]. Therefore, the authors concluded that dynein appears to destabilize microtubules, thereby linking nuclear migration in *A. nidulans* to microtubule dynamics. Recent reports on similar destabilizing activities of vertebrate spindle motors[h,i] have raised the possibility that modifying the dynamics of the cytoskeleton is a common feature of many, if not all, molecular motors. The motor domain of some motors might only be required to reach the site of action at the end of microtubules. Understanding the role of motors in microtubule and, perhaps F-actin dynamics, will be key to elucidating their general cellular role.

Helpful information on kinesins can be found at `http://howard.fhcrc.org/kinesin/` and `http://tubulin.cb.m.u-tokyo.ac.jp/KIF/index.html`

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Organelle positioning and inheritance

During hyphal growth, membranous organelles such as mitochondria, vacuoles and nuclei often remain at a defined position relative to the growing tip. During cell divisions, these organelles are shared out between the mother and the daughter cell and molecular motors participate in these processes.

In *S. cerevisiae*, Myo2p is responsible for vacuole inheritance during cell division[34]. This function has been assigned to the carboxy-terminal domain of Myo2p, whereas other cellular roles might involve different parts of the motor[35]. In contrast to yeast, vacuole organization and transport in filamentous fungi appear to be microtubule-based processes. Cytological analysis of a *U. maydis* mutant deficient in Kin2 (Ref. 29) revealed a role for this conventional kinesin in vacuole organization within haploid yeast-like cells and dikaryotic hyphae[33]. It has been suggested that the proper formation of a sub-apical vacuole is required for hyphal growth independent of nutrient availability. Thus, Kin2 appears to support the *U. maydis* infection process. By contrast, phenotypic analysis of kinesin and dynein *N. crassa* double mutants demonstrates that dynein supports sub-apical vacuole formation[36]. Both these motors move in opposite directions along polar microtubules, indicating that microtubule orientation itself might account for the observed differences between vacuolar transport in *U. maydis* and *N. crassa*.

Nuclear migration is of crucial importance for fungal growth, as it supports the proper inheritance of DNA during cell division, as well as nuclear fusion during karyogamy[37]. Recent studies on the dynamics of the microtubule cytoskeleton revealed a central role for astral microtubules (the equivalent of cytoplasmic microtubules emanating from the spindle pole body, embedded in the nuclear envelope) in nuclear migration.

Extensive studies on several filamentous fungi have confirmed that dynein participates in fungal nuclear migration[38–40]. In contrast to *Dictyostelium* and *Drosophila*, fungal dynein mutants are viable, but have misplaced nuclei and impaired nuclear migration. *In vivo* observation of microtubules using green fluorescent protein suggested that dynamic microtubules interact with cortical dynein, which exerts pulling forces on these microtubules and thereby on the nucleus itself[41]. The localization of dynein to astral microtubules and the spindle pole body in *N. haematoccoca*[42], *S. pombe*[43] and *S. cerevisiae*[44] is therefore consistent with a function in nuclear movement.

Some nuclear motility is still present in Δ*nudA* mutants of *A. nidulans*. It was assumed therefore that additional motor systems for nuclear migration might exist[45]. The identification of kinesins involved in karyogamy (Kar3p)[46] and nuclear positioning [Kip2p (Ref. 47) and Kip3p (Refs 48,49)] in *S. cerevisiae* revealed potential candidates for such a motor system. Extensive mutational analysis of these motors indicated a role for Kip3p in the early nuclear migration

steps prior to mitosis, whereas Kip2p supports the dynein-mediated pathway, probably by stabilizing microtubules (Box 1).

Fungal motors in mitosis

One of the most fascinating aspects of mitosis is the formation of a highly ordered segregation structure, the mitotic spindle. The spindle consists mainly of microtubules. Therefore, it is not surprising that kinesins and cytoplasmic dynein motors are involved in spindle organization and chromosome movement during fungal mitosis. Genetic approaches using *A. nidulans*, *S. pombe* and *S. cerevisiae* have greatly enhanced our understanding of the precise mode of action. Surprisingly, it appears that assembly and organization of the spindle requires counteracting motors, which function to build up tension within the spindle rather than moving a cargo.

