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Interdependence of the actin and the microtubule cytoskeleton during fungal growth

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Cell polarization is a theme in biology conserved from bacteria to man. One of the most extremely polarized cells in nature is the hyphae of filamentous fungi. A continuous flow of secretion vesicles from the hyphal cell body to the tip is essential for cell wall and membrane extension. Microtubules (MTs) and actin, along with their corresponding motor proteins, are involved in the secretion process. Therefore, the arrangement of the cytoskeleton is a crucial step to establish and maintain polarity. Here we review recent findings unraveling the mechanism of polarized growth with special emphasis on the role of the actin and MT cytoskeletons and cell end markers linking the two cytoskeletons. We will mainly focus on *Neurospora crassa* and *Aspergillus nidulans* as model organisms.

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Introduction

Filamentous fungi are highly polarized eukaryotic cells, which continuously elongate their hyphae at the tips. In distal parts, hyphae can initiate new sites of polar growth in the process of branch formation. The establishment and maintenance of polar growth is a fascinating question in biology [1–3]. Some filamentous fungi are pathogen to animals and plants and often growth in the host is accompanied by a dimorphic switch from hyphal growth to yeast-like growth or *vice versa* [4]. Other fungi are useful in biotechnology, such as for enzyme production, and fermentation in food industry due to their high ability for enzyme secretion [5]. Thus, the analysis of polarized growth of filamentous fungi can contribute to medical, agricultural and biotechnological fields.

The actin cytoskeleton

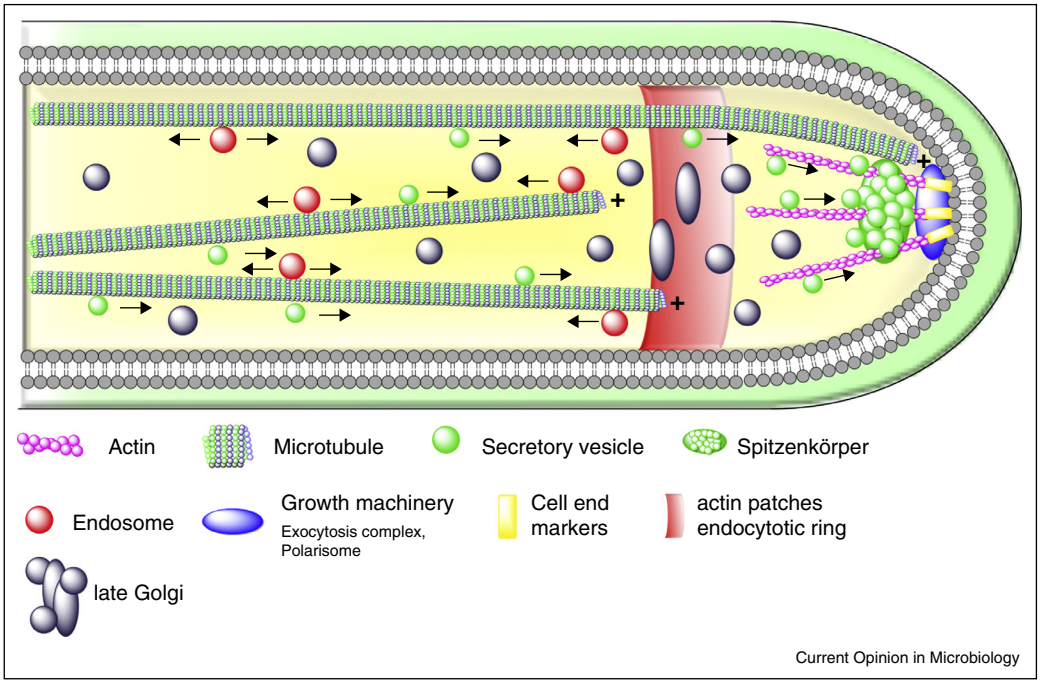
The actin cytoskeleton plays a central role in cell morphogenesis of filamentous fungi [6,7]. There are three high order F-actin structures with distinct functions: actin rings, patches, and cables. The actin rings in cooperation with myosin II function in septum formation [8••,9]. Actin patches are peripheral punctate structures, which localize to regions where also probably the endocytic machinery is located [10•]. The predominant localization of these patches at subapical regions suggests spatial coupling of apical exocytosis and subapical compensatory endocytosis (Figure 1) [11], in addition to endocytic recycling of polarized material at the hyphal tip [12].

