Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Endocytosis and early endosome motility in filamentous fungi

Gero Steinberg

Hyphal growth of filamentous fungi requires microtubule-based long-distance motility of early endosomes. Since the discovery of this process in *Ustilago maydis*, our understanding of its molecular basis and biological function has greatly advanced. Studies in *U. maydis* and *Aspergillus nidulans* reveal a complex interplay of the motor proteins kinesin-3 and dynein, which co-operate to support bi-directional motion of early endosomes. Genetic screening has shed light on the molecular mechanisms underpinning motor regulation, revealing Hook protein as general motor adapters on early endosomes. Recently, fascinating insight into unexpected roles for endosome motility has emerged. This includes septin filament formation and cellular distribution of the machinery for protein translation.

Address
Biosciences, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

Corresponding author: Steinberg, Gero (G.Steinberg@exeter.ac.uk)

Current Opinion in Microbiology 2014, **20**:10–18

This review comes from a themed issue on **Host–microbe interactions: fungi**

Edited by **Jay C Dunlap** and **Jean Paul Latgé**

http://dx.doi.org/10.1016/j.mib.2014.04.001

1369-5274/Published by Elsevier Ltd.

Introduction

The endocytic system comprises several compartments that receive cargo from the plasma membrane for processing and recycling back to the cell surface or its degradation in the lysosomes [1]. Early endosomes (EEs) are a central compartment in the endocytic pathway (Figure 1). They bind the small GTPase Rab5, which, together with its effectors, controls biogenesis, membrane fusion and microtubule-dependent motility in animal cells [2–5]. Motility of animal endosomes supports endocytic sorting, but also participates in long-distance signal transduction, cytokinesis and cell migration [6,7]. In the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, long-distance transport along microtubules does not exist; this may relate to their small size. However, fungal endocytosis was described first in yeasts [8–10], and endocytic recycling supports their polar growth and survival [11–13]. In filamentous fungi, endocytosis supports hyphal growth (see Box 1; overview in [14,15]), but in contrast to the yeasts, filamentous fungi contain EEs that move along microtubules. EEs were first described in *U. maydis* [16] (Table 1). A screen for morphological *U. maydis* mutants revealed a mutation in a soluble *N-ethylmaleimide sensitive* factor attachment protein receptor (SNARE), named Yup1. Temperature-sensitive Yup1^{ts} mutants showed defects in vacuolar sorting of FM4-64, a marker dye for tracking endocytic uptake in fungi [17–21], suggesting that the SNARE functions in the endocytic pathway. Indeed, Yup1 was located on organelles that stained rapidly with shortly after application of FM4-64 [16]. The organelles bind Phox-domains, which specifically interact with EE-characteristic lipids [22] and carry the EE-specific small GTPases Rab4 and Rab5 [23–25]. Thus, there is little doubt that the Yup1-positive organelles are EEs. Rapidly-moving Rab5-positive EEs were also described in *A. nidulans* [26] and *Neurospora crassa* [27]. Such organelles are thus a hallmark of filamentous fungi.

