

MINIREVIEW

Hyphal Growth: a Tale of Motors, Lipids, and the Spitzenkörper^{∇†}

Gero Steinberg*

MPI für Terrestrische Mikrobiologie, Karl-von-Frisch-Straße, D-35043 Marburg, Germany

Filamentous fungi are a large and evolutionarily successful group of organisms of enormous ecological importance (27, 114). Fungi also have a considerable impact on our economy because they serve as bio-factories for the industrial production of proteins (90, 130) and because many fungi are human and plant pathogens that pose a threat to public health and agriculture (1, 105, 124).

The basic unit of a filamentous fungus is the hypha, which usually consists of a chain of elongated cells that expand at the apex of the tip cell (10, 41, 115). Hyphal tip growth is characterized by the initial establishment of one growth site, which is followed by its continuous maintenance. This growth mode is different from budding in the yeast *Saccharomyces cerevisiae*. Here a short period of polarized apical growth is followed by extended isotropic growth, which allows delivery of cell wall material over the entire bud surface and which leads to the almost spherical daughter cell (28, 68). In contrast, hyphal growth results in an elongated tip cell, which raises new requirements. Among these is the long-distance transport between the subapical part and the apex of the tip cell. It is thought that microtubule (MT)-based motors, including kinesin-1 and kinesin-3, deliver vesicles and growth supplies over long distances to the hyphal tip (see below). However, in agreement with the described differences between hyphal growth and budding, these motors are not even encoded by the genome of *S. cerevisiae*.

Hyphal growth is accompanied by the secretion of exoenzymes that participate in lysis of the substrate or are involved in the synthesis of the fungal cell wall (3, 42). The cell wall can be considered as an extracellular matrix consisting mainly of a meshwork of polysaccharides and manno-proteins that shelters the cell and resists the internal turgor pressure to maintain the shape of the hypha (112).

It is currently thought that growth of fungal hyphae is mediated by cytoskeleton-based polar exocytosis at the hyphal tip and cytoplasmic expansion forces that push the cytoplasm against the flexible apical wall (summarized in reference 7). This model is in part based on the phenotypic similarities between fungal cells and plant cells that grow at their tips, such as pollen tubes or root hairs (36). However, phylogenetic comparison of rRNA or conserved proteins demonstrates that

fungi are more closely related to animals (6, 131), and there is evidence for amoeboid motility of fungal cells (50). In this article, I will summarize recent results from different fields of fungal cell biology that are instrumental for understanding hyphal tip growth. This includes research on the Spitzenkörper, the polarisome, the role of the cytoskeleton in endo- and exocytosis, the cellular function of sterol-rich plasma membrane domains, and the mechanism that generates the force for extension of the hypha. Finally, I will integrate all of these results from different fungal systems in a model for hyphal tip growth, and I will end the review by providing directions for future research.

ROLE OF THE SPITZENKÖRPER IN HYPHAL GROWTH

Understanding the mechanisms of hyphal growth is still a major challenge in fungal cell biology. Presently, the most convincing explanation of hyphal elongation is provided by a combination of the steady-state model of Wessels (112, 138) and the vesicle supply center model of Bartnicki-Garcia (7, 9) (Table 1).

According to the steady-state model, fibers of the cell wall, such as chitin or glucan chains, are synthesized at the hyphal apex. In the apex, these fibers are not yet cross-linked and the wall is still flexible. As the tip expands, subapical chitin crystallizes and becomes covalently bound to β -1,3-glucans, thus solidifying the cell wall in the older parts of the growing hypha. Although still controversial, it is widely thought that the hyphal cytoplasm exerts pressure on the wall (see below), thereby powering the expansion of the plastic apex during hyphal tip growth.

The vesicle supply center model is based on the accumulation of vesicles within the hyphal apex, which was initially identified as a dark body in light microscopy and termed the Spitzenkörper (15). Subsequently, such temporary and dynamic accumulation of microvesicles (30 to 40 nm) and macrovesicles (70 to 120 nm; Fig. 1; see Movie S1 in the supplemental material) surrounding a core region enriched in F-actin (48, 55) was found in a broad range of fungal species (40, 45). The Spitzenkörper is important for hyphal growth (8, 15, 39), and its position in the growing hypha determines the directionality of growth (40, 102). Based on the “vesicle supply center” model, it is thought that the Spitzenkörper receives Golgi-derived vesicles that release exocytic vesicles in a controlled manner, thereby generating an exocytosis gradient that determines the shape of the hyphal apex (9). An important aspect of this model is that the Spitzenkörper itself moves forward, which is thought to determine the direction and rate of hyphal

* Mailing address: MPI für Terrestrische Mikrobiologie, Karl-von-Frisch-Straße, D-35043 Marburg, Germany. Phone: 49-6421-178-530. Fax: 49-6421-178-599. E-mail: Gero.Steinberg@mpi-marburg.mpg.de.