The very dynamic actin cables are generally very difficult to visualize. However, recently specific markers, such as Lifeact and tropomyosin were developed [9,13••,14]. Actin cables are present at the apex of hyphae and are thought to serve as tracks for myosin V-dependent secretory vesicle transport to the tip (Figure 1) [6,8••,13••]. The 'basic' growth machinery involved in the formation of actin cables, vesicle transport and exocytosis, such as formin, the polarisome, myosin V and the exocyst complex are relatively conserved among eukaryotic cells and localize to the apex of hyphae (see references in [1,15]). Before fusion with the cell membrane, the secretion vesicles accumulate at the hyphal tip in a structure called 'Spitzenkörper' [16,17], a special structure in filamentous fungi, which determines growth direction of the hyphae [18] (Figure 1). The exact composition and organization is still not completely understood, although one model proposes that the Spitzenkörper acts as vesicle supply center for growing tips (see Riquelme *et al.* in this issue [19]).

The microtubule cytoskeleton

Microtubules (MTs) play a crucial role during mitosis, but also have additional functions in interphase in filamentous fungi. They are important for the distribution of nuclei or other organelles and serve as tracks for vesicles such as endosomes. Thus they are important for rapid hyphal growth [2,11,20••,21,22]. However, they are not

Figure 1

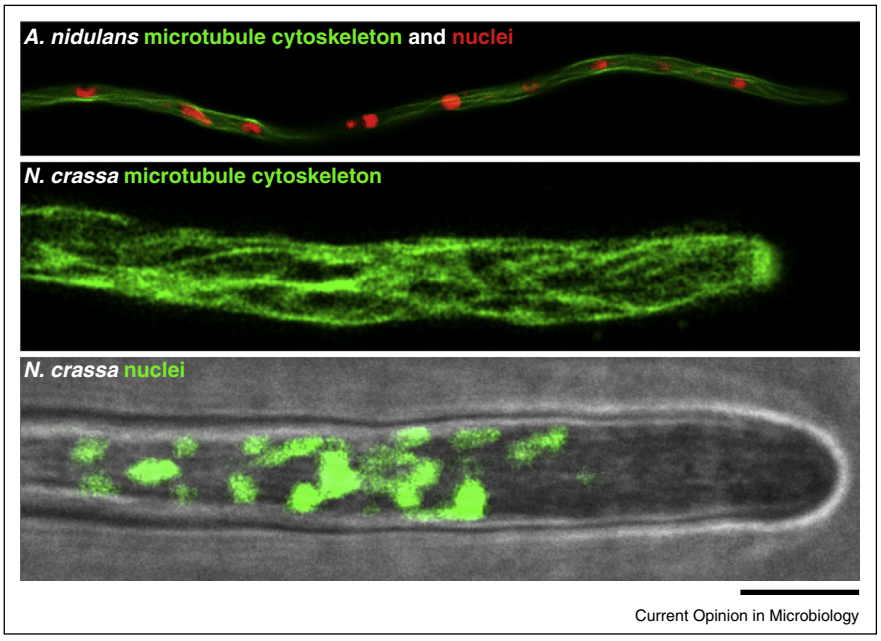


Scheme of an *A. nidulans* hyphal tip showing organelles, cytoskeletons and polarity factors, based on the localization of proteins tagged with fluorescent proteins.

essential for spore germination, but only for site selection of germination [23,24].

The rather stable minus end of MTs is located at the MT-organizing center (MTOC), whereas the plus end is

Figure 2



Microtubule and nuclear arrangement in *A. nidulans* and *N. crassa*. **(a)** The *A. nidulans* microtubule cytoskeleton was labeled with GFP::TubA (α-tubulin), and nuclei were labeled with DsRed::StuA(NLS). Scale bar equals 12 μm. **(b)** The microtubule cytoskeleton was labeled with Bml::sGFP (β-tubulin) in *N. crassa*. Scale bar equals 10 μm. **(c)** *N. crassa* nuclei were labeled with hH1::sGFP (Histone H1) merged with the corresponding bright field image. Scale bar equals 10 μm.

