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MicroReview

Polarized growth in fungi – interplay between the cytoskeleton, positional markers and membrane domains

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Summary

One kind of the most extremely polarized cells in nature are the indefinitely growing hyphae of filamentous fungi. A continuous flow of secretion vesicles from the hyphal cell body to the growing hyphal tip is essential for cell wall and membrane extension. Because microtubules (MT) and actin, together with their corresponding motor proteins, are involved in the process, the arrangement of the cytoskeleton is a crucial step to establish and maintain polarity. In *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe,* actin-mediated vesicle transportation is sufficient for polar cell extension, but in *S. pombe,* MTs are in addition required for the establishment of polarity. The MT cytoskeleton delivers the so-called cell-end marker proteins to the cell pole, which in turn polarize the actin cytoskeleton. Latest results suggest that this scenario may principally be conserved from *S. pombe* to filamentous fungi. In addition, in filamentous fungi, MTs could provide the tracks for long-distance vesicle movement. In this review, we will compare the interaction of the MT and the actin cytoskeleton and their relation to the cortex between yeasts and filamentous fungi. In addition, we will discuss the role of sterol-rich membrane domains in combination with cell-end marker proteins for polarity establishment.

Introduction

The establishment of polarity is a fundamental process in biology. Polarized growth is realized in fungi and is the dominant growth form of filamentous fungi. In single-cell yeasts, such as in budding yeast *Saccharomyces cerevisiae* and in fission yeast *Schizosaccharomyces pombe*, polarized growth is restricted to certain times during the cell cycle, whereas in filamentous fungi, such as *Aspergillus nidulans* or *Neurospora crassa*, cell extension is a continuous and indefinite process (Snell and Nurse, 1994; Pringle *et al*., 1995; Riquelme *et al*., 2003). Filamentous fungi are widely distributed in nature and can cause severe problems as contaminants of food and feed as well as pathogens of plants and animals. Many laboratories are trying to obtain a detailed understanding of the process, because the molecular analysis of polarized growth may lead to the identification of targets for new antifungal drugs. A second important aspect is that filamentous fungi are widely used in biotechnology. It is assumed that heterologously produced hydrolytic enzymes are secreted through the same machinery as the enzymes required for polarized growth (Seiler *et al*., 1997; Pel *et al*., 2007). Here, the understanding of the molecular components might help to increase the production of secreted enzymes or open up new avenues for the production of heterologous proteins.

Polarized growth is studied by genetic, molecular biological, biochemical and cell biological methods. This research field has benefited more than others from the combination of the still ongoing improvement of the microscopic techniques and the development of fluorescent reporter proteins in recent years. Fantastic work has been performed in several laboratories leading to many breakthroughs in *S. pombe* and *S. cerevisiae,* and significant progress in understanding polarized growth in filamentous fungi.

Several overviews have recently summarized different aspects of polarized growth (Chang and Peter, 2003; Nelson, 2003; Xiang and Plamann, 2003; Harris and Momany, 2004; Harris *et al*., 2005; Virag and Harris, 2006a; Fischer, 2007; Steinberg, 2007). In this review, we will focus mainly on the latest findings on the role of the cytoskeleton, and its dependence on and interaction with protein complexes at the growing cell cortex.

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On the role of microtubules and the actin cytoskeleton in S. cerevisiae*,* S. pombe *and* A. nidulans

Microtubules (MTs) grow and shrink in a tread-milling manner if they are polymerized *in vitro*. In contrast, MTs are rather stable at the minus end *in vivo* and are dynamic mainly at the plus end, which exhibits alternating rounds of growth and shrinkage. In most higher eukaryotic cells, MTs distribute radially, and are nucleated from a perinuclear centrosome or MT-organizing centre (MTOC) with their plus ends facing the cell periphery (Keating and Borisy, 1999). In *S. cerevisiae*, the MTOC is localized in the nuclear envelope and named spindle pole body (SPB) (Jaspersen and Winey, 2004). Only few MTs are found in interphase cells and they are disassembled as the mitotic spindle is formed. *S. pombe* has both the SPB and perinuclear MTOCs (Sawin and Tran, 2006) and, in the filamentous fungus *A. nidulans* SPBs, cytoplasmic MTOCs and MTOCs associated with septa are responsible for the formation and maintenance of the MT array (Veith *et al.*, 2005). MTs are oriented along the long axis in the cigar-shaped cells of *S. pombe* as well as in the extremely elongated compartments of *A. nidulans* (Höög *et al.*, 2007). Understanding the regulation of MT formation and their dynamics is one of the main foci of recent research. Proteins called plus-end tracking proteins (+TIPs), because they associate and remain at growing MT plus ends, regulate MT dynamics and are very important for MT–cortex interactions (Akhmanova and Hoogenraad, 2005; Xiang, 2006). In *S. cerevisiae,* these interactions with the cell cortex play crucial roles in positioning of the mitotic spindle; in *S. pombe,* they signal polarity information to the cell cortex; and in *A. nidulans,* they are involved in both nuclear migration and polarity determination (Fig. 1 and Table 1) (Nelson, 2003).