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TABLE 1. Models for hyphal tip growth

Model	Basic concept	Major supportive observations/results	Reference
Vesicle supply center	Post-Golgi vesicles are gathered in an apical vesicle supply center. This center regulates growth by generating a gradient of exocytosis of enzymes, such as lysins. Its active tip-ward movement, in combination with turgor pressure, determines hyphal shape and elongation rate	Growing hyphae contain a Spitzenkörper, and its displacement alters hyphal growth and direction and initiates new growth sites. Hyphal growth can be mathematically simulated	9
Steady state	Polar exocytosis delivers wall-forming enzymes (e.g., chitin synthases and glucanases). Newly added wall material is noncrystalline and plastic, and thus can be expanded by turgor pressure. The wall solidifies as it progresses towards the subapex, thereby resisting turgor pressure and shaping the hypha	Chitin is noncrystalline at the tip, but in older and subapical regions of the wall, chitin forms more rigid microfibrils that become covalently cross-linked to β -1,3-glucans	138
Amoeboid ^a	A membrane cytoskeleton supports integrity of the tip and regulates tip extensibility. The hyphal tip expands by the force produced by the cytoskeleton. The cell wall is considered to be an extracellular matrix that confers shape to the hypha	The fungal cytoplasm contracts and is able to form pseudopodia	50

^a The basic concept of a fungal cell being an amoeba in a tube was first introduced by Reinhardt in the late 19th century (99). Note that hyphal growth is most likely based on all three models.

growth (7). However, the mechanism of this assumed Spitzenkörper motility is not known.

In addition to membranes, the Spitzenkörper contains ribosomes (40, 56), suggesting that translation of mRNA and pro-

tein synthesis happen in the hyphal apex. This notion is strongly supported by a recent report that putative mRNA-binding protein Rrm4 (12) is taken to the hyphal tip by so far unidentified MT-based motors (11, 12). The Golgi apparatus reaches into the hyphal tip (40) and concentrates in the apical 5 to 10 μ m of the hypha (103). The endoplasmic reticulum also concentrates near the apex of hypha (135), which further indicates that the protein synthesis machinery becomes polarized in order to support hyphal tip growth.

ENDOCYTOSIS AND HYPHAL GROWTH

In the “vesicle supply center” model, the Spitzenkörper consists of Golgi-derived exocytic vesicles. However, there are indications that the Spitzenkörper also plays a role in endocytosis. Pioneering studies of the endocytic pathway in the yeast *Saccharomyces cerevisiae* have shown that the amphiphilic styryl dye FM4-64 is internalized by endocytosis and is delivered to the fungal vacuole via the endocytic pathway (127). Surprisingly, similar studies in filamentous fungi have demonstrated that FM4-64 transiently stains the Spitzenkörper (20, 31, 48, 53), which indicates that endocytic vesicles cluster in the hyphal tip. Indeed, endocytic recycling via early endosomes is essential for proper hyphal morphology and pathogenicity in the corn smut *Ustilago maydis* (33, 134 [also see below]). Therefore, it appears that the hyphal apex not only is a site of exocytosis but presumably also participates in membrane recycling processes that support tip growth.

It is well established that the Spitzenkörper supports tip growth of fungal hyphae, but its exact composition and the cargo of the vesicles within the accumulation are still elusive. The Spitzenkörper is enriched in F-actin (55) and also contains formins in *Aspergillus nidulans* and *Candida albicans* (20, 111). Formins nucleate F-actin and are part of a protein complex, the polarisome, that polarizes the actin-associated secretion machinery (overview in reference 64). This suggests some parallels between the polarisome and the Spitzenkörper (48).

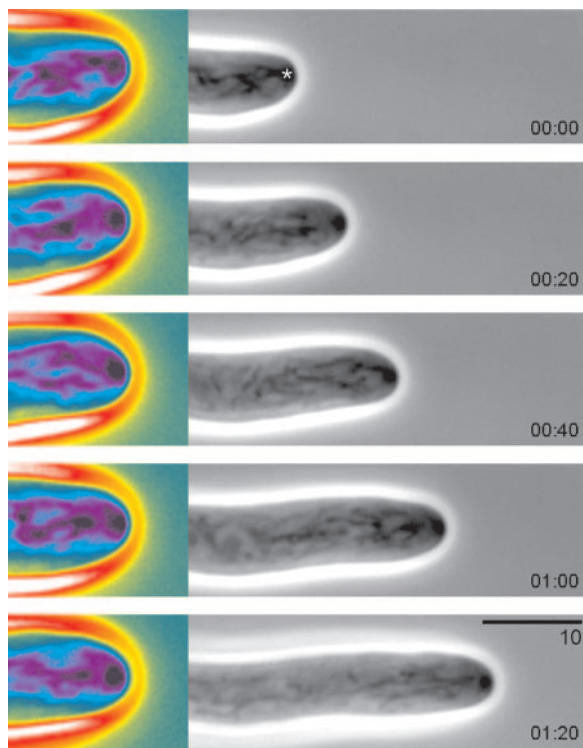


FIG. 1. Tip growth and the Spitzenkörper in *Neurospora crassa* strain 74A. Rapid hyphal elongation through 1% low-melting agarose is accompanied by a dynamic accumulation of vesicles in the apex (dark in phase-contrast micrographs, blue in pseudo-colored image). Elapsed time is given in minutes:seconds. Bar, 10 μ m. The images are taken from Movie S1 in the supplemental material.

However, meticulous localization studies in *Candida albicans* have demonstrated that the polarisome proteins Spa2 and Bud6 do not exactly colocalize with the FM4-64-stained Spitzenkörper (20). Nevertheless, the integrity of the Spitzenkörper depends on the polarisome (20); therefore, the polarisome might participate in F-actin polarization, which might in turn guide Spitzenkörper vesicles to the hyphal apex.