Kinesins of the *bimC* family were first identified in nuclear division mutants of *A. nidulans* (*bimC*)[50] and *S. pombe* (*cut7*)[51] and subsequently in *S. cerevisiae* (*KIP1* and *CIN8*)[47] and several animal systems[52]. It is now evident that, in all systems investigated, *bimC*-like motors are required for spindle pole body or centrosome separation at the onset of mitosis, as well as for the assembly and maintenance of the spindle architecture[52]. Surprisingly, the mitotic phenotypes of fungal *bimC*-like mutants can be rescued by the deletion of other kinesins (*KAR3*-like motors[53–55]). It therefore appears that *KAR3*-like and *bimC*-like motors have counteracting functions. Unlike all other kinesins, *KAR3*-like motors (Kar3p, *S. cerevisiae*[46]; KlpA, *A. nidulans*[54]; Pkl1, *S. pombe*[55]) move towards the 'minus' ends of microtubules and locate at the spindle poles where their major role could be to destabilize microtubules, thereby maintaining spindle integrity, a function in which Kar3p overlaps with Kip3p (Box 1). This suggests that their counteracting partners could also alter microtubule dynamics. However, *bimC*-like motors appear to be tetramers with two motor domains at each end[52,56], and they are located in the mid-zone of the anaphase spindle[57]. This suggests that *bimC* kinesins separate the spindle poles by crosslinking polar microtubules (Fig. 4).

In vertebrate cells, cytoplasmic dynein is located at the spindle and participates in spindle assembly and chromosome segregation. By contrast, fungal dynein is located outside the mitotic nucleus in the growing tip[45,58] where it is thought to pull on astral microtubules to support nuclear migration or support the transport of exocytotic vesicles. However, a screen for *A. nidulans* genes that are synthetic lethal with dynein revealed several mitotic genes[59], suggesting that dynein has an additional role in mitosis. A mitotic function for dynein was also concluded from careful analysis of mitosis in dynein-deficient mutants of *N. haematoccoa*[40]. This led to a model for the cellular function of dynein, suggesting that this motor supports nuclear migration and spindle elongation by pulling on astral microtubules emanating from the spindle pole body[40,59] (Fig. 4).

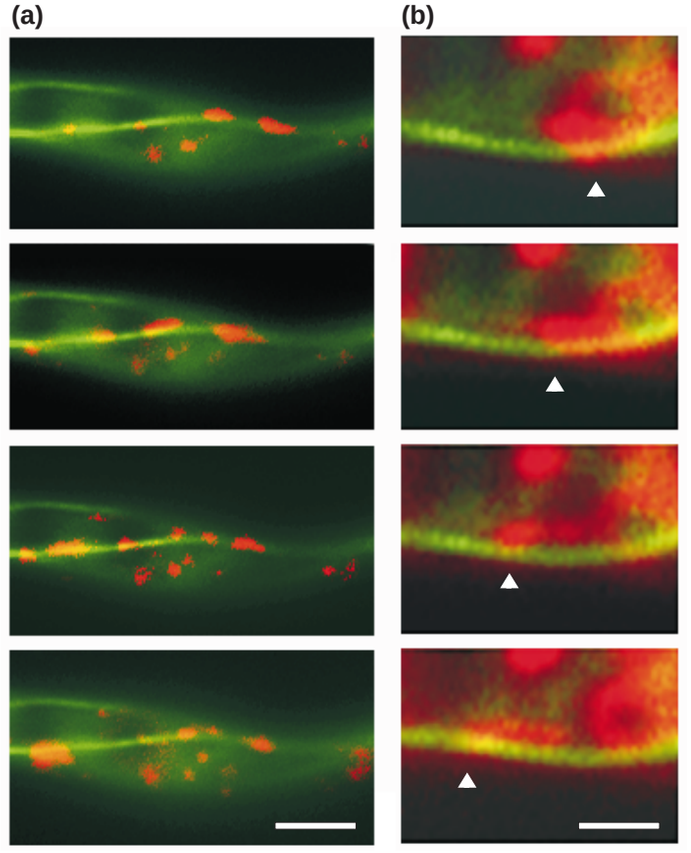

Fig. 3. Movement of endosomes along green fluorescent protein (GFP)-labelled microtubules in haploid *Ustilago maydis* cells. Endosomal membranes were stained with the lipophilic dye FM4-64, which contained GFP-tagged α tubulin[67]. **(a)** Endosomal vesicles undergo fission and fusion and move rapidly along microtubules. Time interval between frames = ~1 sec; scale bar = 3 μm. **(b)** Vesicle membranes incorporated the dye and move along GFP-labelled microtubules, a process that is probably driven by kinesin- or dynein-like motors. Time interval between frames = 0.2 sec, scale bar = 1 μm.