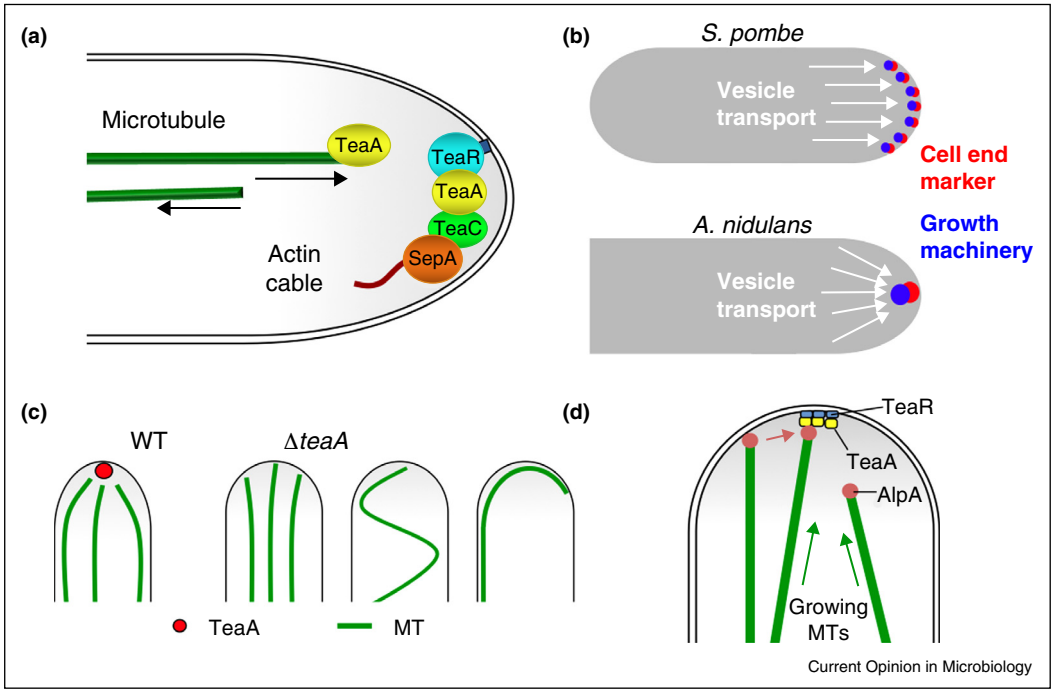
facing to the cell periphery with alternating growing and shrinking phases. In filamentous fungi, spindle pole bodies (SPB) serve as MTOCs [25]. They contain γ-tubulin, first discovered in *Aspergillus nidulans*, which is required for nucleation of MTs [25,26]. Furthermore, there is good evidence that areas close to the septa act as MTOCs in *A. nidulans* (sMTOCs) [27–29]. The composition of those MTOCs remains elusive.

In the tip compartment of *A. nidulans*, most MTs are oriented with their dynamic plus ends toward the hyphal tip [30]. Nuclei migrate probably along MTs until they reach a certain position. The entire hypha looks therefore very organized with equally spaced nuclei (Figure 2) (suppl. movie 1). Similar situations are found in other fungi. However, the organization and perhaps also the mechanisms of organelle distribution appear to be quite different in *Neurospora crassa*. Hyphae of *N. crassa* contain more than 100 nuclei in the apical compartment and many more MT filaments than *A. nidulans* (Figure 2) (suppl. movies 2, 3) [31]. This could be the reason for the much faster growth of *N. crassa* (8.4 ± 5 μm/min) than *A. nidulans* (0.5 ± 0.2 μm/min) [21,32•]. In *N. crassa* another very interesting phenomenon can be observed, namely a massive bulk flow of the cytoplasm. This bulk flow moves nuclei but also the entire MT cytoskeleton forward. The exact mechanism is not well understood yet [32•]. Another big difference is the regulation of mitosis. Whereas nuclear division is nearly synchronized in *A. nidulans* it is not synchronized in *N. crassa* [33,34].

Two classes of MT-dependent motors, the minus end-directed dynein and the plus end-directed kinesins, are involved in positioning of organelles and transport of membranes. Whereas genomes of filamentous fungi contain a single dynein motor, they usually encode 10–12 kinesins [35]. The function of kinesin-3 and the dynein motor in the transport of early endosomes has been extensively studied (see Steinberg in this issue) [2,36,37]. Studies of *A. nidulans* kinesin-3 implicated indirect evidence for the existence of a subpopulation of detyrosinated MTs [29]. However, a final proof for the existence of posttranslationally modified tubulin in fungi is still missing.