F-ACTIN IS ESSENTIAL FOR HYPHAL GROWTH

Hyphal elongation is supported by an impressive rate of intracellular transport of vesicles to the tip, which makes filamentous fungi among the fastest-growing cells, with rates of $\sim 20 \mu\text{m}/\text{min}$ (17). For example, it was estimated that rapid growth of *Neurospora crassa* requires $\sim 38,000$ vesicles fusing with the tip per minute (19). Pharmacological studies on numerous fungal species demonstrated that F-actin is essential for tip growth (see references 2, 34, and 49). This is due to important roles of F-actin in fungal secretion and endocytosis (overview in references 83 and 96). Formins are concentrated at the hyphal tip (20, 111). These actin-binding proteins support actin assembly at the plus ends (barbed end) of the actin filaments (95). Consequently, the accumulation of formins at the hyphal tip suggests that F-actin orients its plus ends towards the hyphal growth region. Almost all myosins move their cargo towards F-actin plus ends (137). Thus, myosin-driven membrane traffic along F-actin could support the formation of the Spitzenkörper, indicating that myosin-based transport processes participate in membrane traffic to the growing tip.

MYOSIN-5, A SECRETION MOTOR FOR HYPHAL GROWTH

The sequenced genomes of filamentous fungi encode four classes of myosins, including myosin-1, myosin-2 (conventional myosin), myosin-5, and the fungus-specific myosin-17, which contains a chitin synthase domain (classification based on reference 52). Considering the organization and importance of F-actin in hyphae, it is likely that myosin-based transport of secretory vesicles along microfilaments supports fungal growth. Indeed, myosin-5 motors are thought to deliver secretory vesicles to the growth region in *Saccharomyces cerevisiae* (43, 59, 107) and *Schizosaccharomyces pombe* (81, 82, 139). In the plant pathogen *Ustilago maydis*, a functional fusion protein of myosin-5 and the green fluorescent protein (GFP) localizes to a spot in the apical dome of the hyphae (133) (Fig. 2). This spot most likely represents the Spitzenkörper. In addition, faint GFP-myosin-5 signals show rapid anterograde motility (see movie at <http://www.mpi-marburg.mpg.de/downloads/>), which indicates that myosin-5 is involved in tip-ward delivery of membranes. Consistently, myosin-5 is essential for filamentous growth and pathogenicity in this corn smut fungus (108, 133), as well as in the human pathogen *Candida albicans* (140). Myosin-5 from chickens is a large protein complex motor that is able to take at least 40 to 50 steps along F-actin, which is estimated to allow 1.5- to 2- μm displacements (74). In contrast, Myo2p, a myosin-5 in *Saccharomyces cerevisiae*, is not processive, and in vitro studies have indicated that at least five motors have to cooperate to move a secretory vesicle (98). How numerous myosin motors can cooperate on the surface of

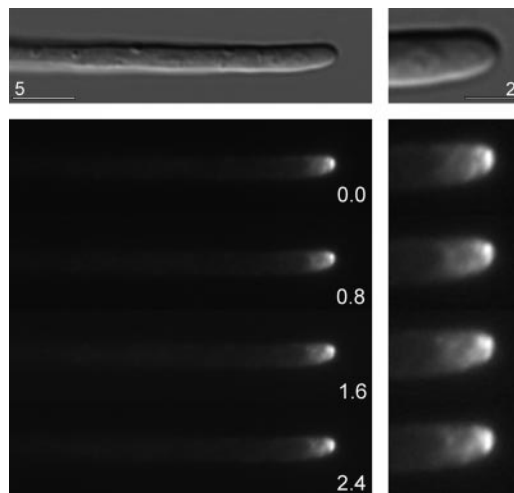


FIG. 2. GFP-labeled myosin-5 in a hypha of a *myo5* null mutant of *Ustilago maydis*. The functional fusion protein concentrates in the hyphal tip, where it is dynamically rearranged. Note that faint GFP-Myo5 signals show directed motility in the subapical regions, which is best seen in the movie available at <http://www.mpi-marburg.mpg.de/downloads/>. Shown is strain AB33 Δ Myo5_GFP₃Myo5, in which the *b*-transcription factor that triggers filamentous growth is under the control of inducible promoters (14). In addition, the endogenous *myo5* gene is deleted and a triple GFP fused to Myo5 is ectopically integrated. Elapsed time is given in seconds. Bars, 5 and 2 μm .

a myosin-5-delivered vesicle that is only 80 to 100 nm in diameter (59) is not known. However, these data taken together indicate that myosin-5 motors very likely act as membrane transporters in secretion during tip growth.

MYOSIN-1 AND FUNGAL ENDOCYTOSIS

Whereas myosin-5 is apparently involved in exocytosis, myosin-1 motors support fungal growth by promoting endocytosis. The first evidence for such a role of myosin-1 was found in *Saccharomyces cerevisiae*, where deletion of myosin-1 motors impaired endocytosis and almost abolished growth (38). In *Aspergillus nidulans* (73, 88) and in *Candida albicans* (86, 87), myosin-1 is essential for hyphal growth. In both fungal species, myosin-1 activity is required to mediate the endocytotic uptake of the endocytic marker dye FM4-64 into the vacuole (86, 142). Surprisingly, a mutant allele in MYOA, the myosin-1 in *A. nidulans*, which has almost no ATPase activity, is still able to support hyphal growth (70). This indicates that the motor activity of myosin-1 is not required for its cellular function and that it does not “walk” along actin filaments to mediate endocytosis.