The molecular machinery for early endosome motility

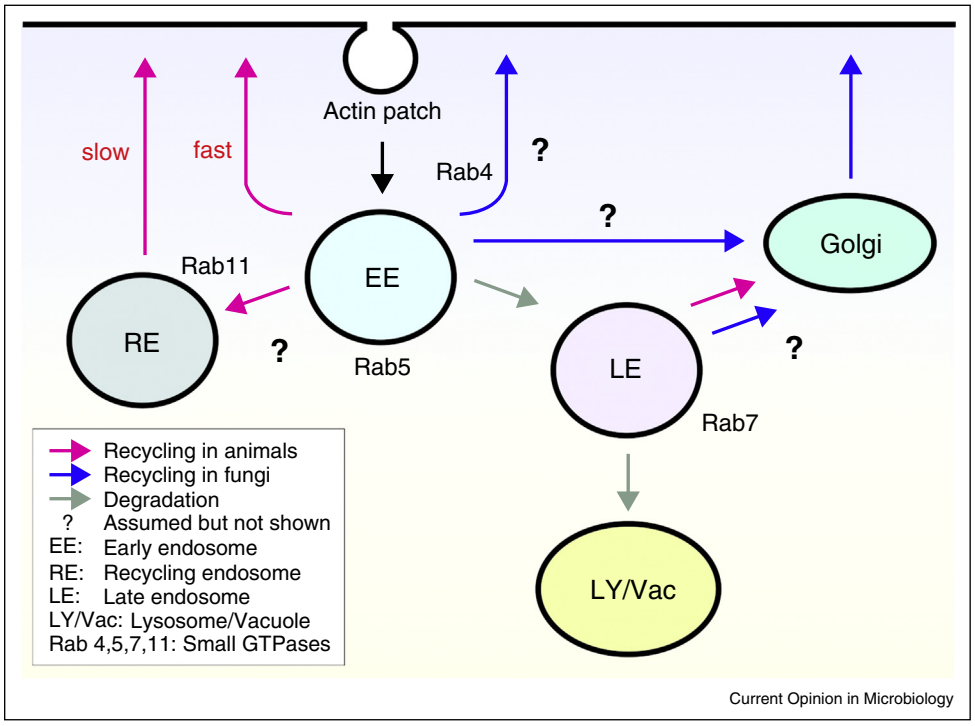
Endosome motility is a microtubule-dependent process

Microtubules are polymers of tubulin dimers that support long-range motility of organelles and vesicles in eukaryotic cells. Numerous studies have suggested that Golgi-derived secretory vesicles support hyphal growth [28], and they are likely to be transported to the hyphal tip by motors [29]. This notion was supported by the discovery of the motor protein kinesin-1 in Asco-, Basidio- and Zygomycetes [30–33]. Subsequent studies confirmed a role for this microtubule-dependent motor in delivering chitin synthase-containing secretory vesicles [34], but also indicated a role in endocytic uptake of the marker dye Lucifer Yellow [20]. This suggested that microtubules support endocytosis. Indeed, the Yup1-positive EEs were shown to move bi-directionally along microtubules [16]. A similar behaviour was reported in *A. nidulans* [26], *A. oryzae* [35] and *N. crassa* [27].

EE loading onto dynein occurs at microtubule plus-ends

Microtubules have an intrinsic polarity, with most polymerization occurring at the plus-end, whereas the minus-end is usually blocked by a nucleation site. Molecular motors utilise this polarity and kinesins move their cargo to plus-ends, whereas dynein transports organelles towards minus-ends. In filamentous fungi, EE motility is bi-directional and is mediated by kinesin-3 and dynein [27,36–40]. Dynein accumulates at the plus-ends of microtubules [36,41] and the motor kinesin-1 is required for this plus-end targeting [36,42]. EE binding to dynein, for retrograde transport to sub-apical parts of the hyphal cell, occurs mainly at the microtubule plus-ends

Figure 1



Schematic overview of endocytic pathways in animal cells and fungi. Endocytosis begins with the uptake of material into endocytic vesicles. In fungi, these vesicles are surrounded by F-actin [61,73]. The first endocytic compartment is early endosomes (EEs), which carry the small GTPase Rab5 [5,24]. In animal cells, recycling back to the plasma membrane involves EEs and the associated GTPase Rab4 (fast recycling) and recycling endosomes (RE) that carry the small GTPase Rab11 [74]. The pathways of recycling in fungi are not clear (indicated by '?'), but may involve late Golgi-associated membranes [75]. While travelling towards the vacuole/lysosome, EEs mature into late endosomes (LE), which involves a replacement of Rab5 by Rab7 [64•,76].

[36,38,43,44]. This led to the idea that the microtubule plus-end is a 'dynein loading zone' [36]. However, it is important to note that dynein loading also takes place in sub-apical parts of the cell [44]. It was suggested that dynein accumulation at plus-ends increases the efficiency of dynein interacting with EEs [38]. Indeed, quantitative light microscopy studies demonstrated that 50-60 dynein motors concentrate at apical plus-ends. Experimentally lowering this number, by interfering with the mechanism of anchorage, reduced the loading of EEs to the motor [43]. Under these conditions, the number of EEs that run too far and 'fall off' at the microtubule end increases ~5 times. This suggests that the accumulation of dynein at plus-ends serves as a 'buffer stop' that keeps the organelles on the microtubule track [43]. This concept assumes a stochastic interaction of dynein and EEs. However, additional dynein regulators, such as NudF/Lis1 [45], have been shown to be required for retrograde EE motility [36,39•]. Fungal Lis1 concentrates at plus-ends [36,41], but does not co-travel with retrograde moving dynein or EEs [36,39•]. This led to the suggestion that Lis1 is an initiation factor for retrograde EE motion [39•].