The deletion of conventional kinesin (kinesin-1) in different fungi decreased the growth rate, and caused defects in Spitzenkörper stability, protein secretion and pathogenicity [38–41,42••]. These results suggest a possible conserved role in vesicle transport similar to higher eukaryotic cells. Secretory vesicles are thought to be transported by kinesin-1 along MTs for long distances toward hyphal tips in filamentous fungi, although the localization of the ER and the Golgi close to hyphal tips raises questions about the function and cargoes of kinesin-1 [43,44]. Therefore, long distance transport of secretion vesicles could be less important and actin-dependent movement could be rather

Figure 3



(a) Scheme of the function of cell end markers in *A. nidulans*. **(b)** Comparison of the localization of cell end markers and the growth machinery in *S. pombe* and *A. nidulans*. **(c)** Behavior of MTs at hyphal tips in *A. nidulans* wild type and Δ*teaA* strains. **(d)** Scheme of the interaction between TeaA at the hyphal tip cortex and AlpA at MT plus ends.

sufficient for polarized growth. Indeed, hyphal extension can occur for a long time without functional MTs, but is immediately stopped if the integrity of the actin cytoskeleton is disturbed [7,21]. Although the dependency of MT and cytoskeletons could be diverse in different fungi, vesicle movement and delivery to the tip plasma membrane likely depends on the cooperation of actin and MT-dependent motors [8••,42••,45••].

Interaction of microtubules and actin through cell-end markers

Cell-end markers link the MT and the actin cytoskeletons and function as polarity markers at hyphal tips in *A. nidulans* and probably in other fungi (Figures 3a and 4) [3]. One of the cell-end markers, TeaA, is delivered by growing MTs to the tip, and is anchored at the membrane through the interaction with the prenylated TeaR [24,46]. At the hyphal tips, TeaA interacts with additional components, which ultimately recruit the formin SepA. SepA polymerizes actin cables required for exocytosis and polarized growth [47,48••]. Thus, MTs transmit positional information for actin cytoskeleton formation through the delivery of the cell end markers to the tip of hyphae. Therefore, cell-end marker deletion strains show defects in growth direction, which leads to curved or zigzag growing hyphae [46].

The role of MTs in transmitting positional information through delivery of cell end markers to the growth machinery was discovered in *Schizosaccharomyces pombe* [49–51]. The main components are conserved in filamentous fungi (Figure 4). A Spitzenkörper, however, can only be observed in filamentous fungi but not at cell ends of fission yeast. This difference could be due to different growth speeds [52]. Another possible reason is that the cell-end markers concentrate at the apex of hyphae in *A. nidulans*, whereas the cell-end markers localize at multiple sites along cell ends in fission yeast (Figure 3b) [53]. The positive feedback loop defined through the interdependence of TeaA and TeaR could be important for their concentration, but not sufficient because this mechanism is conserved in *S. pombe* as well [46,51,54]. MTs in *A. nidulans* elongate toward the tips and tend to converge in the apical region [30], which is not

Figure 4

	TeaA	TeaR (TeaA receptor)
Ascomycota		
Eurotiomycetes		
Aspergillus nidulans	TeaA, AN4564	TeaR, AN4214
Aspergillus fumigatus	EDP53916	EDP55945
Aspergillus oryzae	XP_001822022	XP_001816930
Penicillium oxalicum	PDE_05396	PDE_02684
Sordariomyceta		
Neurospora crassa	NCU00622	NCU03667
Fusarium oxysporum	EGU82635	EGU88823
Trichoderma reesei	EGR47516	ETR99536
Magnaporthe grisea	MGG_02875	MGG_06768
Saccharomycotina		
Saccharomyces cerevisiae	Kel1, YHR158C	Not found
Candida albicans	EEQ43114	Not found
Yarrowia lipolytica	XP_503123	Not found
Taphrinomycotina		
Schizosaccharomyces pombe	Tea1, NP_588351	Mod5, NP_595317
Basidiomycota		
Agaricomycotina		
Coprinopsis cinerea	CC1G_01765	CC1G_07192
Cryptococcus neoformans	CNAG_01149	CNAG_01856
Ustilaginomycotina		
Ustilago maydis	UM15019	UM01554
Zygomycota		
Mucormycotina		
Mucor circinelloides	EPB85432	Not found

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Orthologues of cell end markers in different fungi.

observed in *S. pombe*. The central position of TeaA at the tip correlated with the convergence of the MT plus ends to a single point. In the absence of TeaA MTs often contacted the membrane off the center of the apex (Figure 3c) [46,48••].