In animal and yeast cells, the internalization of endocytic vesicles at the plasma membrane is driven by local actin polymerization (29, 60, 75). Indeed, the myosin-1 tail is able to induce actin polymerization (37, 65). It is therefore possible that the motor domain mediates localization of myosin-1 to sites of endocytosis, namely the hyphal tip and septa (86, 143), whereas the tail supports internalization and motility of endocytic vesicles by triggering actin polymerization. Consequently, the effect of deletion of myosin-1 on hyphal growth and morphology can be explained by a role of the motor in endocytotic

recycling of cell wall remodeling enzymes required for proper cell wall formation and tip growth. Although such recycling of enzymes in hyphal growth is currently speculative, endocytic recycling of receptors has recently been shown in *Ustilago maydis* (33). Whether myosin-1 is indeed involved in receptor recycling remains to be determined.

STEROL-RICH LIPID RAFTS IN FUNGAL ENDOCYTOSIS AND ACTIN POLARIZATION

Surprisingly, Myo1p, the myosin-1 in *Schizosaccharomyces pombe*, is required to concentrate sterols at the growing tip (122). Sterol-rich membrane domains, termed lipid rafts, were first identified in animal cells, where they appear to be of particular importance in polarized cell types, such as axons or epithelial cells (overview in reference 113). Lipid rafts are specialized membrane domains rich in sphingolipids and cholesterol/ergosterol. They help to concentrate lipid-binding proteins, such as glycosylphosphatidylinositol (GPI)-anchored proteins and caveolins, at specific regions of the plasma membrane (overview in reference 97). There is biochemical evidence for detergent-resistant fungal lipid rafts in *Saccharomyces cerevisiae* (4, 44). In this yeast, sphingolipids provide ~30% of the plasma membrane (89) and are important for the early steps of endocytosis (84, 91). It has been suggested that the ergosterol composition of the plasma membrane determines its rigidity and therefore is instrumental for the formation of primary endocytic vesicles (84). In mating projections of *Saccharomyces cerevisiae*, it was found that membrane-bound receptors slowly diffuse away from the tip but are repolarized by endocytic uptake and delivery back to the tip (125). This raises the possibility that lipid rafts support polarized fungal growth by slowing down the diffusion rate and by promoting apical endocytosis and subsequent endocytic recycling (see above).

Lipid rafts are rich in sphingolipids and can therefore be visualized by using the polyene antibiotic filipin, which specifically stains 3- β -hydroxysterols (24). The use of filipin revealed that sterol-rich domains are concentrated at the growing tips of mating projections in *Saccharomyces cerevisiae* (5, 94), *Schizosaccharomyces pombe* (129), and *Cryptococcus neoformans* (85; overview in reference 128). Furthermore, filipin-labeled membrane domains were found in the tip of hyphae of *Candida albicans* (72) and *Aspergillus nidulans* (69). Inhibition of sphingolipid biosynthesis by myriocin in *Candida albicans* (72) and *Aspergillus nidulans* (18) reduces tip growth and increases branching. This loss of polarity is accompanied by a rearrangement of actin patches, which suggests that lipid rafts are essential for a polarized F-actin cytoskeleton (18). This notion is further supported by the disappearance of formin SepA from the hyphal tip in *Aspergillus nidulans* mutants defective in sterol biosynthesis (69). Studies with *S. cerevisiae* have demonstrated that actin polarization and actin cable formation require Mss4, which is an essential phosphatidylinositol-4-phosphate 5-kinase that synthesizes phosphatidylinositol (4,5)-bisphosphate in the plasma membrane (23). Interestingly, this localization and the activity of Mss4 were abolished by the inhibition of sphingolipid biosynthesis (62). This suggests a complex interplay of sphingolipids, lipid kinases, and the machinery for actin polarization within apical lipid rafts of fungi. Thus, sterol-rich membrane domains might support hyphal

