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Where to grow and where to go☆

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ABSTRACT

Filamentous fungi grow as very elongated tubular cells that extend by membrane extension and cell-wall biosynthesis. Membrane and enzyme delivery depend on secretory vesicles that travel along microtubules, accumulate in a structure called the *Spitzenkörper* and then move along actin cables towards the apical membrane. Whereas vesicle fusion and membrane insertion are well studied, less is known about the mechanisms with which the zones of vesicle fusion and hence the growth zones are defined. One mechanism by which polarity is established and maintained is the polar localization of *cell-end marker proteins* (CEMPs). They form multiprotein complexes with formin as F-actin polymerase. CEMP delivery depends on microtubules, and hence CEMPs coordinate the microtubule with the actin cytoskeleton. Actin filaments capture microtubule ends, and this positive feedback loop quickly establishes active growth sites. However, CEMP complexes are self-limiting, because fusing vesicles disturb local growth zones and Ca^{2+} influx pulses lead to F-actin disassembly. This model emerged from studies in *Schizosaccharomyces pombe* and *Aspergillus nidulans*. Surprisingly, deletion of CEMP-coding genes is not lethal. *S. pombe* mutants form T-shaped cells and *A. nidulans* germlings grow less straight. In comparison, CEMP-mutants had a strong phenotype in *Arthrobotrys flagrans*, a nematode-trapping fungus, which produces ring-like trapping structures. CEMP-mutants fail to form adhesive rings and instead form sticks. CEMP overexpression caused a hyperbranching phenotype. Hence, CEMPs are involved in polarity maintenance and play critical roles during modulations of polarity. Here, we are going to discuss the functions of CEMPs and their connections to other polarity determinants.

1. Introduction

Polarity establishment and control are crucial processes from virus to bacteria, to eukaryotic microbes, and from plants to animals. Polarity means a break of symmetry and usually is followed by gradient formation (Glazenburg and Laan, 2023). Like in all asymmetric systems, break of symmetry requires energy, which in the case of eukaryotes is often provided by cytoskeleton-dependent processes. In many cases, polarity needs to be established in a spatially and temporally well-controlled manner. Positive-feedback loops are one possibility to quickly assemble polarity sites, and negative-feedback loops are mechanisms to limit polar processes in time. However, plant pollen tubes, nerve cells and filamentous fungi are examples where polarity is maintained over long times. Fungi grow by apical extension through membrane expansion and cell-wall extension. Given their amenability to cell-biological, genetic, molecular biological and biochemical methods, they are ideal organisms to understand the principles of polarity establishment and maintenance (Riquelme et al., 2018).

In this review, we aim at summarising the roles of cell-end marker proteins (CEMPs) to develop new hypotheses for their functions and their interplay with other polarity determinants rather than giving a comprehensive overview of all observations and possible interconnections to other cellular processes.

2. Cell-end marker proteins are conserved from yeast to filamentous fungi

Most filamentous fungi normally grow at their apices, where new cell wall is synthesised and membrane is inserted into the apical membrane (Bartnicki-Garcia and Lippman, 1969). Secretory vesicles are generated at the ER and Golgi apparatus, travel along microtubules driven by conventional kinesin and are unloaded at an apical organelle, the *Spitzenkörper*, from where they move along actin filaments powered by myosin V until they fuse in a SNARE- and exocyst-dependent pathway

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with the membrane (Pinar et al., 2022; Requena et al., 2001; Pantazopoulou et al., 2014; Taheri-Talesh et al., 2008; Taheri-Talesh et al., 2012; Pinar et al., 2019; Sánchez-León et al., 2011; Ramos-Garcia et al., 2009; Peñalva et al., 2020; Peñalva et al., 2017; Schuster et al., 2012). Exocytosis is restricted to the apical dome, and the area for active secretion is delineated by endocytosis in a zone a few microns away from the tip, the endocytic collar or endocytic ring (Martzoukou et al., 2018; Hernandez-Gonzalez et al., 2018; Bartnicki-Garcia et al., 2018; Delgado et al., 2025; Zhang et al., 2011; Taheri-Talesh et al., 2008; Upadhyay and Shaw, 2008). The importance of the actin cytoskeleton for polarised delivery of secretory vesicles can be easily shown by interference with the integrity of the actin cytoskeleton with anti-actin drugs. Hyphae become wider; their tips start swelling, followed by tip bursting **(**Fig. 1 **A)**. Disassembly of the microtubule cytoskeleton blocks tip extension in *A. nidulans* germlings and reduces growth speed significantly in hyphae (Horio and Oakley, 2005). In *Neurospora crassa,* cell organisation appears to be quite different as compared to *A. nidulans,* and cytoplasmic streaming and bulk flow are very important for growth (Riquelme et al., 2011; Bartnicki-Garcia et al., 2000; Ramos-Garcia et al., 2009).

Polarity establishment and maintenance is essential for hyphal elongation, spore germination, branch formation, hyphal fusion or generation of reproductive structures. Cell-end marker proteins (CEMPs) were first discovered in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (Mata and Nurse, 1997; Philips and Herskowitz, 1998; Snell and Nurse, 1994)**(**Fig. 1 **B)**. *S. pombe* grows at both ends, but after fission, new ends are generated which need to establish new polarity sites (NETO, *new end take off*). Hence, *S. pombe* is an excellent model to investigate polarity establishment and maintenance. To identify molecular components involved in both processes, *S. pombe* was subjected to a mutagenesis approach followed by visual screening for morphological mutants (Snell and Nurse, 1994; Mata and Nurse, 1997). In order to account for the possibility that mutagenesis of polarity genes could be lethal, the screen was set up as a screen for temperature-sensitive mutants. One of the mutants was named *tea1* (*tip-elongation aberrant*). The corresponding protein contains several Kelch and coiled-coil domains and appears to form large protein complexes. In subsequent studies, several other components were discovered and revealed the picture that Tea1 is transported along microtubules by the kinesin motor Tea2, accumulates at the microtubule plus ends and highjacks towards the growing tip (+TIP) (Busch et al., 2004; Browning et al., 2000; Browning et al., 2003; Behrens and Nurse, 2002). There it interacts with a prenylated protein, Mod5, that anchors the protein complex to the membrane (Snaith and Sawin, 2003; Dodgson et al., 2013). Prenylated proteins are post-translationally modified normally in the ER and transported with vesicles (Wang and Casey, 2016). This is probably also the case for Mod5. *S. pombe* contains another Kelch-domain protein similar to Tea1, known as Tea3, which interacts with the Mod5/Tea1 complex but is not commonly found in other fungi (Snaith et al., 2005). Another CEMP, Tea4/Wsh3, possesses an SH3 domain and is recruited by Tea1 (Tatebe et al., 2005). Tea4/Wsh3 interacts with the formin For3 to polymerise actin, thereby linking microtubule cytoskeleton dynamics to the spatial control of actin filament formation (Martin et al., 2005). Additionally, Tea4/Wsh3 recruits the phosphatase Dis2 and DYRK-kinase Pom1, which modulate Cdc42 activity by influencing GAP (GTPase-activating protein) and GEF (guanine nucleotide exchange factor) localisation (Tatebe et al., 2008; Kokkoris et al., 2014). Yeast two-hybrid screening also revealed interaction between Tea4/Wsh3 and the MAP kinase Win1, a component of the stress-activated Spc1 MAPK pathway (Tatebe et al., 2005). Notably, under stress conditions, the Δ*tea4* mutant phenotype becomes more pronounced, suggesting that the CEMP complex may play a role in establishing cell polarity in response to environmental cues.