New cellular roles for fungal motors in RNA transport and meiotic recombination

In budding yeast, mother cells are able to switch their mating type; switching of daughter cells is repressed by Ash1p, a regulator of the endonuclease HO (a homothallic mating-switch endonuclease). A second class V myosin, Myo4p, is responsible for daughter-specific localization of *ASH1* mRNA (Refs 60,61). This indicates that motors transport mRNA, thereby generating RNA gradients within the cell[62].

Recently, an unexpected role for the microtubule motors Kar3p and cytoplasmic dynein in *S. cerevisiae* and *S. pombe* meiosis, respectively has been described. In Kar3p and dynein mutants, meiotic recombination is significantly reduced. In the case of Kar3p mutants of *S. cerevisiae*, this could be the result of defects in microtubule-dependent chromosome pairing within the meiotic spindle[63]. In *S. pombe*, misalignment of homologous chromosomes, which results in reduced recombination, could be owing to a lack of dynein-dependent nuclear oscillations during meiosis[43].

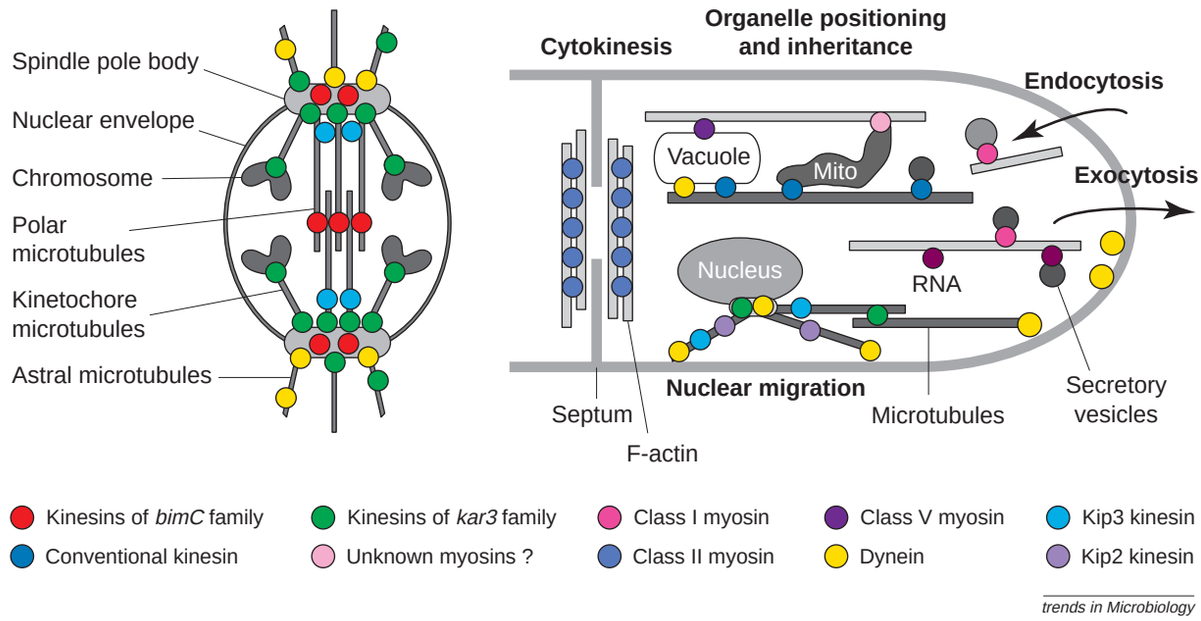

Fig. 4. Localization and/or assumed site of action of fungal motors. **(a)** Mitosis. *kar3*-kinesins influence the dynamics of spindle microtubules and counteract *bimC*-like motors, which appear to crosslink polar microtubules. In addition, Kar3p might function within the chromosomal kinetochore (see Box 1). Anaphase is supported by cytoplasmic dynein that exerts pulling forces on astral microtubules and, in conjunction with Kip2p and Kip3p, probably modifies microtubule dynamics. Note that the localization of Kip3p within the spindle is not known. **(b)** Polar growth and cytokinesis. Motors are involved in a wide spectrum of organelle transport. In *Saccharomyces cerevisiae*, F-actin is involved in mitochondrial motility[3], but the putative myosin has not yet been identified. Note that the figure summarizes data from several fungi.