A recent study showed that a functional connection between TeaA and the MT polymerase AlpA is required for proper regulation of MT growth at hyphal tips [48••]. AlpA is a member of the XMAP215/Dis1 family whose conserved TOG domains, which contain multiple HEAT repeats, are known to bind tubulin from yeast to human [55]. XMAP215 from *Xenopus laevis* catalyzes the addition of tubulin dimers to the growing plus ends [56•,57]. *A. nidulans* AlpA decorates MT filaments and accumulates at MT plus ends [58]. Deletion of *alpA* resulted in a drastic reduction of the MT array and dynamics. MT *in vitro* polymerization assays with purified tubulin from porcine brains and recombinant AlpA has revealed the activity of AlpA as a MT polymerase [48••]. The MT growth speed *in vitro* was comparable with that of XMAP215 of *X. laevis* and approximately 4-fold higher than that of Alp14, the orthologue in *S. pombe* [56•,59•]. The rate of MT polymerization *in vivo* in *A. nidulans* leading hyphae is approximately 3-fold higher than in *S. pombe*, consistent with the ratio *in vitro* [60,61]. However, AlpA-dependent MT growth speed *in vitro* was approximately only half of the one determined *in vivo* (6.3 ± 0.2 μm/min compared to 13.2 ± 3.4 μm/min). Therefore, other +TIPs are likely to enhance the AlpA activity for MT growth *in vivo*.

As a difference to *S. pombe*, *A. nidulans* TeaA is involved in the convergence of MT plus ends at the tip apex, suggesting specific interactions of the MT plus end with the cortex (Figure 3c). One possibility is an interaction between TeaA and AlpA [48••]. MT polymerization assays *in vitro* showed that TeaA increased the catastrophe frequency of MTs in the presence of AlpA, and TeaA reduced the *in vitro* AlpA activity significantly. From these results it was concluded that AlpA promotes MT growth at MT plus ends until MTs reach the hyphal tip, where TeaA blocks the AlpA activity and induces MT catastrophe (Figure 3d).

The interdependence of TeaA and MTs could act as a positive feedback loop to concentrate TeaA at the apex, resulting in well-focused vesicle secretion. Vesicle delivery to the tip membrane depends on the cooperation of actin and MT-dependent motors [8••,42••,45••]. If secretory vesicles need to be transferred from MTs to actin cables, MT convergence at the tip and close association to the actin cytoskeleton could guarantee an effective transfer and thus efficient vesicle secretion. The process might be related to the function of actin cables in budding yeast to guide astral microtubule plus-ends for spindle orientation during mitosis [62].

The cell-end markers are generally conserved in fungi, although orthologs of TeaR are not found in Hemiascomycetes (Figure 4). Obvious orthologues are not found outside of the fungal kingdom, although the establishment and maintenance of cell polarity requires the interplay between the actin and MT cytoskeletons and landmark proteins at the cortex also in other eukaryotic cells [63,64]. In higher eukaryotes, IQGAP and/or APC at MT plus ends, site-specific activated Rho GTPase and a downstream effector formin are involved in the MT-induced cortical cell polarity.

On the role of the actin and the microtubule cytoskeleton in pathogens

In many cases, pathogenic fungi are dimorphic and switch between yeast and hyphal forms. Typically, the filamentous form is invasive with exceptions like *Histoplasma capsulatum* in which the yeast form is the virulent form [65]. That means that the establishment and maintenance of polarity to develop the hyphal form is essential for growth in the host. In the case of plant pathogens such as *Magnaporthe oryzae*, the actin and septin cytoskeletons and polarity markers play key roles in pathogenic development [66•].

In the case of the human pathogen *Candida albicans*, MTs and associated proteins are necessary for the morphological changes associated with virulence [67]. The Rho-family small GTPase, Cdc42, and Ca^{2+} influx are required for the rearrangement of the actin cytoskeleton for polarized hyphal growth and the tropic responses of hyphae to environmental cues, such as thigmotropism and galvanotropism [68•].

Conclusion

The fungal cytoskeleton plays a crucial role in polarity establishment, maintenance and polar growth. Comparisons of the cell biology among different fungi reveals conserved roles of cell cytoskeletons but also species-specific differences. Of special interest for future research will be the study of dynamic changes of the polarity machinery providing the basis for numerous morphogenetic changes during cell differentiation and especially during pathogenic development. Fungi are able to produce a variety of different cell types and structures, such as specialized hyphae, reproductive structures, or in case of pathogenic and symbiotic fungi, foot structures, hyphopodia, appressoria, penetration hyphae, haustoria or arbuscules. All these special structures require massive changes of the polarity machinery, which are only at the beginning to be understood. The analysis of dynamic changes of the cytoskeletons in dimorphic fungi and pathogenic development is an exciting research field with a potential to open new avenues for antifungal treatments.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mib.2014.04.005.

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- •• of outstanding interest

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