Kinesin-3 is the motor for tip-directed motility of EEs

The human genome encodes 45 kinesin motors of which 8 belong to the kinesin-3 family [46]. Kinesin-3-like motors are absent from the genomes of *S. cerevisiase* and *Schizosaccharomyces pombe*. This coincides with the absence of microtubule-based EE motility in these yeasts. Early studies in *U. maydis* identified kinesin-3 as the anterograde motor for EE motility [36,37]. Subsequently, kinesin-3 and its role in EE motility was discovered in *A. nidulans* [39•,40] and *N. crassa* [27]. While kinesin-3 appears to be the major motor for EE motility, a role in secretion was also reported [47]. Kinesin-3 opposes dynein in mediating bi-directional motility of EEs. In animal cells [48] and *A. nidulans* [39•], dynein and kinesin motors are bound simultaneously to the cargo. In *U. maydis*, however, anterograde kinesin-3-delivered EEs do not bind dynein [44]. In this fungus, a turn in EE-transport direction is achieved in two ways. Firstly, each EE carries ~3–5 kinesin-3 motors; this may allow 'hopping' from one microtubule to another, without interrupting transport. As most EE motility occurs along microtubule bundles, consisting of anti-polar oriented microtubules [49], 'hopping' causes a change in transport

Box 1 A historical perspective on endocytosis in filamentous fungi

Filamentous fungal growth is characterized by apical extension of the hyphal cell. This process requires the constant supply of membranes and proteins, such as cell wall-forming enzymes, to the growing hyphal tip. The Spitzenkörper, an apical vesicle accumulation found in many fungal species [78–81], is thought to be of key importance to tip growth. It was suggested that the Spitzenkörper consists of Golgi-derived exocytic vesicles that fuse with the expanding apex to fuel tip growth [82]. This view was firstly challenged by the work of Hoffmann and Mendgen [18], who stained the Spitzenkörper of *Uromyces fabae* with the lipophilic marker FM4-64. At this time, the dye was already well-established as a tracer for the endocytic pathway in the yeasts *Saccharomyces cerevisiae* [21]. Therefore, staining of the Spitzenkörper with FM4-64 suggested that endocytic uptake of membranes participates in tip growth. Subsequent studies in *Ustilago maydis* [16,20], *Magnaporthe grisea* [83], *Aspergillus nidulans* [19], *Neurospora crassa* and 6 additional other fungi [17] confirmed the uptake of FM4-64 into fungal cells. This strongly suggested that endocytsis is common in filamentous fungi. However, FM4-64 or other endocytic marker dyes were not taken up in some fungal species [84,85]. In addition, fungal hyphae are continuously growing and endocytic vesicle uptake against the internal turgor pressure was difficult to envisage [85]. Thus, just a decade ago, the existence of endocytosis in fungal hyphae was a matter of debate [86]. To-date, elegant experimental studies in the fission yeast have shown that initial endocytic steps are slowed down by turgor pressure, but this is overcome by the energy-dependent assembly of the actin cytoskeleton [87]. In addition, overwhelming evidence coming from bioinformatic analysis of fungal genomes [86,88], live observation of fluorescent proteins involved in endocytosis [35,62], combined with mutant studies in *U. maydis*, *A. nidulans*, *A. oryzae*, *Candida albicans*, *Ashbya gossypii* and *N. crassa* [16,58–63,89–96], leaves no doubt that endocytosis and endocytic recycling is of central importance for hyphal growth (for more comprehensive overview see [14,15]).

direction. Secondly, kinesin-1 delivers dynein to the microtubule plus-end [36], where it accumulates and from where it is released to travel 'freely' towards minus-ends [43]. This dynein is competent to bind anterogradely-moving EEs, which turns the transport direction from anterograde to retrograde [44]. This is achieved by single dynein motors that probably inactivate the excess of kinesin-3 (see below). Finally, it should be noted that Ascomycete kinesin-3 moves cargo along a subset of detyrosinated, less dynamic microtubules [27,40]. The biological reason for this merits further investigation.