Conclusion

Recently, much attention has focused on the identification and functional understanding of molecular motors in fungi. In particular, the characterization of their mitotic roles has revealed similarities between fungal model systems and provided some general principles on their function. Moreover, intensive work on nuclear migration has greatly enhanced our understanding of dynein and kinesin motors in this important process. Of course, many additional components participate in nuclear migration, and the challenge now is to uncover the regulatory and structural network within which molecular motors function. By contrast, our understanding of molecular motors in fungal membrane traffic is restricted mainly to actin-based transport in *S. cerevisiae*. In particular, microtubule-associated transport requires further experimentation and, almost certainly, new microtubule-dependent organelle motility processes and their underlying transport machinery will be discovered. In addition, even well studied motors could surprise us with unexpected structural or functional aspects. Therefore, combined approaches using genetics, cytology and biochemistry in suitable fungal model systems, such as the ascomycetes *S. pombe*, *A. nidulans*, *N. crassa* and *N. haematococca*, as well as new model organisms such as the basidiomycete *U. maydis*, will all help to further our understanding of microtubule-dependent membrane traffic processes in fungi.

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Adventures of a pore-forming toxin at the target cell surface

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The past three years have shed light on how the pore-forming toxin aerolysin binds to its target cell and then hijacks cellular devices to promote its own polymerization and pore formation. This selective permeabilization of the plasma membrane has unexpected intracellular consequences that might explain the importance of aerolysin in *Aeromonas* pathogenicity.

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Aerolysin is one of the major toxins secreted by the Gram-negative bacterium *Aeromonas hydrophila*, as well as other *Aeromonas* species that are human pathogens involved in food-borne infections[1]. In a mouse toxicity model, mutant strains deficient in aerolysin production were found to be less virulent than wild type[2]. Additionally, specific neutralizing antibodies to aerolysin have been detected in animals surviving *Aeromonas* infection.

Aerolysin is released from *A. hydrophila*, via a type II secretion system, as a soluble precursor, proaerolysin[1], which undergoes a monomer-to-dimer transition at high concentrations such as those found during crystallization processes[3,4]. The structure of proaerolysin indicates that it consists of two lobes[3] (Fig. 1): the amino-terminal domain 1 (Ref. 5) and a less stable, large carboxy-terminal region that can be divided into three domains, domains 2–4 (Fig. 1). Domain 1 shares structural homology with the S2 and S3 subunits of the *Bordetella pertussis* toxin and is similar to a fold found in C-type lectins[6] (blue in Fig. 1). In addition, proaerolysin shares strong sequence homology with *Clostridium septicum* alpha toxin, which has a similar mode of action[7]. Interestingly, the proaerolysin sequence contains no hydrophobic stretches predicted to be capable of membrane insertion.

Receptor binding and activation

Once released by the bacterium, proaerolysin binds to high-affinity receptors on the target cell. Instead of recognizing one specific receptor protein, proaerolysin interacts with a specific post-translational modification, a glycosylphosphatidylinositol (GPI) anchor[8–12]. This anchor is added, in the endoplasmic reticulum (ER), to the carboxy terminus of newly synthesized proteins bearing a GPI-anchoring signal. The GPI anchor then targets these proteins to the plasma membrane[13]. All anchors have the same backbone structure consisting of ethanolamine-HPO_4-6Manα1-2Manα1-6Manα1-4GlcNH_2α1-6-myo-inositol-1HPO_4 linked to a lipid moiety. The mannose residues can be modified and the inositol ring can be acylated in certain cell types. Binding of proaerolysin appears to involve mainly the glycan core and probably specific modifications of the central mannose residues[10]. To what extent the protein moiety of the receptor influences binding remains to be established. However, it

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