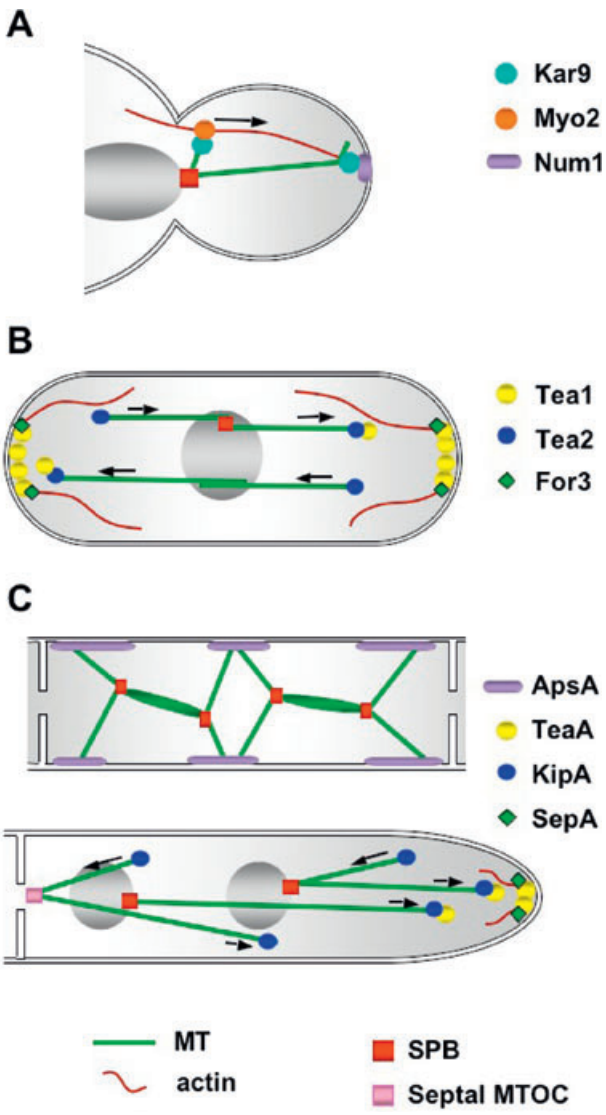

Fig. 1. Scheme of the MT and actin cytoskeleton in *S. cerevisiae*, *S. pombe* and *A. nidulans*.
A. In *S. cerevisiae*, actin cables capture MT plus ends and thereby regulate MT localization and shrinkage to orient the mitotic spindle.
B. On the other hand, interphase MTs in *S. pombe* determine actin cable localization through the deposition of the cell-end marker protein Tea1, which recruits formin.
C. In *A. nidulans*, early results suggest conservation of the *S. cerevisiae* machinery during mitosis (upper panel) and conservation of the *S. pombe* machinery for polarized growth (lower panel). See text for details.

Saccharomyces cerevisiae

In *S. cerevisiae*, cortical capture of astral MTs (MTs formed from the two SPBs during mitosis) establishes spindle polarity. During mitosis, Kar9 directs one SPB towards the bud by linking astral MTs to the actin cytoskeleton (Fig. 1). Kar9 initially localizes to the old SPB, and is transported by the kinesin Kip2 (kinesin-7) along astral MTs to the plus ends (Liakopoulos *et al.*, 2003; Maekawa *et al.*, 2003; Moore *et al.*, 2006). The asymmetric localization of Kar9 depends on its interaction with +TIPs, such as Bim1 and Bik1 (EB1 and CLIP-170 in higher eukaryotes respectively), and the cyclin-dependent kinase Cdc28 (Pearson and Bloom, 2004; Moore and Miller, 2007). Once the growing MTs reach the cortex, Kar9 interacts with class-V myosin Myo2, which in turn pulls Kar9 together with the attached MT along an actin cable towards the growing tip. This leads to proper spindle orientation from the mother cell to the bud (Yin *et al.*, 2000; Hwang *et al.*, 2003). For spindle elongation and movement into the bud, pulling forces mediated by astral MT sliding along the bud cortex are required. In this process, the cortical anchor protein Num1 captures the astral MT plus ends (Farkasovsky and Küntzel, 1995; 2001; Heil-Chapdelaine *et al.*, 2000) and cytoplasmic dynein, also accumulated at the MT plus ends, becomes activated

Table 1. Homologue proteins in three fungi.

	S. cerevisiae	*S. pombe*	*A. nidulans*
+TIPs			
CLIP-170	Bik1	Tip1	ClipA
EB1	Bim1	Mal3	AN2862.3
APC	Kar9	None	None
XMAP215	Stu2	Alp14	AlpA
Dynein (heavy chain)	Dyn1	Dhc1	NudA
Lis1	Pac1	?	NudF
Cortical anchor	Num1 (nuclear migration)[a]	Mcp5 (nuclear oxillation in meiosis)[a]	ApsA (nuclear distribution)[a]
Kinesin-7	Kip2 (nuclear migration defect)[b]	Tea2 (T-shape or bent cell)[b]	KipA (Curved hyphae)[b]
Cell-end marker	Kel1, Kel2 (cell fusion defects during mating)[b]	Tea1, Tea3 (T-shape or bent cell)[b]	TeaA (Zig-zag hyphae)[b]
Cortical receptor	?	Mod5 (T-shape or bent cell)[b]	TeaR (Curved hyphae)[b]
Formin	Bni1, Bnr1	For3, Fus1, Cdc12	SepA
Polarisome	Spa2	SPAC3G9.05	SpaA
	Bud6	Bud6	BudA
Cdc42	Cdc42	Cdc42	ModA
Rac1	None	None	RacA

a. Protein functions or **b.** mutant phenotypes are shown in branckets.

once it contacts the cortex. Kip2 kinesin transports Kar9, dynein and Bik1 to the MT plus end (Carvalho *et al.*, 2004). Bik1 and Pac1 (dynein activator LIS1 homologue) play roles in targeting and activation of dynein at MT plus ends (Lee and Oberle, 2003; Sheeman *et al.*, 2003). In summary, actin cables capture MT plus ends through the interaction of class-V myosin with Kar9, and thereby regulate MT localization and shrinkage to orient the mitotic spindle. MTs are not necessary for polarized growth of the emerging bud.

Schizosaccharomyces pombe

In *S. pombe*, the molecular function of astral MTs in spindle alignment and elongation remains unclear, and a functional counterpart of Kar9 has not yet been identified. Nevertheless, a Num1 homologue, Mcp5, exists and appears to perform similar functions as in *S. cerevisiae*, the contact between astral MTs and the cortex (Yamashita and Yamamoto, 2006). However, in contrast to *S. cerevisiae,* Mcp5 shows meiosis-specific expression and localizes at the cell cortex during meiosis. Deletion of the gene caused a lack of nuclear oscillations during the meiotic prophase. In contrast to *S. cerevisiae*, in *S. pombe*, interphase MTs have a function in signalling polarity information to the cell ends. MT plus ends normally keep elongating until they reach the cell ends, and then shrink. This intrinsic characteristic of MTs is used to transport and deliver the cell-end marker protein, Tea1 (tip elongation aberrant), to the cell ends (Mata and Nurse, 1997). Tea2 (kinesin-7) transports Tea1 to MT plus ends (Browning *et al.*, 2000; 2003). The proteins were indentified by screening for strains with bent and T-shaped cells (Snell and Nurse, 1994). Tea1 is crucial for the formation of a protein complex that organizes the actin cytoskeleton (see blow). Secretion vesicles are then transported along the actin filaments to support cell growth.