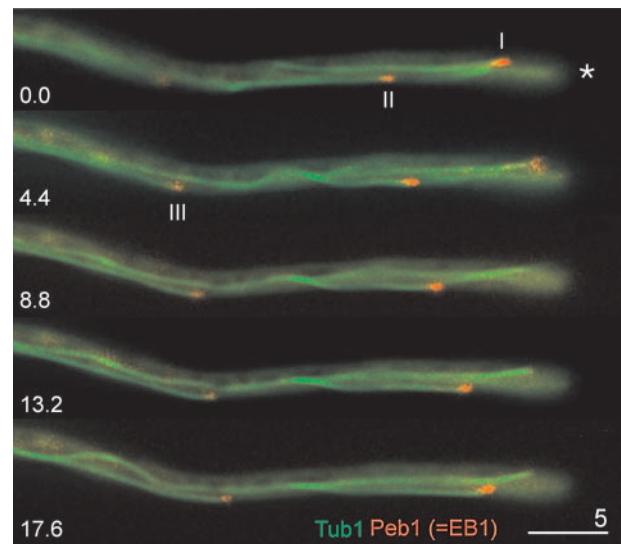


FIG. 3. Orientation of microtubules in a hypha of *Ustilago maydis*. A functional fusion protein of the EB1-homologue Peb1 and RFP binds to the plus end of elongating microtubules (indicated by roman numbers). About 90% of all plus ends are directed to the hyphal tip (indicated by an asterisk). Elapsed time is given in seconds. Bar, 5 μ m. Shown is strain AB33Peb1RFP_GT, in which MTs are labeled by ectopically expressed GFP- α -tubulin, and which contains the endogenous plus end-binding EB1-homologue Peb1 (121) fused to RFP. The images are taken from a movie available at <http://www.mpi-marburg.mpg.de/downloads/>.

growth in two ways: (i) by facilitating apical endocytosis and (ii) by providing an apical scaffold that helps to organize the cytoskeleton, thereby supporting F-actin-based secretion.

MICROTUBULES IN FUNGAL TIP GROWTH

The role of MTs in filamentous growth has long been a matter of debate. However, evidence has accumulated that MTs are essential for hyphal growth (34, 54, 57, 108), and MTs and associated motors are required for the integrity of the Spitzenkörper (20, 63, 66, 109). MTs are polar structures that grow at their plus ends by addition of tubulin dimers; the minus ends are usually less active (22). The transport machinery utilizes the polarity of MTs: dyneins move towards the MT minus end, and kinesins take their cargo towards the MT plus end (126). Specific proteins, such as members of the EB1 family, which bind to the growing MT plus end (76, 123), regulate polymerization of tubulin. Therefore, fluorescently labeled EB1-like proteins can be used to determine the orientation of MTs in living fungal cells (67, 108, 121). In hyphae of *Ustilago maydis*, EB1-like Peb1 fused to the monomeric red fluorescent protein (RFP) localizes to the plus end of GFP-labeled MTs (108). Quantitative analysis of the motility of Peb1-RFP has revealed that 80 to 90% of all plus ends grow towards the hyphal apex (108) (Fig. 3; see supplemental movie at <http://www.mpi-marburg.mpg.de/downloads/>). MTs are oriented similarly, as indicated by the observation of plus-end-binding kinesin motors fused to GFP (63). In vitro studies have shown that fungal kinesin motors transport their cargo towards the MT plus ends (116, 119). Considering the orientation of the

MTs in the hypha, it is likely that kinesins takes secretory vesicles to the hyphal tip (108).

MICROTUBULE-DEPENDENT KINESINS SUPPORT POLARIZED HYPHAL GROWTH

The analysis of fungal genome sequences has revealed that filamentous fungi contain 1 cytoplasmic dynein and 10 to 11 kinesin motors (106, 108). However, only dynein (92, 104) and three kinesin motors, kinesin-1, kinesin-3 (67, 108), and kinesin-7 (63), have been shown to be essential for filamentous growth. In animal cells, kinesin-1 (conventional kinesin) and kinesin-3 (Unc104/Kif1A) are prominent organelle transporters (13, 51), and these motors are also encoded by filamentous fungi (32, 46, 66, 106, 116, 119, 136, 141), whereas they are absent from the yeast *Saccharomyces cerevisiae*. This suggests that both motors could play a role in long-distance transport in fungal hyphae. A motor for exocytosis of the expected large number of secretory vesicles (e.g., up to 38,000 per min [see above]) should be abundant. Indeed, the concentration of kinesin-1 is high enough for the protein to be purified from cell extracts of the ascomycete *Neurospora crassa* (119), the zygomycete *Syncephalastrum racemosum* (116), and the basidiomycete *Ustilago maydis* (120).

Mutant analysis has revealed that kinesin-1 also participates in Spitzenkörper organization in *Neurospora crassa* (109) and *Ustilago maydis* (66), and there is preliminary evidence that kinesin-1 functions in the secretion of invertase (G. Steinberg, unpublished). Furthermore, kinesin-1 and myosin-5 colocalize at the Spitzenkörper, and they cooperate to maintain polarity of hyphae in *Ustilago maydis* (108). Consequently, it is most likely that kinesin-1 is a major player in apical secretion during polarized growth of fungal hyphae.

Since kinesin-1 and kinesin-3 null mutants have similar growth defects, kinesin-3 might also participate in hyphal tip growth (108). Indeed, kinesin-3 null mutants are impaired in acid phosphatase secretion (108), and a functional GFP–kinesin-3 fusion protein accumulates in the hyphal apex and rapidly moves bidirectionally over long distances. However, compared to myosin-5 and kinesin-1 (see above), the apical localization of kinesin-3 is less focused and more dynamic (108). Therefore, this motor might transport organelles or vesicles other than the vesicles of the Spitzenkörper. Indeed, there is good evidence that MTs, kinesin-3, and dynein play a role in traffic of early endosomes in yeast-like and hyphal cells of *Ustilago maydis* (67, 134, 136). These studies have shown that kinesin-3 mediates anterograde (tip-directed) transport, whereas minus-end-directed dynein could support retrograde traffic. Interestingly, the switch from kinesin-3-based anterograde transport to retrograde and dynein-dependent motility of early endosomes occurs in the hyphal tip (67) (Fig. 4; see Movie S2 in the supplemental material).