In *S. cerevisiae* polar growth is established during a short time during budding. Two CEMPs, Kel1 and Kel2 share sequence similarity to Tea1

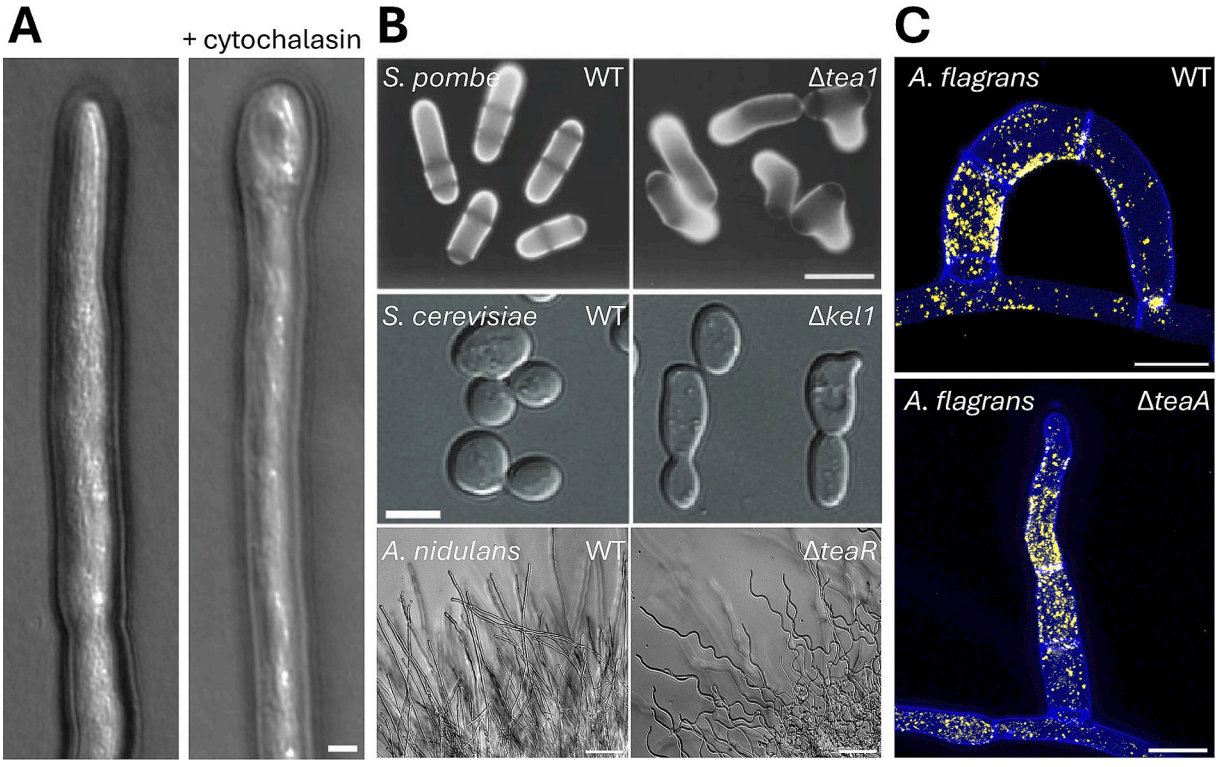

Fig. 1. Cell-end marker proteins (CEMPs) are conserved from yeast to filamentous fungi. **A** Comparison of a hyphal tip of *A. flagrans* without and in the presence of cytochalasin A. Scale bar, 2 µm. **B** Deletion of CEMP encoding genes in *S. pombe, S. cerevisiae*, and *A. nidulans*. **C** Trap morphology in *A. flagrans* and the Δ*teaA* mutant. To demonstrate that both structures are physiologically traps, the trap-specific, small-secreted protein, NipA, is visualised as a RFP-fusion protein (yellow) (Emser et al., 2024). Pictures in **B** and **C** were taken from (Mata and Nurse, 1997; Kriegler et al., 2025; Garcia et al., 2021). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and were discovered in a genetic screen for overexpression mutants with roles in mating (Philips and Herskowitz, 1998) **(**Fig. 1**B)**. Deletion of *kel1* leads to an impairment of cell fusion during mating, but vegetative cells additionally display a morphological phenotype with elongated and misshapen cells. Kel1 mediates cell fusion through a Fus2 and Cdc42-dependent pathway (Smith and Rose, 2016). Bud14 is the orthologue of Tea4/Wsh3 and interacts with Kel1 and Kel2 to recruit the formin Bnr1, similar to *S. pombe* (Cullen and Sprague Jr., 2002). However, no orthologue of Mod5 has been identified yet.

In filamentous fungi, polar growth is the dominant growth form, and the question was if yeast CEMPs are conserved. First inspections of fungal genomes of *N. crassa* and *Aspergillus* species revealed no evidence for the presence of *S. pombe* or *S. cerevisiae* cell-end marker proteins (Galagan et al., 2005; Galagan et al., 2003). Especially a Mod5 orthologue seemed to be missing, suggesting fundamental differences between single-cell and filamentous fungi. Hence, the existence of the *S. pombe* CEMP machinery was discovered by chance in *A. nidulans*. To analyse the role of the 10 different *A. nidulans* kinesin proteins, organelle movement was followed in respective mutant strains (Suelmann et al., 1997; Requena et al., 2001). In the case of the *kipA* mutation (*tea2* in *S. pombe*), microscopic analyses revealed that some nuclei in a hypha were in focus, whereas others were not, suggesting that hyphae grew in different planes under the cover slip. Indeed, *kipA*-deletion caused a meandering phenotype of growing hyphae, suggesting molecular mechanisms for maintenance of polarity similar to *S. pombe* and *S. cerevisiae* (Konzack et al., 2005) **(**Fig. 1 **B)**. In *S. pombe* Tea2 delivers Tea1 to the MT plus ends. After the analysis of the kinesin motor, a Tea1 orthologue, TeaA, was characterised, where the Kelch repeat structure followed by coiled-coil domains is conserved (Takeshita et al., 2008). A Mod5 orthologue could not be identified in the *A. nidulans* genome by sequence comparison. Since Mod5 is a prenylated protein with the fourth-last amino acid being a cysteine, systematic inspection of all putative proteins of *A. nidulans* revealed Mod5 candidates from which one uncharacterised protein, with a similar size than Mod5, was chosen for further analyses. It was named TeaR (Tea receptor) and displayed very weak overall sequence conservation to Mod5 besides the critical cysteine close to the C-terminus. Deletion of *teaR* indeed causes a meandering hyphal phenotype (Takeshita et al., 2008). The CEMPs also in *A. nidulans* recruit the formin, SepA. Hence, CEMPs appear to be conserved from yeast to filamentous fungi. In *A. nidulans* CEMP complexes are inserted in special sterol-rich membrane areas (Takeshita et al., 2008; Takeshita et al., 2012). It was also shown that CEMP complexes consist of about 50 molecules of TeaR, are highly dynamic and change positions frequently, similar to the CEMP complex dynamics in *S. pombe* (Ishitsuka et al., 2015; Dodgson et al., 2013). In *A. nidulans,* TeaA interacts with the MT polymerase AlpA (XMAP215) once a MT end reaches the cortically anchored TeaA complex (Takeshita et al., 2013). TeaA then inhibits the AlpA activity and hence stops MT elongation after reaching the hyphal tip. In the absence of TeaA MTs curve around the apex and grow towards the rear of the hyphae. The microtubule-plus ends interact through the Kar9 orthologue MigA with the myosin V motor MyoE and are actively pulled towards the CEMP complexes (Manck et al., 2015). Hence, microtubules deliver CEMPs to the cortex, polymerise actin filaments, which in turn capture more microtubules. This is a positive-feedback loop, which guarantees quick assembly of polarity sites.

One intriguing question is which role(s) CEMPs may play if hyphal morphology changes drastically, as is the case, for example during trap formation in nematode-trapping fungi (NTF). NTF, like *Arthrobotrys flagrans,* provide a remarkable example of how the polarity machinery adapts to meet the organism's changing requirements (Wernet et al., 2022; Wernet et al., 2023). Under favourable nutritional conditions, *A. flagrans* lives saprotrophically but changes to a predatory lifestyle when starving (Fischer and Requena, 2022). The morphogenetic transitions associated with the shift from a saprotrophic to a predatory lifestyle are triggered by volatile nematode-derived molecules and autocrines from the fungus (Yu et al., 2021; Hsueh et al., 2017; Hu et al., 2024). *A. flagrans* produces ring-shaped, adhesive traps. Ring-like structures are rather rare in biology, and in the case of *A. flagrans* require a continuous change of polarity followed by hyphal fusion for ring closure. Absence of TeaA or TeaC impairs trap production and alters trap morphology, as the fungus loses its ability to bend the hyphae into functional rings (Kriegler et al., 2025) **(**Fig. 1 **C)**. Although the shape of the trap differs from wild-type traps, they retain their stickiness and are still able to capture nematodes. Interestingly, deletion of the putative TeaR receptor protein does not impair the ability to bend traps but instead results in traps with a smaller diameter. Furthermore, meandering hyphae are more pronounced in the *teaR*-deletion strain as compared to the *teaA*- and *teaC*-deletion strains. Conversely, overexpression of TeaA leads to a hyperbranching phenotype, likely due to the formation of additional polarity centres along the hyphae.