The regulation of MT dynamics is essential for the signalling of polarity information. Two +TIPs, Tip1 (CLIP-170) and Mal3 (EB1), are important for suppressing MT catastrophe (the growth to shrinkage transitions) (Brunner and Nurse, 2000; Busch and Brunner, 2004). In Δ*tip1* cells, MTs initiate catastrophe anywhere the MT plus ends contact the cortex and, in Δ*mal3* cells, MTs undergo catastrophe even before they reach the cortex. As a result, these mutants have shorter MT bundles. The mutants with abnormal MTs show defects in polarized growth and exhibit bent or T-shaped cells. Tea2, which transports Tea1 to MT plus ends, also transports Tip1, and thus Δ*tea2* cells also exhibit same defects in MTs and polarized growth (Browning *et al.*, 2000; 2003; Busch *et al.*, 2004).

Aspergillus nidulans *and other filamentous fungi*

In *A. nidulans,* MTs are required for nuclear migration and positioning as in *S. cerevisiae.* An *apsA* mutant, lacking the *S. cerevisiae* Num1 homologue, exhibits a defect in nuclear distribution (Fischer and Timberlake, 1995; Suelmann *et al.*, 1998; Veith *et al.*, 2005). Although alignment of mitotic spindles is not required in syncytial fungal compartments, deletion of *apsA* leads to a lack of spindle oscillations. This indicates that mitotic spindles are held in place through contacts with astral MTs on each side of the spindle and the cortex. How and whether interphase nuclear distribution is regulated by MT–cortex interactions are still unclear. Because a Kar9 homologue cannot be detected in the genomes of Aspergilli, it is also not clear whether the actin cytoskeleton is involved in MT–cortical interactions.

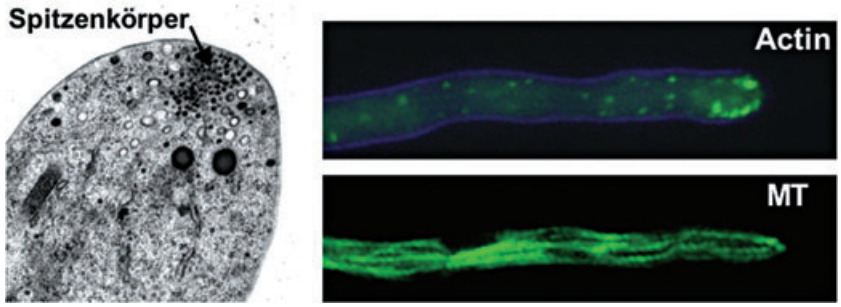

Fig. 2. The Spitzenkörper, the actin and the MT cytoskeleton in *A. nidulans*. left: transmission electron micrograph of a hyphal tip. The small vesicles accumulating in the Spitzenkörper (SPK) are visible. Actin (right, upper picture) and MTs (right, lower picture) visualized in a hyphal tip as GFP fusion proteins. The actin-GFP distribution shows only the presence of actin patches. Actin cables are only rarely visible in *A. nidulans* (Araujo-Bazan *et al.*, 2008; Taheri-Talesh *et al.*, 2008). The left picture was provided by B. Richardson (Athens, GA).

It was reported recently that interphase MTs also play a role in signalling polarity information to the hyphal tips in *A. nidulans*, as described in *S. pombe*. MTs in *A. nidulans* have mixed polarities in hyphal compartments, but the tip compartment contains two to eight MTs, most of which are oriented with their plus ends towards the tip and merge at one point (Fig. 2) (Konzack *et al.*, 2005; Sampson and Heath, 2005). Proteins such as Tea1 and Tea2 appear to be conserved in *A. nidulans* as TeaA (Tea1) and KipA (Tea2) respectively (Konzack *et al.*, 2005; Takeshita *et al.*, 2008). Although overall sequence similarity between Tea1 and TeaA is only 27%, the architecture of the two proteins is similar. KipA localizes to MT plus ends and regulates the position of TeaA at hyphal tips (Fig. 3) (see below). Knockout mutants in the respective genes show defects in maintenance of polarized growth and exhibit curved or zigzag-shaped hyphae.

The regulation of MT dynamics by +TIPs, ClipA (CLIP-170) and KipA is different in *A. nidulans* from that in *S. pombe*. In Δ*clipA* mutants, more MTs fail to undergo long-

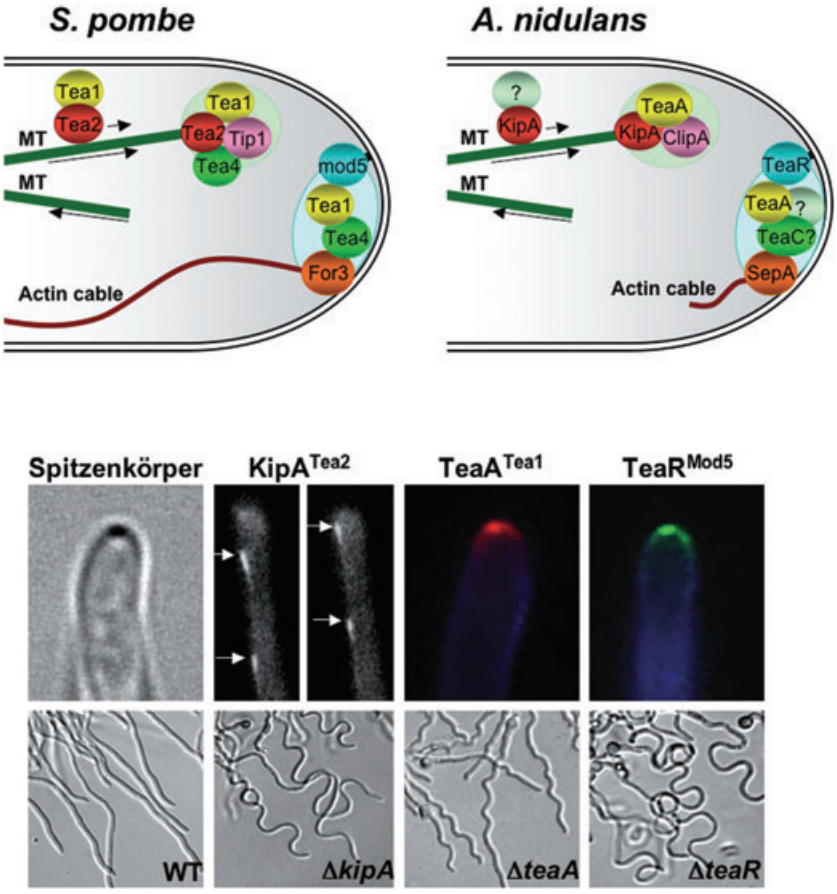

Fig. 3. Scheme of the transport of cell-end markers in *S. pombe* and *A. nidulans*, localization of the corresponding proteins as GFP or mRFP fusion proteins and phenotypes of the corresponding mutants, as indicated. Taken from Konzack *et al.* (2005) and Takeshita *et al.* (2008). For details see the text. TeaC is the homologue of Tea4. GFP-KipA spots moved towards the tip (time, left at 0 and right after 12 s). mRFP1-TeaA or GFP-TeaR produced under native promoter control localized to one point at the tip and along the tip membrane (upper panels). Differential interference contrast images of wild type, Δ*kipA*, Δ*teaA*, Δ*teaR* strains. Δ*kipA* and Δ*teaR* strains exhibited curved hyphae and Δ*teaA* strains exhibited zigzag hyphae (lower panels).