A recent report demonstrates that MT-based transport might also be involved in mRNA transport. Studies with *U. maydis* have demonstrated that Rrm4, a protein that contains RNA recognition motifs (12), rapidly moves bidirectionally along MTs (11). Mutants with deletion in *rrm4* show severe defects in hyphal growth, but have no phenotype in yeast-like cells (11). Thus, it was suggested that long-distance MT-based transport of Rrm4 takes mRNA from the subapical nucleus to

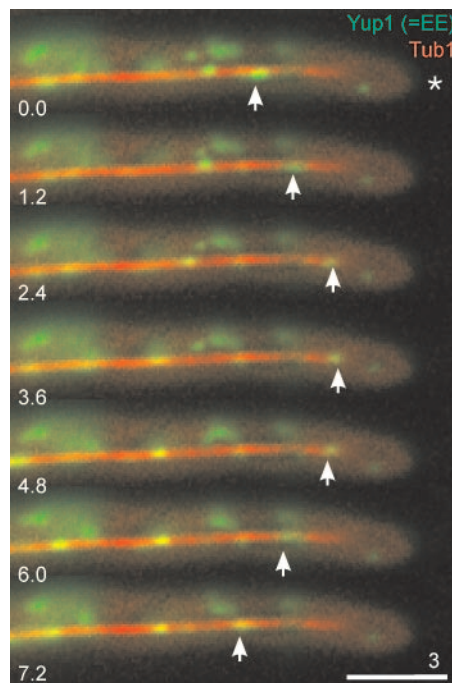


FIG. 4. Motility of early endosomes along microtubules in *Ustilago maydis*. Organelles are marked by Yup1, a tSNARE that locates to early endosomes (134). In hyphae, endosomes (Yup1-GFP; green) move along RFP- α -tubulin-containing microtubules (Tub1; red) to the tip, where they remain for a short period and return to subapical regions (arrow) (67). Elapsed time is given in seconds. Bar, 5 μ m. Shown is strain AB33Yup1GFP_RT, in which MTs are labeled by ectopically expressed GFP- α -tubulin and which contains an ectopically expressed endosomal tSNARE Yup1 fused to GFP (134). The images are taken from Movie S2 in the supplemental material.

the hyphal tip, where it might be translated at apical ribosomes that are concentrated within the Spitzenkörper (40, 56).

Finally, it is important to notice that kinesins can also support fungal growth by organizing the MT cytoskeleton (117). In *A. nidulans* KipA, a kinesin-7 localizes at plus ends of MTs and appears to mediate proper contact of MT tips with the growth region (63), a function that is essential to direct the hyphal growth machinery. However, such a role of kinesin-7 was not found in the basidiomycete *U. maydis* (108), which raises doubt about a general role of these kinesins in MT organization. Taken together, it is very likely that a small set of kinesin motors support hyphal tip growth by delivering a diverse spectrum of cargo along MTs to the extending apex.

DRIVING FORCE FOR HYPHAL TIP EXPANSION: COMBINATION OF TURGOR PRESSURE AND AMOEBOID MOTILITY OF THE CYTOPLASM?

The described mechanism of tip growth is mainly based on a combination of the steady-state model of hyphal growth (138) and the vesicle supply center model (7, 9). In this model, the cytoskeleton supports delivery of membranes to the expanding hyphal tip and might also determine the rate of growth by moving the Spitzenkörper forward. Although still controversial, it is currently assumed that the cellular turgor pressure provides the driving force for this tip expansion (138). In partic-

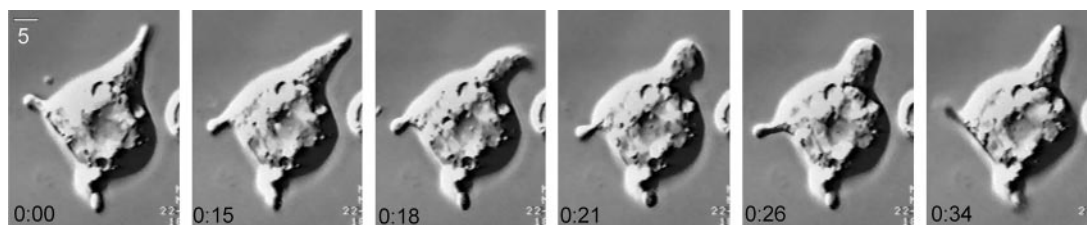


FIG. 5. Motility of a wall-less *Neurospora crassa* mutant cell in phosphate-buffered saline. Cells taken from solid medium show amoeboid movement in osmotically stabilizing buffer. The formation of cellular extensions in the absence of a cell wall indicates that internal cytoplasmic forces are generated that might involve the cytoskeleton. Elapsed time is given in minutes:seconds. Bar, 5 μm . Shown is strain fz;sg;os-1 ("slime"; FGSC 1118), which is defective in (1-3)- β -D-glucan synthase. The images were taken from the movie available at <http://www.mpi-marburg.mpg.de/downloads/>.