CEMPs have also been investigated in *Magnaporthe grisea* and *Ustilago maydis* (Valinluck et al., 2014; Rogers et al., 2024; Qu et al., 2022). In *M. grisea*, Tea4 plays a crucial role in actin polarisation during appressorium formation and regulates branch formation. In *U. maydis*, deletion of *tea4* results in abnormal cell morphology and reduces pathogenicity in the absence of cell fusion. Notably, the Tea4 protein in basidiomycetes contains a nuclear localisation signal (NLS).

A survey of several genomes of different fungal lineages revealed the presence of proteins with sequence and structural similarity to Tea1/TeaA/Kel2 and Tea4/TeaC/Bud14, which are indicative of CEMPs, across all fungal lineages **(**Fig. 2**)**. This similarity is more pronounced in ascomycota and basidiomycota than in chytridiomycota. To identify Mod5/TeaR proteins, we used the same strategy as for TeaR of *A. nidulans,* where the proteome was filtered for proteins containing a CAAX-motif, followed by an analysis of domain composition and protein size. Mod5 and TeaR contain a CAAX motif and no other functional domains **(**Fig. 2**)**. Some species appear to lack some CEMPs, like *S. cerevisiae* a TeaR orthologue, or *Entomophthora muscae,* a TeaA orthologue. However, absence of evidence is not necessarily evidence for absence. Whether sequence similarities are too low to be detected or whether other proteins take over the function in those organisms cannot be revealed without further analyses and experiments.

In conclusion, it appears that many or all fungi contain CEMPs to coordinate polar growth, which may be fine-tuned depending on the organism's lifestyle and ecological needs.

3. Dynamic cell extension – Instability for stability

Polar growth appears to be the result of a positive-feedback loop. However, without limitation, this mechanism would lead to very localised growth rather than extension of the entire tip. The limitation of local growth comes from increasing numbers of vesicles, which first accumulate at the CEMP polarity site and then fuse with the membrane induced by Ca^{2+} influx. Ca^{2+} also causes actin catastrophe and thereby destruction of the vesicle tracks and the tracks for microtubule capture (Takeshita et al., 2017). Hence, the positive loop guarantees quick assembly of CEMP complexes and the negative-feedback loops limit vesicle fusion spatially and to a short period of time **(**Fig. 3**)**. The Ca^{2+} influx may not be a negative-feedback loop in the strict sense, because it is not known yet if and how CEMP assembly or vesicle fusion are interlinked with Ca^{2+} oscillations. Nevertheless, the mechanisms guarantee that cell extension occurs at small areas rather than uniformly at the entire apex (Ishitsuka et al., 2015). Likewise, in *S. pombe* and *A. nidulans* cell-walls, a mosaic-like pattern of cell-wall thickness was observed, where thinner and thicker cell-wall areas alternated (Davi et al., 2018; Chevalier et al., 2023). Unfortunately, this pattern has not yet been related to the location of CEMPs.

One intriguing question is why the instability of the CEMP complexes was maintained in evolution or why local growth patches have an advantage over uniform cell-wall extension. To illustrate the problem which filamentous fungi face if they extend their hyphae, one can think

	Class	Species	TeaA	TeaC	TeaR
Ascomycota	Eurotiomycetes	*Aspergillus nidulans*	XP_662168.1 1474 aa Kelch Coiled-coil	XP_658703.1 721 aa SH3	XP_050467330.1 524 aa CAAX-motif
	Orbiliomycetes	*Arthrobotrys flagrans*	XP_067495653.1 E: 0.0, 1506 aa	XP_067486226.1 E: 2e-90, 718 aa	XP_067489801.1 E: 7e-26, 549 aa
	Saccharomycetes	*Saccharomyces cerevisiae*	KZV10896.1 E: 3e-78, 1164 aa	KZV13474.1 E: 2e-11, 709 aa	–
	Schizosaccharo-mycetes	*Schizosaccharo-myces pombe*	CAA20875.1 E: 3e-56, 1147 aa	CAA19002.2 E: 2e-30, 821 aa	CAA19170.1 522 aa
	Sordariomycetes	*Neurospora crassa*	XP_965762.3 E: 0.0, 1491 aa	XP_011393719.1 E: 1e-124, 792 aa	XP_961443.1 E: 3e-42, 541 aa
	Sordariomycetes	*Magnaporthe oryzae*	XP_003720856.1 E: 0.0, 1504 aa	XP_011393719.1 E: 1e-124, 792 aa	XP_030982482.1 E: 4e-43, 583 aa
Basidiomycota	Ustilagino-mycetes	*Ustilago maydis*	AVL25102.1 E: 2e-108, 1698 aa	XP_011386992.1 E: 8e-38, 1684 aa	KIS70384.1 E: 6e-05, 608 aa
	Agaricomycetes	*Schizophyllum commune*	KAL1737553.1 E: 2e-146, 1478 aa	KAL1718566.1 E: 3e-33, 1345 aa	KAI5886859.1 565 aa
	Tremellomycetes	*Cryptococcus neoformans*	OWZ61961.1 E: 6e-96, 1531 aa	OWZ70547.1 E: 2e-33, 1508	AAW42363.2 709 aa
Zoo-pago	Entomophthoro-mycetes	*Entomophthora muscae*	KAJ9065492.1 E: 2e-17, 1219 aa	–	KAJ9055759.1 486 aa
Glo-mero	Glomeromycetes	*Rhizophagus irregularis*	GET58069.1 E: 2e-86, 1575 aa	EXX62187.1 E: 1e-39, 1351 aa	UZO26834.1 340 aa
Chytridiomycota	Chytridiomycetes	*Polyrhizophydium stewartii*	KAL2914784.1 E: 1e-18, 813 aa	KAL2916387.1 E: 2e-30, 1271 aa	KAL2919465.1 493 aa
	Chytridiomycetes	*Gaertneriomyces semiglobifer*	KAI9014631.1 E: 6e-18, 1028 aa	–	–
	Chytridiomycetes	*Batrachochytrium salamandrivorans*	KAH9253039.1 E: 2e-24, 888 aa	KAH6575176.1 E: 3e-27, 964 aa	KAJ1344093.1 365 aa

Fig. 2. CEMPs are found across the fungal kingdom. CEMPs from *A. nidulans* were used as query sequences for BLAST searches across different fungal lineages to identify orthologues. The respective protein's NCBI accession number, *E*-value (E:), and amino acid length (aa) are provided. Kelch repeats (red), SH3 domains (yellow), and CAAX-motifs (orange) were identified using NCBI (National Centre for Biotechnology Information), while coiled-coil regions (green) were identified with Waggawagga (Simm et al., 2015). CEMPs from species that have not yet been experimentally analysed were selected based on sequence and structural similarities, but require further investigation and experimental validation. TeaR orthologues in all species outside the Ascomycota were identified through proteome-wide analysis of CAAX motif-containing proteins lacking known domains. In many cases, multiple candidate proteins met the selection criteria, and final selection was only based on protein size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of a steel water pipe which needs to be elongated although it contains water under high pressure. Softening the tip by heat and adding new metal is a very delicate process because softening too much will lead to bursting of the tip, and adding too much metal will inhibit further extension. To develop a hypothesis for fungal growth, one needs to consider that the mechanism of expansion of the rigid cell wall is not fully understood. It has been shown that in *N. crassa* and *A. nidulans* at least two types of vesicles deliver enzymes for cell-wall biosynthesis, chitin synthases and enzymes for glucan synthesis (Verdín et al., 2009; Sánchez-León et al., 2011). If hyphae grow fast, freshly produced cell walls are likely to be soft enough and allow hyphal extension. However, this should be a very fine-tuned equilibrium, because delayed biosynthesis of chitin and glucans would result in a too-thin cell wall, unable to resist the turgor pressure. On the other hand, more cell-wall synthesis