range growth towards the tip but MTs that reach the tips are less likely to undergo catastrophe (Efimov *et al*., 2006). Moreover, in Δ*kipA* mutants, MTs elongate to the tips but do not merge in one point (Konzack *et al*., 2005), whereas the *S. pombe tip1* (CLIP-170) or *tea2* mutants have shorter MTs. Whereas Tea2 is required for Tea1 and Tip1 transport in *S. pombe*, in *A. nidulans*, *kipA* deletion only partially affects MT plus-end localization of ClipA at an elevated temperature and KipA is not required for TeaA accumulation at the hyphal tip but is needed for correct TeaA positioning (Efimov *et al*., 2006; Takeshita *et al*., 2008). These differences remain unexplained so far.

A third role for MTs in *A. nidulans* and other filamentous fungi is probably an involvement in vesicle transport. Hyphal tip growth in filamentous fungi is a complex and regulated process, which involves the synthesis and plasticity of the cell wall, transport and extension of the membrane, diverse cytoplasmic movements, turgor pressure and certain ion gradients (Torralba *et al*., 2001; Virag and Harris, 2006a). A structure named the Spitzenkörper localized in the apical dome of the hyphae is involved in polarized growth (Fig. 2) (Girbardt, 1957). It represents an accumulation of vesicles and determines growth direction of fungal hyphae (Grove and Bracker, 1970; Riquelme *et al*., 1998). The exact structure and organization are still not completely understood. It is thought to act as vesicle supply centre (VSC) for growing tips. According to a model, vesicles are delivered to the Spitzenkörper via MT-dependent transportation and from the Spitzenkörper to the cortex via actin-dependent vesicle movement. Already, early ultrastructural studies with *Fusarium acuminatum* supported this model. Vesicles were observed closely associated with MTs and, after MT disassembly with the anti-MT agent benomyl, intracellular vesicle transport appeared to be inhibited (Howard and Aist, 1980; Howard, 1981). However, these experiments did not unambiguously show that MTs are required as tracks for vesicles, because the observations were done in fixed cells. In addition, anti-MT drugs not only disassemble cytoplasmic MTs but also inhibit mitosis. Thus, secondary effects could also occur and account for the observed changes in the appearance of the Spitzenkörper and growth inhibition. More direct evidence for the involvement of MTs in long-distance vesicle movement comes from the study of the role of MT-dependent kinesins. For instance, the deletion of conventional kinesin (kinesin-1) in *A. nidulans* or *N. crassa* reduced the growth rate significantly (Seiler *et al*., 1997; Requena *et al*., 2001). Especially in *N. crassa*, the kinesin-1 mutant showed defects in Spitzenkörper stability and protein secretion (Seiler *et al*., 1997; 1999), suggesting a possible role in vesicle transportation similar to the role in neurons. Similarly, Unc104-like kinesins (kinesin-3) in *A. nidulans* and the dimorphic fungus *Ustilago maydis* also have roles in hyphal growth (Schuchardt *et al*., 2005) (N. Zekert, pers. comm.). These kinesins are not essential for growth but are necessary for fast extension of hyphae. However, it should be noted that also the kinesin motors could cause secondary effects, which are responsible for the observed growth rate reduction. For instance, it was shown in *A. nidulans* that conventional kinesin is required for dynein targeting to growing MT plus ends (Zhang *et al*., 2003). Another aspect is that MT dynamics could affect the position of other proteins and organelles by pushing the cytoplasmic matrix. Near the tip, most MTs are growing towards the cortex and could thereby generate a forward-directed cytoplasmic matrix flow (Sampson and Heath, 2005; Mouriño-Pérez *et al*., 2006). Stronger evidence for the importance of secretion for polarized growth came recently from a study on exocytosis (Taheri-Talesh *et al*., 2008). In this paper, the authors used GFP-tagged markers for exocytotic vesicles and studied the secretion in live cells. They showed that exocytotic vesicles accumulate in the Spitzenkörper, and are transported from there to the membrane. Recycling of the proteins occurs by endocytosis, which is localized in a zone a few micrometres behind the growing tip. Thus exocytosis and endocytosis are linked processes and are both required for tip extension. The importance of endocytosis for polarized growth was also shown by Araujo-Bazan *et al.* (2008).

Whereas actin-dependent vesicle secretion is necessary for growth in all fungi, the importance of MTs appears to be different. In *Candida albicans*, a fungus that can switch between budding and filamentous growth, as well as in the constitutively filamentously growing *Ashbya gossypii,* MTs are not necessary for filamentous growth (Alberti-Segui *et al*., 2001; Rida *et al*., 2006). The importance of the actin cytoskeleton in *S. cerevisiae*, *C. albicans* and *A. gossypii* may be also reflected in the fact that they have two to three formins, that actin cables are well established in the cell and that Cdc42, small GTPase and master regulator of actin organization are essential for polarity establishment (Wendland and Philippsen, 2001; Bassilana and Arkowitz, 2006). In contrast, the filamentous fungi *A. nidulans* and *N. crassa* have only one formin, actin cables are rarely observed (Torralba *et al*., 1998; Virag and Griffith, 2004; Araujo-Bazan *et al*., 2008; Taheri-Talesh *et al*., 2008) and Cdc42 deletion does not show severe morphological phenotypes in *A. nidulans* (Virag *et al*., 2007). The poorer actin cytoskeleton in filamentous fungi may suggest that the actin cytoskeleton is necessary but not sufficient for hyphal growth and that MTs are required in addition.

The role of molecular motors for polarized growth

Three classes of cytoskeleton-dependent motor proteins, kinesins, dynein and myosin, are involved in the transport

of proteins, vesicles and organelles. According to the latest nomenclature, kinesins are grouped into 14 families (kinesins 1–14) and one orphan family (Lawrence *et al.*, 2004). The number of kinesins in fungi ranges from six in *S. cerevisiae*, to nine in *S. pombe*, 10 or 11 in *N. crassa* and *A. nidulans* respectively (Schoch *et al.*, 2003; Rischitor *et al.*, 2004). In comparison, fungi contain a single cytoplasmic dynein (Yamamoto and Hiraoka, 2003). In yeasts, myosins of three families (myosins I, II and V) are conserved, and myosin V transports secretion vesicles to polarization sites.