ular in plant infection, the fungal cell uses considerable turgor pressure to overcome physical barriers, such as the outer wall of the plant epidermis (58, 80, 132). However, tip growth can occur in the absence of cytoplasmic turgor pressure (78, 79), and it was suggested that additional cytoskeleton-based mechanisms might exert force on the tip (47, 50, 77). Assuming that turgor is the only cytoplasmic force exerted on the wall, the fungal cytoplasm must be considered to be an inactive bag that is held in shape by the rigid cell wall, a view that is supported by the observation that spherical protoplasts are formed when the fungal cell wall is removed. However, the fungal cytoplasm is able to contract during growth of hyphae (100, 101). Furthermore, amoeboid motility is observed in a wall-less *Neurospora crassa* "slime" mutant (25) that is defective in the synthesis of the cell wall compound (1,3)- β -D-glucan (21). This mutant produces cell-wall-deficient fungal cells that are unable to form hyphae, but should behave like wild-type cells of *Neurospora crassa* in all other respects. Surprisingly, cells of this mutant strain in iso-osmotic liquid form pseudopodia-like ex-

tensions that extend into the buffer and move with "waving" motions (Fig. 5; see supplementary movie at <http://www.mpi-marburg.mpg.de/downloads/>) (50). Cells placed on protamine sulfate-coated coverslips (for experimental details, see reference 118) occasionally form thin cytoplasmic extensions that "crawl" over the surface at $\sim 4.5 \mu\text{m min}^{-1}$ (Fig. 6; see Movie S3 in the supplemental material). Unfortunately, these cytoplasmic extensions are extremely sensitive to any experimental manipulation, and therefore the role of the cytoskeleton in their formation is presently elusive. However, these results clearly demonstrate that the fungal protoplast is far from being a passive bag. Instead, it might be considered to be an amoeba that crawls within an extracellular tube, a concept initially introduced by Reinhardt (99). This amoeboid model should also be considered in an integrative concept of hyphal tip growth.

SUMMARY AND FUTURE DIRECTIONS

Hyphal tip growth is a typical feature of filamentous fungi and is an essential requirement for fungal pathogenicity (71, 133). When considering the recent progress in fungal cell biology summarized in this article, a picture of the mechanism of tip growth emerges (Fig. 7). Sterol-rich membrane rafts appear to mediate the localization of the polarisome at the tip of the hypha. Formins, components of the polarisome, foster actin polymerization and anchor the plus ends of actin filaments at the growing tip. Myosin-5 motors utilize this actin orientation to deliver exocytic vesicles to the plasma membrane. Myosin-5 and F-actin might also be involved in maintaining the integrity of the Spitzenkörper. The Spitzenkörper also contains ribosomes, which might receive the mRNA by MT-based transport from the nucleus to the hyphal tip. The Golgi apparatus, which is concentrated in the apical 5 to 10 μm of the hypha, releases secretory vesicles that carry newly synthesized proteins to the apex. These Golgi-derived vesicles are most likely delivered to the Spitzenkörper by the activity of molecular motors along fibers of the cytoskeleton. Studies with *Ustilago maydis*, *Aspergillus nidulans*, and *Candida albicans* have demonstrated that only three classes of kinesins (classes 1, 3, and 7) and three types of myosins (classes 1, 5, and 17) are involved in polarized growth. It emerges that individual motors perform multiple functions. For example, kinesin-3 supports secretion, but also mediates tip-ward traffic of early endosomes, which take up endocytosed material from primary endocytic transport vesicles and sort their content back to the surface or to the vacuole

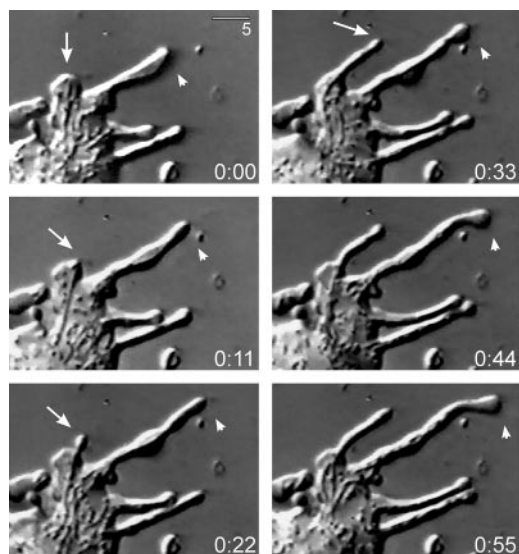


FIG. 6. Motility of a wall-less *Neurospora crassa* mutant cell attached to a protamine sulfate-coated coverslip. The cell is able to push out cytoplasmic extensions that remain attached and thereby "crawls" over the surface (arrow and arrowhead). Elapsed time is given in minutes:seconds. Bar, 5 μm . Shown is strain fz;sg;os-1 ("slime"; FGSC 1118), which is defective in (1-3)- β -D-glucan synthase. The images were taken from the Movie S3 in the supplemental material.