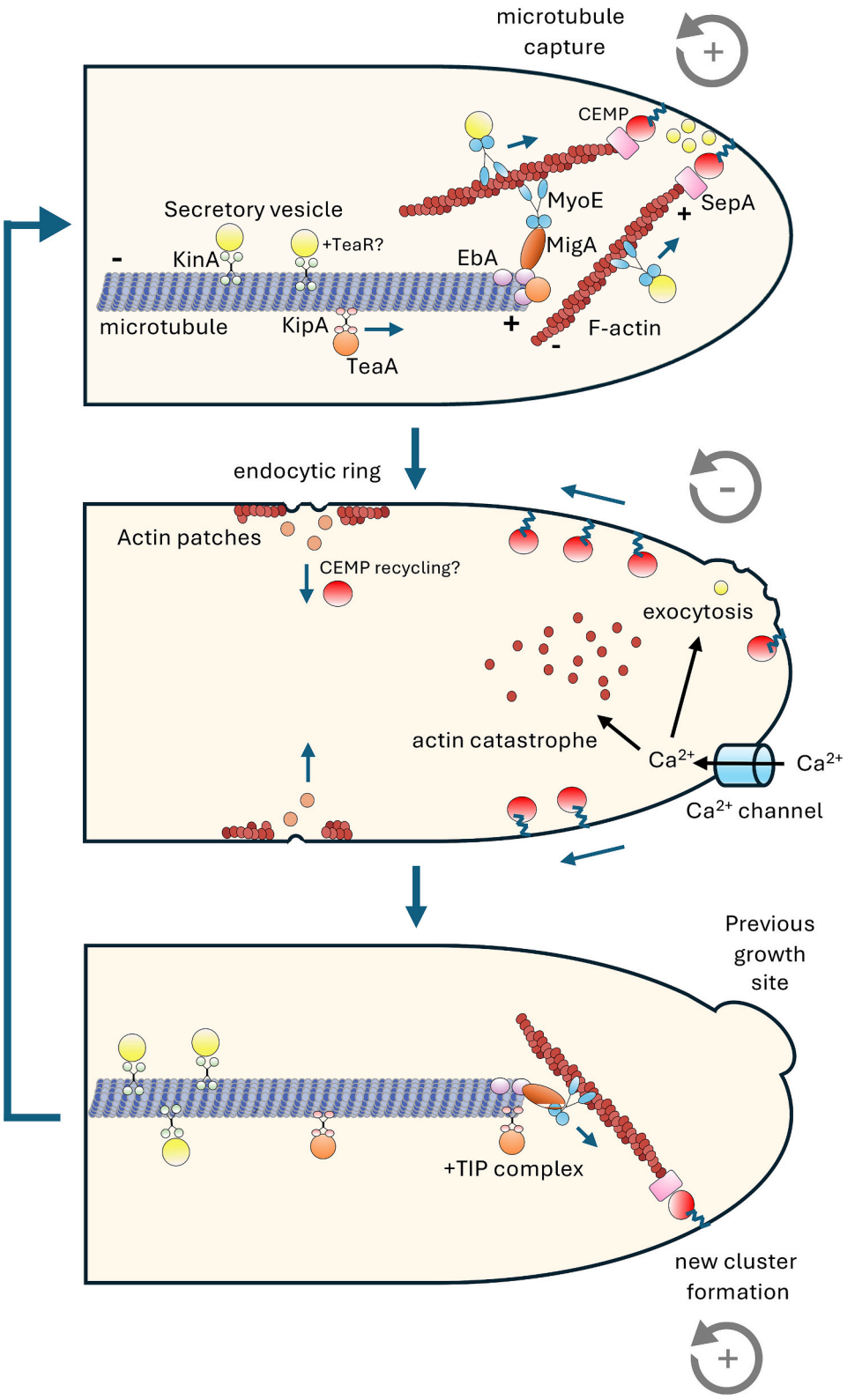

Fig. 3. Role of CEMPs in establishment of local growth sites. Upper panel: CEMPs are transported along microtubules either directly as cargo of the kinesin VII KipA (TeaA), or in the case of the prenylated TeaR receptor at vesicles. KipA and TeaA accumulate at microtubule plus ends and are thus microtubule plus end tracking proteins (+TIP). A small CEMP complex at the membrane assembles actin filaments along which microtubule plus ends are pulled to merge at one point and deliver more CEMPs. This leads to more actin filaments. Secretory vesicles are transported along microtubules with conventional kinesin as a motor before they are reloaded onto actin filaments. Vesicles containing cell-wall biosynthesis enzymes accumulate around the CEMP site. **Middle and lower panels:** Pulsed Ca^{2+} influx induces fusion of the secretory vesicles with the membrane and actin catastrophe. The cell wall will be synthesised at that place. Membrane integration disturbs the integrity of the CEMP complexes, which then dissolve. Some of them will be internalised in the endocytic ring, and others give rise to new actin filament formation.

than required for hyphal extension would result in a thickened cell wall, too rigid to be expanded. An alternative to the only action of cell-wall synthesis enzymes is the additional action of cell-wall softening enzymes, such as chitinases or endoglucanases. In this regard, it has been shown that EglD, a β-1,3-endoglucanase with an expansin-like domain, is mainly localised in germinating spores (Bouzarelou et al., 2008). Deletion of *eglD* showed no effect on the cell wall, but knockout of another similar endoglucanase, *eglC*, resulted in hyphae more resistant to cell wall lytic enzymes (Choi et al., 2005). Regarding chitinases, ChiA has been observed in regions of apical growth, although subapically and at incipient branching points (Yamazaki et al., 2008). Likewise, glycoside hydrolases with glycosyltransferase activity (Bgt-1 and Bgt-2) were also found in the subapical region where they probably contribute to remodeling of the cell wall (Martinez-Nunez & Riquelme, 2015). On the other hand, preliminary observations using AFM microscopy in our laboratory indicated that the elasticity of the cell wall increases towards the tip, suggesting that the *de novo* formed wall is softer than the mature one (data not shown). These observations have been corroborated by indirect imaging methods (Chevalier et al., 2023). In summary, there is increasing evidence that the *softening* enzymes are involved in cell-wall remodelling and maturation but do not play important roles in continuous polar growth. Although the action of such enzymes is very plausible, there is, to our knowledge, no experimental evidence for crucial roles of those activities. Of course, even if they may not play a role at actively growing tips, they should play roles during germination of spores and during branch formation, when mature and rigid cell walls need to be softened to allow germtube emergence. Again, this sounds plausible, but conclusive experimental evidence is yet missing.

Despite the fact that the mechanism of hyphal expansion has not been entirely understood, there is evidence that uncontrolled fusion of vesicles all along the hyphal apex is deleterious for the hypha. This is the case if the actin cytoskeleton is destroyed (Fig. 1). In comparison, the CEMP model of cell-wall synthesis restricts growth to small areas and short times. The advantage of small "soft" areas may be explained by Blaise Pascal's (1623–1662) law of the hydraulic pressure, which describes that under the same pressure, a small surface requires less force to remain stable than a large surface. This is reflected in the formula $F_1/a_1 = F_2/a_2$, where F is the force and a is the area/surface. If after disassembly new CEMP complexes form arbitrarily along the apex and, due to the positive-feedback loop quickly assemble new active growth sites, there is a chance that several CEMPs assemble in close proximity, giving rise to larger areas of cell extension, which could even lead to cell bursts if the areas were too big. However, these are probably very rare occasions, and hence dynamic instability of CEMPs guarantees stability of polar growth.

4. Interplay between cell-end markers and other polarity determinants

The model of local cell extension driven by CEMP complexes may nicely help to explain the extension of a rigid cell wall of a pressurized tube but raises the immediate question of why deletion of any of the CEMP-coding genes does not lead to more drastic phenotypes. To understand this, the connection to other polarity markers has to be considered (Fig. 4). Cdc42 is one of the most critical proteins involved in polarity (Johnson, 1999). It is a small GTPase of the Rho family, which is spatiotemporally tightly regulated. Its activity depends on the cycling of bound guanine nucleotides, GTP or GDP. When bound to GTP, Cdc42 is active. Guanine nucleotide exchange factors (GEFs) activate Cdc42 by promoting the exchange of GDP for GTP, while GTPase-activating proteins (GAPs) negatively regulate it by accelerating GTP hydrolysis (Scheffzek and Ahmadian, 2005). When Cdc42 is in its active form, it will coordinate many factors, like the cytoskeleton and membrane trafficking machinery, to build a polar growth site (Harris, 2011). In yeast, unlike *A. nidulans*, deletion of *cdc42* is lethal, but filamentous fungi have a Rac1 homologue which can partly substitute for Cdc42. Like the CEMPs, Cdc42 will localise to the spot of polar growth and at septa in a sort of cluster formation (Bendezu and Martin, 2012). This process undergoes an oscillation cycle where positive and negative-feedback loops contribute to the formation and regulation of these aggregates, just like for the CEMPs. Recent studies demonstrated that CEMPs in *S. pombe* are indirectly controlling Cdc42 activity by recruiting the Pom1 DYRK-kinase which leads to the recruitment of the GEF Gef1 and the exclusion of the GAP Rga4 at the polar sites (Tay et al., 2018). Therefore, Rga4 will accumulate at the side of the cell and inhibit the activation of Cdc42, resulting in the typical bipolar morphology of *S. pombe*.