Three kinesin families (1, 3 and 7) are involved in polarized growth in fungi. As outlined above, members of the kinesin-7 family (Kip2 in *S. cerevisiae*, Tea2 in *S. pombe,* KipA in *A. nidulans*) are used to deliver proteins to the MT plus ends. Kip2 transports Bik1 (CLIP-170), Kar9 (for spindle polarity) and dynein, while Tea2 transports Tip1 (CLIP-170) and Tea1 (for cell polarity). KipA is not essential for ClipA (CLIP-170) and TeaA transport, likewise, dynein accumulation at the MT plus end is independent of KipA but depends on the kinesin-1 family protein conventional kinesin (KinA) (Zhang *et al.*, 2003). Surprisingly, kinesin-7 family kinesins in *U. maydis* have no critical role in polarized growth (Schuchardt *et al.*, 2005).

Members of the kinesin-1 family (conventional kinesin) play important roles in filamentous growth, probably in the transportation of vesicles (Seiler *et al.*, 1997; Requena *et al.*, 2001). However, additional roles were reported, for example, defects on mitochondrial distribution were observed in kinesin-1 mutants of *N. crassa* and *Nectria haematococca,* and defective vacuolar distribution was found in the corresponding *U. maydis* mutant (Lehmler *et al.*, 1997; Steinberg *et al.*, 1998; Wu *et al.*, 1998; Steinberg, 2000). Members of the kinesin-1 family do not exist in *S. cerevisiae*, whereas in *S. pombe* such a kinesin functions in Golgi membrane recycling (Brazer *et al.*, 2000). Hence, it appears that kinesin-1 can bind to different cargoes and, thus, be involved in different cellular processes.

Other kinesins with a role in polarized growth are those of the kinesin-3 family. This motor does not exist in *S. pombe* or *S. cerevisiae* but in *U. maydis*, *N. crassa* and other filamentous fungi. In *U. maydis*, it is involved in endosome transport and necessary for hyphal growth (Wedlich-Söldner *et al.*, 2002a). In *A. nidulans*, one motor of this family is clearly involved in polarized growth but, as in *U. maydis*, double deletion of kinesin-1 and kinesin-3 is not lethal (Schuchardt *et al.*, 2005; N. Zekert, pers. comm.). In comparison, two related kinesin-3 motors in *N. crassa* act together on mitochondrial distribution (Fuchs and Westermann, 2005). Our understanding of vesicle and organelle transport towards the tip is still quite limited and it seems that different motors play different roles in different fungi.

Cytoplasmic dynein has various roles in nuclear migration and organelle transport in fungi (Yamamoto and Hiraoka, 2003; Xiang and Fischer, 2004). The role in nuclear migration has been best studied in *S. cerevisiae*. Dynein mediates the contact of astral MTs to the cortex and slides the MTs on the contact sites by moving along the MTs towards the minus end. Consequently, the nucleus moves to the bud neck and the opposing pulling forces along the cell axis contribute to spindle pole separation (Bloom, 2001; Yamamoto and Hiraoka, 2003). In filamentous fungi, dynein mediates organelle and vesicle transport (Xiang and Plamann, 2003). In *N. crassa*, dynein is involved in retrograde transport of vesicles and a dynein mutant showed defects in the organization and stability of the Spitzenkörper (Seiler *et al.*, 1999; Riquelme *et al.*, 2002). In *U. maydis*, it functions in endoplasic reticulum (ER) organization and endosome transport (Wedlich-Söldner *et al.*, 2002a, b). Moreover, dynein and its regulator accumulated at MT plus ends within the hyphal tips possibly ensure that endosomes reach the tips and contribute to tip growth by endocytic membrane recycling (Lenz *et al.*, 2006).

Myosin function is also studied well in *S. cerevisiae*. Actin cables are nucleated from the bud tip to the mother cell during bud growth and one myosin V, Myo2, transports vesicles and other organelles, such as the Golgi, mitochondria, vacuoles and peroxisomes (Pruyne *et al.*, 2004). Some mRNA molecules such as *ASH1* are transported by another myosin V, Myo4 (Bobola *et al.*, 1996; Shepard *et al.*, 2003). In *S. pombe*, a new daughter cell grows at the previous cell end in a monopolar manner, and then initiates growth at the previous cell division site in a bipolar manner. This phenomenon is named NETO (new end take-off) (Mitchison and Nurse, 1985). Actin cables grow towards the growing cell ends, only towards the old ends before NETO and towards both ends after NETO, and Myo52, a myosin V, is responsible for polarized secretion of vesicles along actin cables and hence membrane enlargement and secretion of cell wall-synthesizing enzymes (Montegi *et al.*, 2001; Win *et al.*, 2001; Mulvihill *et al.*, 2006). Although in filamentous fungi, the function of myosin V is largely unclear, these myosins are required for filamentous growth and pathogenicity in *U. maydis* and *C. albicans* (Weber *et al.*, 2003; Woo *et al.*, 2003; Schuchardt *et al.*, 2005).

If we accept the model of long-distance MT-dependent vesicle transportation and subsequent accumulation in an organelle called VSC or Spitzenkörper and actin-dependent short-distance transportation from the VSC towards the surface, one interesting yet open question is whether different motor proteins (kinesin, dynein and myosin) are always attached to the vesicles or whether they associate with the vesicles as required (Fig. 2).

Cell-end markers and polarity determination

In *S. cerevisiae*, the decision to initiate a new polarized growth site depends on intrinsic factors and is determined by the last budding site. In *S. pombe*, cell growth occurs first at the previous cell division site, and interphase MTs are used to establish the polarity site. In both yeasts, mutation analysis revealed some proteins that act as cortical landmarks. Once the polarity site is marked at the cell cortex, the landmarks regulate localization and activation of cascades of small GTPases (Cdc42 and other Rho-type GTPase) (Chang and Peter, 2003). In *S. cerevisiae*, activated Cdc42 regulates multiple downstream effectors and establishes cell polarity, organizes the actin cytoskeleton and the septin ring, and directs membrane traffic and the formation of membrane compartments (Pruyne and Bretscher, 2000; Park and Bi, 2007). In general, polarity site selection is not essential for polarized growth, but the polarity-establishing machinery is essential for cell polarity.