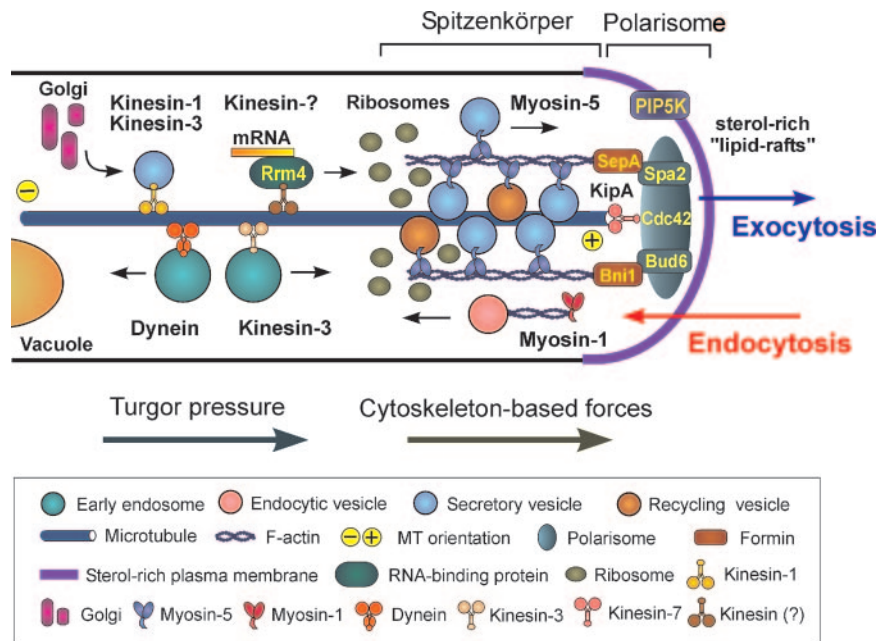


FIG. 7. A model of hyphal tip growth. Sterol-rich membrane domains are concentrated at the hyphal apex, where they might facilitate endocytosis. Myosin-1 participates in initial steps of endocytosis by polymerizing actin, which “rocks” vesicles away from the membrane. In addition, lipid rafts could provide a scaffold for apical proteins, such as phosphatidylinositol-4-phosphate 5-kinase (PIP5K) (62), that generate a functional microdomain, which might recruit components of the polarisome (Bud6 and Spa2) and Cdc42 (20, 61). Spa2 and Bud6 bind formins (26, 35), which themselves participate in nucleation and polarization of the F-actin cytoskeleton (64, 95). Consequently, tip-ward delivery of exocytic vesicles becomes possible. MTs might be anchored at the tip via kinesin-motors (63), which supports long-distance kinesin-1- and kinesin-3-based transport (108) that delivers vesicles, but also might take mRNA to the tip (11), where translation at apical ribosomes (40, 56) and further processing in the endoplasmic reticulum and the apical Golgi (103) happen. Note that the endoplasmic reticulum also concentrates in the hyphal tip (135), but was left out for simplicity. Short-distance transport might be mediated by myosin-5 motors (108, 133). Vesicles are stored in the Spitzkörper, which determines hyphal growth by controlled exocytosis. Cytoplasmic forces that could be generated by turgor pressure and cytoskeleton-based dynamics, both pushing the flexible apex forward. Note that the presented model is based on data from several model systems, including *Fusarium acuminatum*, *S. cerevisiae*, *Ashbya gossypii*, *A. nidulans*, *C. albicans*, and *U. maydis*.

for degradation. Endocytic vesicles might be formed at the sterol-rich apical domains, and myosin-1-dependent actin polymerization appears to be involved in the initial steps of endocytosis.

Although recent progress in several areas of fungal cell biology has begun to elucidate the mechanism of hyphal tip growth, much needs to be learned. Among the open questions is the role of lipid rafts in hyphal tip growth. It appears that sterol-rich rafts polarize the cytoskeleton. How sphingolipids themselves are concentrated at the hyphal tip is not understood, but there is evidence for involvement of actin and myosin-1 (72, 122). Furthermore, neither the cargo of motor proteins nor their functional interplay is known. Apparently, motor proteins depend on each other and form functional networks (67, 108), but how their activity is fine-tuned is unknown. It is almost certain that unexpected functions of the cytoskeleton, such as the role of kinesin and dynein in equal distribution of a POD6/COT1 kinase complex in *Neurospora crassa* (110) or in transport of mRNA-binding proteins (11), await discovery. Whereas mRNA-binding proteins, and therefore most likely mRNA, are taken to the tip by MT-based mechanisms, the Golgi apparatus concentrates in the hyphal tip by means of the actin cytoskeleton (103). Thus, the cytoskeleton polarizes the secretion machinery, and it will be most interesting to elucidate the mechanism of apical protein synthesis in further detail. Finally, whether turgor pressure

alone is the driving force of hyphal elongation is questionable. It appears that the cytoplasm itself has the capacity to push the tip forward. The ability of cell-wall-less mutants of *Neurospora crassa* to form cytoplasmic extensions argues for mechanistic similarities between animal cell migration and fungal tip growth. Directed cell motility in animal cells is based on stable focal adhesion contacts with the substrate and subsequent motor activity and polymerization of the cytoskeleton (93). No fungal orthologues of animal focal adhesion plaque proteins, such as paxillin or vinculin, have been found (unpublished results), which raises questions about a similar “crawling” mechanism of the fungal protoplast within the cell wall tube. Alternatively, fungal motors that are able to slide MTs against each other (16) or along the cellular cortex (30), as summarized in reference 117, might exert force on the plasma membrane. However, the importance of such a cytoplasmic force in hyphal elongation remains to be confirmed.

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