One role of Cdc42 is to coordinate the fusion of secretory vesicles at

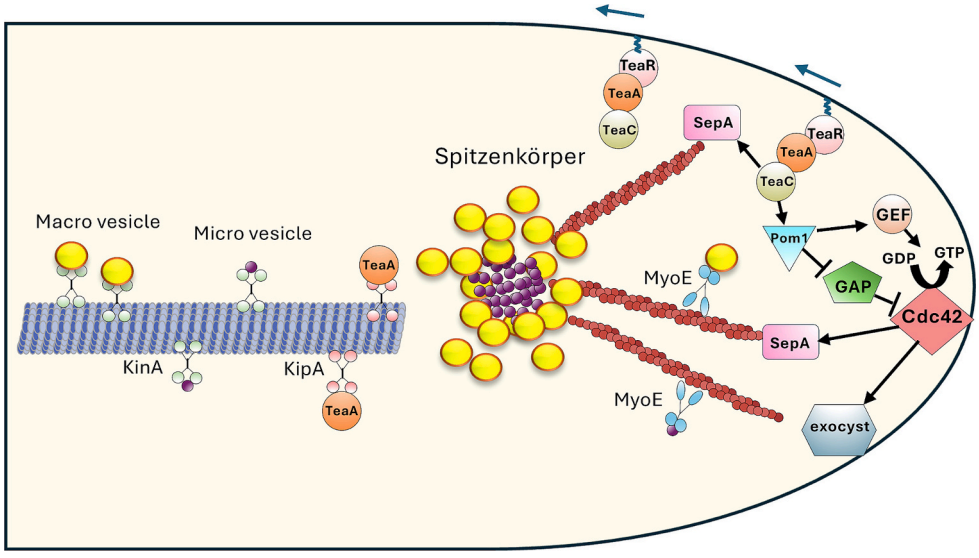

Fig. 4. Interaction of CEMPs with other polarity determinants. Vesicles and CEMPs are transported along microtubules to the tip of a growing hypha. Micro- and macrovesicles accumulate at the Spitzenkörper, where they are distributed to the hyphal tip. The CEMP complex localises at the tip membrane and recruits the formin SepA to promote actin assembly. The following interactions and relations are based mainly on findings in *S. pombe* and need to be validated in other fungi. Interaction of TeaC and Pom1 may regulate the activity of GAP (GTPase-activating protein) and GEF (guanine nucleotide exchange factor), thereby modulating Cdc42 activation at the site of CEMP localisation. Activated Cdc42, in turn, recruits additional formins and activates the exocyst complex, which is essential for vesicle tethering.

the membrane (Miller et al., 2020). Therefore, it is in control of the so-called exocyst complex, which is an octameric protein complex necessary for tethering secretory vesicles to the plasma membrane before exocytosis (Zuriegat et al., 2024; Riquelme et al., 2014). Cdc42 directly interacts with two proteins from the exocyst complex, Sec3 and Exo70, and by that controls the coordination and activity of the complex. The exocyst complex can interact with different plasma membrane (Sso1/Sso2, Sec9) and vesicle (Snc1/Snc2) SNAREs (Soluble NSF Attachment Protein Receptors) to coordinate the tethering and fusion of vesicles. For example, the *S. cerevisiae* chitin synthase 3 (Chs3) is a crucial enzyme for cell-wall biosynthesis and is secreted by the exocyst complex (Zanolari et al., 2011).

In conclusion, the CEMP complex coordinates major polarity proteins like Cdc42, which, in turn, regulate the exocyst complex and other key polarity factors. While the absence of CEMPs does not have an effect as pronounced as the lack of Cdc42, it suggests a more supportive role for the protein complex, particularly evident during hyphal redirection events. To gain further insights into the function of CEMPs, it would be valuable to study Cdc42 dynamics in CEMP deletion strains, especially in the context of hyphal redirection, and observe how these deletion strains respond under various conditions of hyphal redirection.

5. Some fungi or all – Where to go

The above-described model of the role of CEMP appears to apply to *S. pombe, A. nidulans, M. oryzae,* and *U. maydis*. Of course, the analysis of CEMPs is most advanced in *S. pombe,* but the work so far in *A. nidulans* and the other fungi suggests conservation of the machinery and reveals new aspects for CEMP functioning. That does not exclude species-specific rewiring of the components. For example, it was shown that germlings of *A. nidulans* differ from mature hyphae. They grow much faster and microtubules are more important to support hyphal extension than in germlings (Horio and Oakley, 2005). Likewise, the cell wall in germlings is much thicker than in fast-growing hyphae (Chevalier et al., 2023). Not surprisingly, germlings and hyphae differ with the importance of CEMPs. The meandering phenotype of CEMP-deficient strains is more pronounced in germlings than in hyphae. The CEMP machinery is also connected to stress responses, suggesting that environmental cues may be transmitted through CEMPs to hyphal growth (Tatebe et al., 2005).

If CEMPs are important in all fungi, is difficult to predict. There is good evidence that the cellular organisation of hyphae in different fungi is very different, and the question is if CEMPs exist in all fungi - and if yes – if a unified picture for CEMP functioning will emerge. In *N. crassa,* cytoplasmic streaming and bulk flow of cytoplasm appear to contribute significantly to organelle distribution and vesicle transportation, whereas cytoplasmic streaming is not observed in fungi like *A. nidulans* or *M. grisea* (Abadeh and Lew, 2013; Ramos-Garcia et al., 2009). They also behave very differently when growing into narrow channels (Fukuda et al., 2021). One big step forward to unify or deviate the roles of CEMPs will therefore be the analysis of those proteins in *N. crassa*. *N. crassa tea-1*-deletion strains displayed an increased thigmotropic response, but the exact arrangement of CEMPs and their contribution to organising the MT and/or the actin cytoskeleton have not been analysed yet (Stephenson et al., 2014). There are other interesting candidate fungi which appear to have very different tip architectures and different *Spitzenkörper* morphology. The *U. maydis Spitzenkörper* contains uniform-sized vesicles instead of micro- and macrovesicles as in *N. crassa* and in *A. nidulans*, and in zygomycetous fungi vesicles are organised as a vesicle crescent (Fisher and Roberson, 2016; Schuster et al., 2016).

The picture which emerges currently places CEMPs at the growing hyphal tip where it becomes crucial if polarity needs to be adapted to morphological requirements like trap formation in NTFs, but there is emerging evidence that CEMPs play additional roles in other cellular pathways. It has been shown that CEMPs also localise at septa (Kriegler et al., 2025). The current explanation is that also at septa secretion of vesicles for cell wall biosynthesis is required. But this might not be all. There is also good evidence that CEMPs are required for hyphal fusion which led to the discovery of CEMPs in *S. cerevisiae.* Again, this could be due to effects on the remodelling of the cell wall as a prerequisite for cell-wall and cell fusion. However, in *A. flagrans* CEMPs appear to be also involved in the cell-to-cell communication signalling process (Wernet et al., 2023; Kriegler et al., 2025). The role of CEMPs in the initiation of polarity sites is also not yet fully clear. CEMP-deficient mutants are still able to germinate and to branch, suggesting that CEMPs are not essential for setting up new polarity sites. On the other hand, overexpression of TeaA in *A. flagrans* causes a hyperbranching phenotype (Kriegler et al., 2025). This is in agreement with findings in *S. pombe,* where artificial recruitment of Tea4 to defined places at the cell membrane induced bulge formation (Kokkoris et al., 2014). Hence, CEMPs appear to be sufficient but not essential for polarity establishment.

Some of the open questions are probably due to limitations in microscopy because a few molecules of CEMPs may be sufficient for some responses and are probably overlooked with conventional fluorescence microscopy. Implementation of super-resolution techniques but also alternative methods like expansion microscopy may lead to a more comprehensive picture of the role of CEMPs (Reza et al., 2024; Ishitsuka et al., 2015). One interesting question is also if the differences in cell-wall thickness can be correlated with the pattern of CEMPs along the apical membrane (Chevalier et al., 2023).