Interestingly, the initiation of polarized growth in *S. cerevisiae* depends on the genotype at the mating-type (MAT) locus. MAT a or α cells exhibit axial budding, which means that a new daughter bud emerges next to the previous one. On the other hand, diploid MAT a/α cells exhibit a bipolar budding pattern, where a new bud emerges from the opposite pole of the previous daughter (Kron and Gow, 1995). Bud3, Bud4 and Axl2/Bud10 are landmark proteins for the axial budding pattern, and Bud8, Bud9 and Rax2 for the bipolar budding pattern (Madden and Snyder, 1998). In the case of axial budding, the landmark proteins localize to a septin ring. Septins are GTPases, which assemble into a ring structure (septin ring) at the previous bud neck at the end of the cell cycle, and guide new bud formation next to the septin ring. In contrast, the mechanism of landmark protein localization for bipolar budding is not fully understood. Genetic analyses revealed that several other processes besides the septin ring and the timing of *BUD8* and *BUD9* gene expression are involved in the mechanism (Ni and Snyder, 2001; Schenkman *et al*., 2002). In both cases, the landmarks recruit and activate the polarity-establishing machinery, the Cdc42 cascade, at the cell surface. The mediator Rsr1-Bud1 (Ras small GTPase) regulates the link between the landmarks and Cdc42. The landmarks activate Rsr1-Bud1 through the recruitment of its guanine nucleotide exchange factor (GEF) Bud5 (Bender, 1993). Activated Rsr1-Bud1 regulates Cdc42 activity through the recruitment of its GEF Cdc24. Once Cdc42 is activated at the proper site, multiple effectors, such as formins (Bni1, Bnr1), p21-activated kinases (Ste20 and Cla4) and GTPase activating protein (GAP) for the Rab-type GTPase Sec4 (Msb3 and Msb4), lead to the local assembly and orientation of the actin cytoskeleton and vesicle delivery for bud growth. The local Cdc42 activity is amplified in a self-sustaining positive feedback loop (Butty *et al*., 2002; Wedlich-Söldner and Li, 2003).

In *S. pombe,* some of the bud site landmark proteins from *S. cerevisiae* are not conserved. However, other genes were identified by polarity mutant screening (T-shaped or bent cells). Among these were the above-mentioned *tea1* and *tea2* genes, and the novel landmark-encoding *mod5* gene (morphology defective). Mutants of these genes exhibit T-shaped or bent cells as a result of the mislocalization of the polarity site away from the centre of the cell end. Mod5 plays a very important role, because it anchors Tea1 at the cell pole (Fig. 3). Mod5 harbours a CAAX (cysteine, two aliphatic amino acids followed by any amino acid) prenylation motif at the C terminus. The cysteine is covalently prenylated, which anchors the protein in the membrane (Snaith and Sawin, 2003). Tea1 and Mod5, also named cell-end markers, accumulate interdependently at the growing cell ends and contribute to the spatial distribution of actin cables. At the cell ends, Tea1 interacts with a number of additional components, and a large protein complex is formed that includes the formin For3, which nucleates the actin cable assembly, and Bud6, an actin-binding protein (Feierbach *et al*., 2004; Martin *et al*., 2005). Bud6 in *S. cerevisiae* stimulates formin activity and Bud6 in *S. pombe* is required for proper For3 localization (Feierbach *et al*., 2004; Moseley and Goode, 2005). After cell division, Tea1 is delivered to the new end by MTs, and For3 and Bud6 localize there after Tea1 is anchored. Therefore, Tea1 contributes to cell polarity and actin cable organization through the interaction with For3 and Bud6. Their interactions link the MT with the actin cytoskeleton in fission yeast. Transition from monopolar to bipolar growth (NETO) depends on the localization of For3 to the new end and, thus, *tea1* and *bud6* mutants display defects in NETO. Besides these components, Tea4, which links Tea1 and For3, and Tea3, a Tea1-related, Kelch repeat-containing protein, are also necessary for NETO (Arellano *et al*., 2002; Martin *et al*., 2005). Tea3 binds independently to Tea1 and Mod5, and is required for Tea1 anchorage specifically at non-growing cell ends (Snaith *et al*., 2005). Although the contribution of Cdc42 on For3 localization in *S. pombe* is not well understood, relief of autoinhibition of For3 by Cdc42 and/or Bud6 is necessary for For3 localization (Martin *et al*., 2007). Bud6 is also directly or indirectly recruited to the new end by Tea1 and Tea4 (Feierbach *et al*., 2004). Whereas MTs and Tea1 play central roles in the decision of the growth site, they are not required for polarity establishment. This led to models that local self-activation and lateral inhibition are responsible for polarized growth (Castagnetti *et al*., 2007).

S. cerevisiae-type landmarks are poorly or not conserved in *A. nidulans* and other filamentous fungi, leading

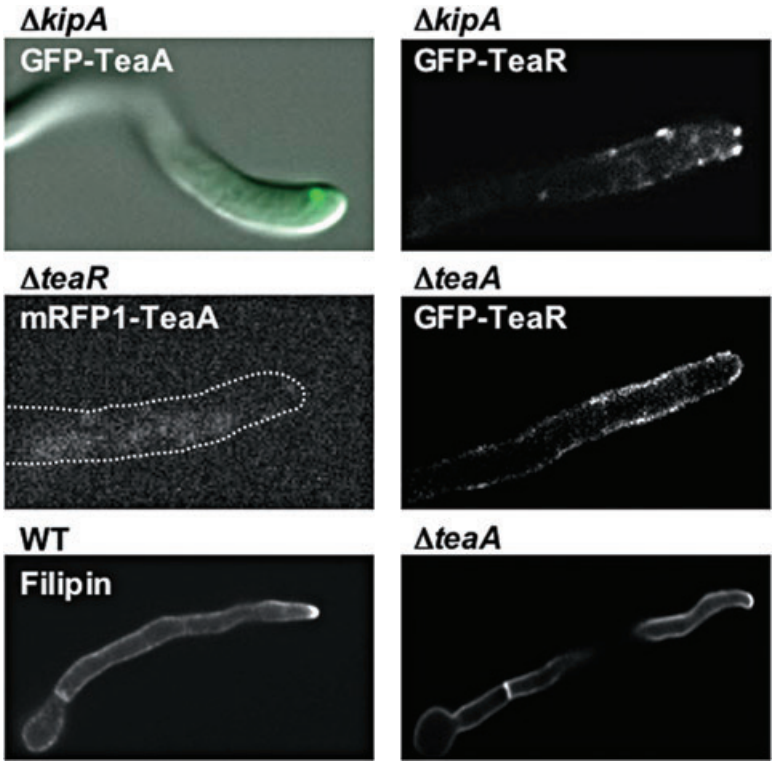

Fig. 4. Interdependence of the localization of cell-end markers and on the KipA motor protein in hyphae of *A. nidulans*. Taken from Takeshita *et al.* (2008). In the Δ*kipA* mutant, GFP1-TeaA still localized to one point at the hyphal tip but often moved away from the centre of the apex (left upper panel) and some GFP-TeaR signal localized at the membrane of the apex and others dispersed along the membrane away from the tip (right upper panel). In the Δ*teaR* mutant, mRFP1-TeaA was not observed at the tip (left middle panel). In the Δ*teaA* mutant, GFP-TeaR lost the preference for the hyphal tip and diffused all along the membrane (right middle panel). wild type (WT) (left lower panel) and the Δ*teaA* mutants (right lower panel) were stained with filipin. Filipin accumulated at the tip and at septa.

to speculations that novel mechanisms could be at work (Harris and Momany, 2004). However, the *S. pombe* cell-end marker Tea1 appears to be conserved in filamentous fungi (Takeshita *et al.*, 2008)(see above). Two Tea1 homologues exist in *S. cerevisiae* (Kel1 and Kel2), where they are involved in cell fusion during mating (Philips and Herskowitz, 1998). Despite the central role of Mod5 for polarized growth in *S. pombe*, a sequence homologue was not identified in *S. cerevisiae* or any filamentous fungus. In recent work, a protein that could act as a membrane anchor for TeaA was discovered in *A. nidulans* and named TeaR (Fig. 3) (Takeshita *et al.*, 2008). TeaR was identified by screening for proteins that harbour a C-terminal prenylation motif. TeaR shows low identity to Mod5 (15.4%) and is conserved in all filamentous fungi whose genomes have been analysed. Deletion of *teaR* produces meandering instead of straight hyphae. This curved hyphal phenotype resembles that of *kipA* mutants. In comparison, *teaA* mutant hyphae display a rather zigzag growth phenotype. Both TeaA and TeaR localize to one point at the tip and along the tip membrane. TeaA was shown to interact with TeaR by split YFP and yeast two-hybrid technology. The localization of both proteins is interdependent, as it is in *S. pombe.* TeaA colocalizes with the formin SepA at hyphal tips. These results suggest conservation of the *S. pombe* polarity site selection mechanism in *A. nidulans*, although the interaction of cell-end marker and formin is still unknown. Homologous protein of Tea4, which links Tea1 and For3 in *S. pombe,* exists in *A. nidulans,* known as TeaC, and its functional analysis is in progress (Higashitsuji *et al.*, unpubl. results). However, the role of KipA appears to be different from that of *S. pombe* Tea2. Whereas Tea2 transports Tea1 to the MT plus end, in *A. nidulans* TeaA still localized to tips in the *kipA*-deletion mutant. Interestingly, KipA is required for correct TeaA and TeaR positioning (Fig. 4). KipA might transport additional landmark and cell-end marker proteins. Although MTs had been demonstrated to be necessary for hyphal tip localization of TeaA (Takeshita *et al.*, 2008), meanwhile the localization of TeaA at MT plus ends was also revealed (Takeshita *et al.*, unpubl. results).

Besides their role in organizing the actin cytoskeleton, the cell-end marker proteins might play a role in organizing the MT cytoskeleton itself. In *A. nidulans*, MT plus ends are centred in the hyphal apex and merge in the TeaA protein spot at the tip (Takeshita *et al.*, 2008). This raises the question about the interaction of MT plus ends with the cell-end markers or landmark proteins. That the integrity of the MT plus-end protein complex is indeed required for cell polarity establishment, besides the transportation of potential cell-end marker proteins, comes from the observation that the lack of the Dis1/XMAP215 protein AlpA causes meandering hyphae in *A. nidulans*, just like the absence of the cell-end marker proteins (Enke *et al.*, 2007). XMAP215 is a processive MT polymerase (Brouhard *et al.*, 2008), and the conserved protein family

promotes MT growth. Indeed, *alpA* deletion in *A. nidulans* led to a reduced number of MTs and reduced dynamics (Enke *et al*., 2007).

Cdc42, its regulators, and its downstream machinery, such as the polarisome, Arp2/3, and exocyst complex, are conserved from *S. cerevisiae* to *A. nidulans* (Harris and Momany, 2004; Virag and Harris, 2006a). The role of polarisome components in *A. nidulans,* BudA and SpaA, corresponding to *S. cerevisiae* Bud6 and Spa2, was analysed and SpaA was shown to be dispensable for Spitzenkörper organization (Virag and Harris, 2006b). It was also shown that a scaffold protein for the polarity-establishing machinery, BemA, corresponding to *S. cerevisiae* Bem1, is required for proper hyphal growth and formin SepA localization (Leeder and Turner, 2008). Surprisingly, whereas Cdc42 is essential in budding and fission yeast, it is not essential in *A. nidulans* and *U. maydis* (Mahlert *et al.* 2006; Virag *et al*., 2007). In addition to Cdc42, another Rho GTPase, a Rac1 homologue, appears to function in hyphal growth of both fungi. The functional relationship of the Cdc42 and Rac1 homologues and the cell-end markers employed for actin cytoskeleton organization and hyphal polarity establishment is an important next question.