Other open questions are related to the interconnection of CEMPs with other cellular processes. Since the phenotype of CEMPs gene deletions is so mild, synthetic lethal screens could be helpful to resolve the network of polarity control at hyphal tips. Another possibility towards a more complete picture of the players could be spatial proteomic analyses. Laser-capture methods have been developed and proteomic analyses significantly improved to address those questions (Zhang et al., 2016).

CEMPs have been shown to play several roles in different fungi. They are involved in polarity maintenance, polarity initiation, branch formation control, hyphal fusion control, mating, and cell-to-cell communication. Whether these roles are specific to some fungi or whether they are conserved in all fungi remains to be resolved and urgently requires comparative studies. It will be the challenge for future research to develop a unified picture for the role of these interesting protein complexes at polarity sites.

CRediT authorship contribution statement

Marius Kriegler: Writing – original draft. **Satur Herrero:** Writing – original draft. **Reinhard Fischer:** Writing – review & editing, Conceptualization.

Declaration of competing interest

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Data availability

No data was used for the research described in the article.

References

Abadeh, A., Lew, R.R., 2013. Mass flow and velocity profiles in Neurospora hyphae: partial plug flow dominates intra-hyphal transport. Microbiology (Reading) 159, 2386–2394.

Bartnicki-Garcia, S., Lippman, E., 1969. Fungal morphogenesis: cell wall construction in Mucor rouxii. Science 165, 302–304.

Bartnicki-Garcia, S., Bracker, C.E., Gierz, G., Lopez-Franco, R., Lu, H., 2000. Mapping the growth of fungal hyphae: orthogonal cell wall expansion during tip growth and the role of turgor. Biophys. J. 79, 2382–2390.
Bartnicki-Garcia, S., Garduno-Rosales, M., Delgado-Alvarez, D.L., Mourino-Perez, R.R., 2018. Experimental measurement of endocytosis in fungal hyphae. Fungal Genet. Biol. 118, 32–36.
Behrens, R., Nurse, P., 2002. Roles of fission yeast tea1p in the localization of polarity factors and in organizing the microtubular cytoskeleton. J. Cell Biol. 157, 783–793.
Bendezu, F.O., Martin, S.G., 2012. Cdc42 oscillations in yeasts. Sci. Signal. 5, pe53.
Bouzarelou, D., Billini, M., Roumelioti, K., Sophianopoulou, V., 2008. EglD, a putative endoglucanase, with an expansin like domain is localized in the conidial cell wall of *aspergillus nidulans*. Fungal Genet. Biol. 45, 839–850.
Browning, H., Hayles, J., Mata, J., Aveline, L., Nurse, P., McIntosh, J.R., 2000. Tea2p is a kinesin-like protein required to generate polarized growth in fission yeast. J. Cell Biol. 151, 15–27.
Browning, H., Hackney, D.D., Nurse, P., 2003. Targeted movement of cell end factors in fission yeast. Nat. Cell Biol. 5, 812–818.
Busch, K.E., Hayles, J., Nurse, P., Brunner, D., 2004. Tea2p kinesin is involved in spatial microtubule organization by transporting tip1p on microtubules. Dev. Cell 16, 831–843.
Chevalier, L., Pinar, M., Le Borgne, R., Durieu, C., Penalva, M.A., Boudaoud, A., Minc, N., 2023. Cell wall dynamics stabilize tip growth in a filamentous fungus. PLoS Biol. 21, e3001981.
Choi, C.J., Ju, H.J., Park, B.H., Qin, R., Jahng, K.Y., Han, D.M., Chae, K.S., 2005. Isolation and characterization of the *Aspergillus nidulans eglC* gene encoding a putative beta-1,3-endoglucanase. Fungal Genet. Biol. 42, 590–600.
Cullen, P.J., Sprague Jr., G.F., 2002. The Glc7p-interacting protein Bud14p attenuates polarized growth, pheromone response, and filamentous growth in *Saccharomyces cerevisiae*. Eukaryot. Cell 1, 884–894.
Davi, V., Tanimoto, H., Ershov, D., Haupt, A., De Belly, H., Le Borgne, R., Couturier, E., Boudaoud, A., Minc, N., 2018. Mechanosensation dynamically coordinates polar growth and cell wall assembly to promote cell survival. Dev. Cell 45 (170–182), e177.
Delgado, M., Pinar, M., Polonio, P., Fandiño, S., Espeso, E.A., Peñalva, M.A., 2025. The actin mocule of endocytic internatlization in *Aspergillus nidulans*: a critical role of the WISH/DIP/SPIN90 fmailiy protein Dip1. *bioRxiv*.
Dodgson, J., Chessel, A., Yamamoto, M., Vaggi, F., Cox, S., Rosten, E., Albrecht, D., Geymonat, M., Csikasz-Nagy, A., Sato, M., Carazo-Salas, R.E., 2013. Spatial segregation of polarity factors into distinct cortical clusters is required for cell polarity control. Nat. Commun. 4, 1834.
Emser, J., Wernet, N., Hetzer, B., Wohlmann, E., Fischer, R., 2024. The small cysteine-rich virulence factor NipA of *Arthrobotrys flagrans* interferes with cuticle integrity of *Caenorhabditis elegans*. Nat. Commun. 15 (1), 5795.
Fischer, R., Requena, N., 2022. Small secreted proteins as virulence factors in nematode-trapping fungi. T. Microbiol. 30, 616–617.
Fisher, K.E., Roberson, R.W., 2016. Hyphal tip cytoplasmic organization in four zygomycetous fungi. Mycologia 108, 533–542.
Fukuda, S., Yamamoto, R., Yanagisawa, N., Takaya, N., Sato, Y., Riquelme, M., Takeshita, N., 2021. Trade-off between plasticity and velocity in mycelial growth. mBio 12 e03196–03120.
Galagan, J.E., Calvo, S.E., Borkovich, K.A., Selker, E.U., Read, N.D., Jaffe, D., FitzHugh, W., Ma, L.J., Smirnov, S., Purcell, S., Rehman, B., Elkins, T., Engels, R., Wang, S., Nielsen, C.B., Butler, J., Endrizzi, M., Qui, D., Ianakiev, P., Bell-Pedersen, D., Nelson, M.A., Werner-Washburne, M., Selitrennikoff, C.P., Kinsey, J.A., Braun, E.L., Zelter, A., Schulte, U., Kothe, G.O., Jedd, G., Mewes, W., Staben, C., Marcotte, E., Greenberg, D., Roy, A., Foley, K., Naylor, J., Stange-Thomann, N., Barrett, R., Gnerre, S., Kamal, M., Kamvysselis, M., Mauceli, E., Bielke, C., Rudd, S., Frishman, D., Krystofova, S., Rasmussen, C., Metzenberg, R.L., Perkins, D.D., Kroken, S., Cogoni, C., Macino, G., Catcheside, D., Li, W., Pratt, R.J., Osmani, S.A., DeSouza, C.P., Glass, L., Orbach, M.J., Berglund, J.A., Voelker, R., Yarden, O., Plamann, M., Seiler, S., Dunlap, J., Radford, A., Aramayo, R., Natvig, D.O., Alex, L. A., Mannhaupt, G., Ebbole, D.J., Freitag, M., Paulsen, I., Sachs, M.S., Lander, E.S., Nusbaum, C., Birren, B., 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422, 859–868.
Galagan, J.E., Calvo, S.E., Cuomo, C., Ma, L.-J., Wortman, J.R., Batzoglou, S., Lee, S.-I., Bastürkmen, M., Spevak, C.C., Clutterbuck, J., Kapitonov, V., Jurka, J., Scazzocchio, C., Farman, M., Butler, J., Purcell, S., Harris, S., Braus, G.H., Draht, O., Busch, S., d'Enfert, C., Bouchier, C., Goldman, G.H., Bell-Pedersen, D., Griffiths-Jones, S., Doonan, J.H., Yu, J., Vienken, K., Pain, A., Freitag, M., Selker, E.U., Archer, D.B., Peñalva, M.A., Oakley, B.R., Momany, M., Tanaka, T., Kumagai, T., Asai, K., Machida, M., Nierman, W.C., Denning, D.W., Caddick, M., Hynes, M., Paoletti, M., Fischer, R., Miller, B., Dyer, P., Sachs, M.S., Osmani, S.A., Birren, B.W., 2005. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438, 1105–1115.
Garcia, I., Orellana-Munoz, S., Ramos-Alonso, L., Andersen, A.N., Zimmermann, C., Eriksson, J., Boe, S.O., Kaferle, P., Papamichos-Chronakis, M., Chymkowitch, P., Enserink, J.M., 2021. Kel1 is a phosphorylation-regulated noise suppressor of the pheromone signaling pathway. Cell Rep. 37, 110186.
Glazenburg, M.M., Laan, L., 2023. Complexity and self-organization in the evolution of cell polarization. J. Cell Sci. 136, jcs259639.
Harris, S.D., 2011. Cdc42/rho GTPases in fungi: variations on a common theme. Mol. Microbiol. 79, 1123–1127.
Hernandez-Gonzalez, M., Bravo-Plaza, I., Pinar, M., de Los Rios, V., Arst Jr., H.N., Peñalva, M.A., 2018. Endocytic recycling via the TGN underlies the polarized hyphal mode of life. PLoS Genet. 14, e1007291.
Horio, T., Oakley, B.R., 2005. The role of microtubules in rapid hyphal tip growth of *Aspergillus nidulans*. Mol. Biol. Cell 16, 918–926.
Hsueh, Y.P., Gronquist, M.R., Schwarz, E.M., Nath, R.D., Lee, C.H., Gharib, S., Schroeder, F.C., Sternberg, P.W., 2017. Nematophagous fungus *Arthrobotrys oligospora* mimics olfactory cues of sex and food to lure its nematode prey. eLife 6, e20023.
Hu, X., Hoffmann, D., Wang, M., Schuhmacher, L., Stroe, M.C., Schreckenberger, B., Elstner, M., Fischer, R., 2024. GprC of the nematode-trapping fungus *Arthrobotrys flagrans* activates mitochondria and reprograms fungal cells for nematode hunting. Nat. Microbiol. 9, 1752–1763.
Ishitsuka, Y., Savage, N., Li, Y., Bergs, A., Grün, N., Kohler, D., Donnelly, R., Nienhaus, G. U., Fischer, R., Takeshita, N., 2015. Superresolution microscopy reveals a dynamic picture of cell polarity maintenance during directional growth. Sci. Adv. 1, e1500947.
Johnson, D.I., 1999. Cdc42: an essential rho-type GTPase controlling eukaryotic cell polarity. Microbiol. Mol. Biol. Rev. 63, 54–105.
Kokkoris, K., Gallo Castro, D., Martin, S.G., 2014. The Tea4-PP1 landmark promotes local growth by dual Cdc42 GEF recruitment and GAP exclusion. J. Cell Sci. 127, 2005–2016.
Konzack, S., Rischitor, P., Enke, C., Fischer, R., 2005. The role of the kinesin motor KipA in microtubule organization and polarized growth of *Aspergillus nidulans*. Mol. Biol. Cell 16, 497–506.
Kriegler, M., Wernet, V., Hetzer, B., Herrero, S., Wei, A., Wäckerle, J., Dewein, I., Fischer, R., 2025. Cell-end marker proteins are required for hyphal ring formation and size determination of traps of *Arthrobotrys flagrans*. J. Cell Sci. (in press). doi: 10.1242/jcs.263744.
Manck, R., Ishitsuka, Y., Herrero, S., Takeshita, N., Nienhaus, G.U., Fischer, R., 2015. Genetic evidence for a microtubule-capture mechanism during polarised growth of *Aspergillus nidulans*. J. Cell Sci. 128, 3569–3582.
Martin, S.G., McDonald, W.H., Yates, J.R.r., and Chang, F., 2005. Tea4p links microtubule plus ends with the formin for3p in the establishment of cell polarity. Dev. Cell 8, 479–491.
Martinez-Nunez, L., Riquelme, M., 2015. Role of BGT-1 and BGT-2, two predicted GPI-anchored glycoside hydrolases/glycosyltransferases, in cell wall remodeling in *Neurospora crassa*. Fungal Genet. Biol. 85, 58–70.
Martzoukou, O., Diallinas, G., Amillis, S., 2018. Secretory vesicle polar sorting, endosome recycling and cytoskeleton organization require the AP-1 complex in *Aspergillus nidulans*. Genetics 209, 1121–1138.
Mata, J., Nurse, P., 1997. *tea1* and the microtubular cytoskeleton are important for generating global spatial order within the fission yeast cell. Cell 89, 939–949.
Miller, K.E., Kang, P.J., Park, H.O., 2020. Regulation of Cdc42 for polarized growth in budding yeast. Microb. Cell 7, 175–189.
Pantazopoulou, A., Pinar, M., Xiang, X., Peñalva, M.A., 2014. Maturation of late Golgi cisternae into RabE(RAB11) exocytic post-Golgi carriers visualized *in vivo*. Mol. Biol. Cell 25, 2428–2443.
Peñalva, M.A., Moscoso-Romero, E., Hernandez-Gonzalez, M., 2020. Tracking exocytosis of a GPI-anchored protein in *Aspergillus nidulans*. Traffic 21, 675–688.
Peñalva, M.A., Zhang, J., Xiang, X., Pantazopoulou, A., 2017. Transport of fungal RAB11 secretory vesicles involves myosin-5, dynein/dynactin/p25, and kinesin-1 and is independent of kinesin-3. Mol. Biol. Cell 28, 947–961.
Philips, J., Herskowitz, I., 1998. Identification of Kel1p, a kelch-domain-containing protein invovled in cell fusion and morphology in *Saccharomyces cerevisiae*. J. Cell Biol. 143, 375–389.
Pinar, M., Arias-Palomo, E., de Los Rios, V., Arst Jr., H.N., Peñalva, M.A., 2019. Characterization of aspergillus nidulans TRAPPs uncovers unprecedented similarities between fungi and metazoans and reveals the modular assembly of TRAPPII. PLoS Genet. 15, e1008557.
Pinar, M., Alonso, A., de Los Rios, V., Bravo-Plaza, I., de la Gandara, A., Galindo, A., Arias-Palomo, E., Peñalva, M.A., 2022. The type V myosin-containing complex HUM is a RAB11 effector powering movement of secretory vesicles. iScience 25, 104514.
Qu, Y., Cao, H., Huang, P., Wang, J., Liu, X., Lu, J., Lin, F.C., 2022. A kelch domain cell end protein, PoTea1, mediates cell polarization during appressorium morphogenesis in *Pyricularia oryzae*. Microbiol. Res. 259, 126999.
Ramos-Garcia, S.L., Roberson, R.W., Freitag, M., Bartnicki-Garcia, S., Mouriño-Peréz, R. R., 2009. Cytoplasmic bulk flow propels nuclei in mature hyphae of *Neurospora crassa*. Eukaryot. Cell 8, 1880–1890.
Requena, N., Alberti-Segui, C., Winzenburg, E., Horn, C., Schliwa, M., Philippsen, P., Liese, R., Fischer, R., 2001. Genetic evidence for a microtubule-destabilizing effect of conventional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. Mol. Microbiol. 42, 121–132.
Reza, M.H., Dutta, S., Goyal, R., Shah, H., Dey, G., Sanyal, K., 2024. Expansion microscopy reveals characteristic ultrastructural features of pathogenic budding yeast species. J. Cell Sci. 137, jcs262046.
Riquelme, M., Bredeweg, E.L., Callejas-Negrete, O., Roberson, R.W., Ludwig, S., Beltran-Aguilar, A., Seiler, S., Novick, P., Freitag, M., 2014. The *Neurospora crassa* exocyst complex tethers Spitzenkorper vesicles to the apical plasma membrane during polarized growth. Mol. Biol. Cell 25, 1312–1326.
Riquelme, M., Yarden, O., Bartnicki-Garcia, S., Bowman, B., Castro-Longoria, E., Free, S. J., Fleissner, A., Freitag, M., Lew, R.R., Mouriño-Peréz, R., Plamann, M., Rasmussen, C., Richthammer, C., Roberson, R.W., Sanchez-Leon, E., Seiler, S., Watters, M.K., 2011. Architecture and development of the *Neurospora crassa* hypha – a model cell for polarized growth. Fungal Biol. 115, 446–474.
Riquelme, M., Aguirre, J., Bartnicki-García, S., Braus, G.H., Feldbrügge, M., Fleig, U., Hansberg, W., Herrera-Estrella, A., Kämper, J., Kück, U., Mouriño-Pérez, R.R., Takeshita, N., Fischer, R., 2018. Fungal morphogenesis: from the polarized growth of