The role of the membrane – sterol-rich lipid microdomains

In both *S. pombe* and *A. nidulans,* Mod5 (TeaR) is necessary for Tea1 (TeaA) positioning, and *vice versa* (Fig. 4). Which other molecules or factors, besides MTs, guide the proteins to their destination? Mod5 and TeaR are assumed to localize to the membrane through their prenyl residue, because their prenylation motifs are essential for their localization and function (Snaith and Sawin, 2003; Takeshita *et al*., 2008). Hence, the membrane environment could be important for their localization. Membranes are no longer considered as homogeneous, and sterols and sphingolipids can cluster into domains within mixtures with glycerophospholipids. These domains, termed lipid rafts, contribute to specific protein localization of, for example, GPI-anchored and lipid-associated proteins, at a specific site, and play important roles in cell signalling and cell polarity (Rajendra and Simons, 2005). In fungi, lipid rafts can be observed as clusters by staining with filipin, a sterol-binding dye (Alvarez *et al*., 2007). These domains, termed sterol-rich membrane domains, are detected at polarized growth sites in several fungi, for example, in the tip of the mating projection in *S. cerevisiae* and *Cryptococcus neoformans,* the growing cell ends and the site of cytokinesis in *S. pombe,* and the hyphal tips and septa in *C. albicans* and *A. nidulans* (Bagnat and Simons, 2002; Nichols *et al*., 2004; Wachtler and Balasubramanian, 2006). The sterol-rich domains contribute to polarized growth in *C. albicans* (Martin and Konopka, 2004). In *A. nidulans,* another correlation of the sterol-rich domains and fomin SepA localization was revealed by analysing mutants such as *mesA*. The corresponding gene encodes a tip-localized membrane protein and its mutation enhances *sepA* defects (Pearson *et al*., 2004). In addition, *barA* and *basA*, which encode a ceramide synthase and a sphingolipid C4-hydroxylase, respectively, were identified by screening for mutants resistant or sensitive to heat-stable antifungal factor. These three mutants exhibited depolarized filipin staining, mislocalized SepA and actin cables, and severe polarity growth defects (Pearson *et al*., 2004; Li *et al*., 2006; 2007).

Cell-end markers or sterol-rich microdomains – which is first

Intact sterol-rich domains are required for cell-end marker localization at the tips in *A. nidulans* (Takeshita *et al*., 2008). Treatment with filipin causes mislocalization of TeaA and TeaR. However, the cell-end markers cannot be the only targets whose localization is regulated by the sterol-rich domains, because the disruption of the sterol-rich domains led to defects in polarity establishment that are not identical to the polarity mispositioning in the cell-end marker deletion mutants (Takeshita *et al*., 2008). The *A. nidulans* mutants with depolarized sterol-rich domains have defects in the localization and activation of SepA, as they do not have actin cables (Pearson *et al*., 2004; Li *et al*., 2006). Sterol-rich domains could also be required for the localization of the Cdc42 cascade, or Cdc42 itself, which localizes to the plasma membrane through post-translational geranyl–geranyl modification. Whereas the sterol-rich domains are necessary for cell-end marker localization, the sterol-rich domains still localized to the hyphal tips in *teaA*- or *teaR*-deletion mutants (Fig. 4) (Takeshita *et al*., 2008). These results suggest that the sterol-rich domains determine cell-end marker localization and not the other way around. If the known cell-end markers do not themselves organize the sterol-rich domains, the question that arises is how the sterol-rich domains are organized and positioned at polarized growth sites. In *S. pombe*, class I myosin is required for proper organization of the sterol-rich domains (Takeda and Chang, 2005), whereas Cdc15 is also required for its organization at cell division sites during cytokinesis (Takeda *et al*., 2004). Septins, which colocalize with the sterol-rich domains at growth sites, are also speculated to have a role in the organization of the membrane domains (Douglas *et al*., 2005). Interestingly, these proteins, class I myosin and septins, are members of the Cdc42 cascade. Indeed, a relationship between the Cdc42 pathway and sterol synthesis has recently been revealed. Ste20, p21-

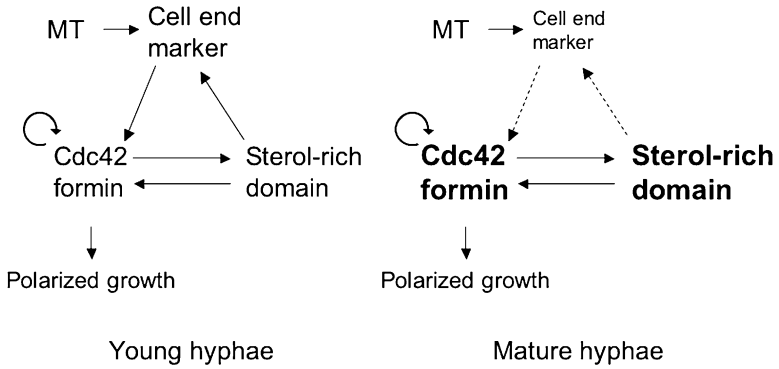

Fig. 5. Scheme of the interdependence of cell-end markers and lipid rafts. See text for details.

activated kinase and effector of Cdc42, interacts with proteins involved in sterol synthesis and regulates cell polarity (Tiedje *et al*., 2007). Homologues of oxysterol-binding proteins, which regulate the synthesis and transport of sterols, function in Cdc42-dependent polarity establishment (Kozminski *et al*., 2006). The Cdc42 cascade is thought to amplify the intensity by positive feedback loops (Wedlich-Söldner and Li, 2003). Sterol-rich domains might increase the local concentration of Cdc42 cascade proteins, and function in such a positive feedback loop. If this were true, cell-end markers, the Cdc42 cascade and sterol-rich domains were in a three-art interaction (Fig. 5). This means that cell-end markers regulate the positioning of the Cdc42 cascade by interaction with formin, that the Cdc42 cascade and sterol-rich domains function cooperatively and amplify the intensity, and that the sterol-rich domains support cell-end marker positioning. Evidence indicates that the sterol-rich domains act upstream of the cell-end markers. In addition, the sterol-rich domains are likely required for the localization of additional factors other than the cell-end markers. However, the following model is also possible. The sterol-rich domains recruit both the Cdc42 cascade and cell-end markers. In addition, MTs independently recruit cell-end markers, which also recruit the Cdc42/formin cascade. Cdc42/formin sets up a feedback loop with sterol-rich domains. These ideas could explain why in *A. nidulans* the defect in growth directionality in the cell-end marker mutants is most prominent in young hyphae. Whereas the position information of cell-end markers might be important for Cdc42 cascade localization in young hyphae, the Cdc42 cascade and the sterol-rich domains in mature hyphae could stabilize their localization interdependently, and polarized growth could be maintained without the position information of cell-end markers.

Conclusion

In the past decade, our knowledge about the molecular components involved in polarized growth in *S. cerevisiae*, *S. pombe* or *A. nidulans* and other fungi has improved tremendously. Nevertheless, the comparison of the mechanism in different organisms shows that our picture of the process is still far from complete. Especially the advance of the understanding of the process in filamentous fungi should allow to identify new proteins determining polarized growth. The example of *kipA* deletion in *A. nidulans* shows that this motor protein apparently does not transport TeaA as in *S. pombe*, but other components, which are required for correct localization of TeaA and TeaR. It will be the challenge for future research to identify such targets and further unravel the mechanism of polarized growth in filamentous fungi.

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