hyphae to complex reproduction and infection structures. Microbiol. Mol. Biol. Rev. 82 e00068–00017.
Rogers, A.M., Taylor, R., Egan, M.J., 2024. The cell-end protein Tea4 spatially regulates hyphal branch initiation and appressorium remodeling in the blast fungus *Magnaporthe oryzae*. Mol. Biol. Cell 35, br2.
Sánchez-León, E., Verdín, J., Freitag, M., Roberson, R.W., Bartnicki-Garcia, S., Riquelme, M., 2011. Traffic of chitin synthase 1 (CHS-1) to the Spitzenkörper and developing septa in hyphae of *Neurospora crassa*: actin dependence and evidence of distinct microvesicle populations. Eukaryot. Cell 10, 683–695.
Scheffzek, K., Ahmadian, M.R., 2005. GTPase activating proteins: structural and functional insights 18 years after discovery. Cell. Mol. Life Sci. 62, 3014–3038.
Schuster, M., Treitschke, S., Kilaru, S., Molloy, J., Harmer, N.J., Steinberg, G., 2012. Myosin-5, kinesin-1 and myosin-17 cooperate in secretion of fungal chitin synthase. EMBO J. 31, 214–227.
Schuster, M., Martin-Urdiroz, M., Higuchi, Y., Hacker, C., Kilaru, S., Gurr, S.J., Steinberg, G., 2016. Co-delivery of cell-wall-forming enzymes in the same vesicle for coordinated fungal cell wall formation. Nat. Microbiol. 1, 16149.
Simm, D., Hatje, K., Kollmar, M., 2015. Waggawagga: comparative visualization of coiled-coil predictions and detection of stable single alpha-helices (SAH domains). Bioinformatics 31, 767–769.
Smith, J.A., Rose, M.D., 2016. Kel1p mediates yeast cell fusion through a Fus2p- and Cdc42p-dependent mechanism. Genetics 202, 1421–1435.
Snaith, H.A., Sawin, K.E., 2003. Fission yeast mod5p regulates polarized growth through anchoring of tea1p at cell tips. Nature 423, 647–651.
Snaith, H.A., Samejima, I., Sawin, K.E., 2005. Multistep and multimode cortical anchoring of tea1p at cell tips in fission yeast. EMBO J. 24, 3690–3699.
Snell, V., Nurse, P., 1994. Genetic analysis of cell morphogenesis in fission yeast - a role for casein kinase II in the establishment of polarized growth. EMBO J. 13, 2066–2074.
Stephenson, K.S., Gow, N.A., Davidson, F.A., Gadd, G.M., 2014. Regulation of vectorial supply of vesicles to the hyphal tip determines thigmotropism in *Neurospora crassa*. Fungal Biol. 118, 287–294.
Suelmann, R., Sievers, N., Fischer, R., 1997. Nuclear traffic in fungal hyphae: *in vivo* study of nuclear migration and positioning in *Aspergillus nidulans*. Mol. Microbiol. 25, 757–769.
Taheri-Talesh, N., Horio, T., Araujo-Bazan, L., Dou, X., Espeso, E.A., Peñalva, M.A., Osmani, A., Oakley, B.R., 2008. The tip growth apparatus of *Aspergillus nidulans*. Mol. Biol. Cell 19, 1439–1449.
Taheri-Talesh, N., Xiong, Y., Oakley, B.R., 2012. The functions of myosin II and myosin V homologs in tip growth and septation in *Aspergillus nidulans*. PLoS One 7, e31218.
Takeshita, N., Higashitsuji, Y., Konzack, S., Fischer, R., 2008. Apical sterol-rich membranes are essential for localizing cell end markers that determine growth directionality in the filamentous fungus *Aspergillus nidulans*. Mol. Biol. Cell 19, 339–351.
Takeshita, N., Diallinas, G., Fischer, R., 2012. The role of flotillin FloA and stomatin StoA in the maintenance of apical sterol-rich membrane domains and polarity in the filamentous fungus *Aspergillus nidulans*. Mol. Microbiol. 83, 1136–1152.
Takeshita, N., Mania, D., Herrero, S., Ishitsuka, Y., Nienhaus, G.U., Podolski, M., Howard, J., Fischer, R., 2013. The cell-end marker TeaA and the microtubule polymerase AlpA contribute to microtubule guidance at the hyphal tip cortex of *Aspergillus nidulans* to provide polarity maintenance. J. Cell Sci. 126, 5400–5411.
Takeshita, N., Evangelinos, M., Zhou, L., Somera-Fajardo, R.A., Lu, L., Takaya, N., Nienhaus, G.U., Fischer, R., 2017. Pulses of Ca^{2+} coordinate actin assembly and exocytosis for stepwise cell extension. Proc. Natl. Acad. Sci. USA 114, 5701–5706.
Tatebe, H., Shimada, K., Uzawa, S., Morigasaki, S., Shiozaki, K., 2005. Wsh3/Tea4 is a novel cell-end factor essentail for bipolar distribution of Tea1 and protects cell polarity under environmental stress in *S. pombe*. Curr. Biol. 15, 1006–1015.
Tatebe, H., Nakano, K., Maximo, R., Shiozaki, K., 2008. Pom1 DYRK regulates localization of the Rga4 GAP to ensure bipolar activation of Cdc42 in fission yeast. Curr. Biol. 18, 322–330.
Tay, Y.D., Leda, M., Goryachev, A.B., Sawin, K.E., 2018. Local and global Cdc42 guanine nucleotide exchange factors for fission yeast cell polarity are coordinated by microtubules and the Tea1-Tea4-Pom1 axis. J. Cell Sci. 131, jcs216580.
Upadhyay, S., Shaw, B.D., 2008. The role of actin, fimbrin and endocytosis in growth of hyphae in *Aspergillus nidulans*. Mol. Microbiol. 68, 690–705.
Valinluck, M., Worawatanadharm, T., Lu, C.Y., Quintanilla Jr., R.H., Banuett, F., 2014. The cell end marker Tea4 regulates morphogenesis and pathogenicity in the basidiomycete fungus *Ustilago maydis*. Fungal Genet. Biol. 66, 54–68.
Verdín, J., Bartnicki-Garcia, S., Riquelme, M., 2009. Functional stratification of the Spitzenkörper of *Neurospora crassa*. Mol. Microbiol. 74, 1044–1053.
Wang, M., Casey, P.J., 2016. Protein prenylation: unique fats make their mark on biology. Nat. Rev. Mol. Cell Biol. 17, 110–122.
Wernet, V., Wäckerle, J., Fischer, R., 2022. The STRIPAK component SipC is involved in morphology and cell-fate determination in the nematode-trapping fungus *Duddingtonia flagrans*. Genetics 220, iyab153.
Wernet, V., Kriegler, M., Kumpost, V., Mikut, R., Hilbert, L., Fischer, R., 2023. Synchronization of oscillatory growth prepares fungal hyphae for fusion. eLife 12, e83310.
Yamazaki, H., Tanaka, A., Kaneko, J., Ohta, A., Horiuchi, H., 2008. *Aspergillus nidulans* ChiA is a glycosylphosphatidylinositol (GPI)-anchored chitinase specifically localized at polarized growth sites. Fungal Genet. Biol. 45, 963–972.
Yu, X., Hu, X., Mirza, M., Wernet, N., Kirschhöfer, F., Brenner-Weiss, G., Keller, J., Bunzel, M., Fischer, R., 2021. Fatal attraction of *Caenorhabditis elegans* to predatory fungi through 6-methyl-salicylic acid. Nat. Commun. 12, 5462.
Zanolari, B., Rockenbauch, U., Trautwein, M., Clay, L., Barral, Y., Spang, A., 2011. Transport to the plasma membrane is regulated differently early and late in the cell cycle in *Saccharomyces cerevisiae*. J. Cell Sci. 124, 1055–1066.
Zhang, J., Xao, X., Fischer, L., J.F., A, Peñalva, M.A., Xiang, X., 2011. The p25 subunit of the dynactin complex is required for dynein-early endosome interaction. J. Cell Biol. 193, 1245–1255.
Zhang, Y., Fischer, R., Teichert, I., Kück, U., 2016. Laser capture microdissection to identify septum-associated proteins in *Aspergillus nidulans*. Mycologia 108, 528–532.
Zuriegat, Q., Abubakar, Y.S., Wang, Z., Chen, M., Zhang, J., 2024. Emerging roles of exocyst complex in fungi: A review. J Fungi (Basel) 10, 614.