Hook proteins link motors to EEs

Motor proteins often interact with their cargo membrane via adapter complexes, which control the affinity of the motor for the cargo and regulate the direction of transport [50]. In animal cells, several adapter complexes for dynein and kinesin-1 have been described, but no adapter for kinesin-3 is known. In fungi, our knowledge is restricted to a single report, which shows that the p25 subunit of the dynactin complex links dynein to EEs [51]. However, neither a kinesin-3 binding partner on EEs, nor any regulatory protein has been described. To identify such regulatory machinery for bi-directional EE motility, genetic screening was undertaken in *A. nidulans* and *U. maydis*, leading to the simultaneous description of Hook proteins as an adapter for dynein on EEs [52••,53••]. Hook proteins were first identified in fruit flies and are intensively studied in human cells, where they appear to link organelles to microtubules [54,55]. In fungi, *hook* null mutants are defective in retrograde EE motility, as the organelles are no longer able to bind dynein [52••,53••]. It was shown biochemically [53••] and by live cell imaging [52••] that the N-terminal part of fungal hook proteins (HookA in *A. nidulans*, [53••]) and Hok1 in *U. maydis*, [52••]) interact with the dynein/dynactin complex, whereas the C-terminal region binds to EEs. In *A. nidulans*, EE motility requires the Rab5-GTPase RabB [57]. Whether hook proteins are anchored to EE membranes via Rab5 GTPases remains to be seen.

Surprisingly, Hok1 in *U. maydis* also controls kinesin-3 binding to EEs. A short and highly conserved part of the N-terminal coiled-coil region of Hok1 is crucial for this, but also for dynein binding to EEs. This suggests that an unidentified Hok1-binding protein bridges between Hok1 and both motors. In *U. maydis*, homologues of the human proteins FTS and FHIP bind to Hok1 and localize to EEs [52••]. The precise role of both proteins in both humans and fungi remains to be unmasked, but they may support motor attachment of Hok1 on EEs. The ability of Hok1 to bind both motors suggests that the Hook/FTS/FHIP complex serves as a coordinator for EE bi-directional motility. Hok1 may control attachment of both kinesin-3 and dynein to the cargo, thereby regulating the transport direction (Figure 2a, [52••]). Interestingly, hook proteins, like kinesin-3, are not present in *S. cerevisiae* and *S. pombe*. As both are found in filamentous fungi and in animals [52••], they appear to form a functional pair, required for long-range, bi-directional motility of cargo along microtubules.

The cellular role of endosome movement

EE motility and the endocytic pathway

Endocytic recycling near the hyphal apex supports fungal tip growth in *A. nidulans*, *A. oryzae*, *N. crassa and Ashbya gossypii* [15,58–63] and receptor exposure during early pathogenic development of *U. maydis* [24]. Recycling appears to depend on fusion of transport vesicles with the EE [16,24], which are also thought to be involved in sorting cargo to the subapical vacuole for degradation. Thus, it is likely that EE motility shuttles the organelles between the expanding tip and the subapical vacuolar system. However, in *A. oryzae* uptake of uric acid-xanthine permease, from the plasma membrane and delivery to vacuoles, occurs in the absence of EE motility [35]. While this finding questions a role of EE motility in vacuolar sorting, a recent report by Penalva [64•] demonstrates that retrograde motility is required to allow fusion of late endosomes with vacuoles. In *A. nidulans*, EEs

Table 1

Scientific milestones in endocytosis research in filamentous fungi

Contribution	Fungal system	Reference
First report of endocytosis in filamentous fungi	*Uromyces fabae*	[18]
First report on a role of microtubules in fungal endocytosis	*Ustilago maydis*	[20]
Identification of motile early endosomes that move along microtubules; first indication of a role of endocytic recycling in fungal morphology	*Ustilago maydis*	[16]
Establishment of FM4-64 as a general tracer for endocytosis in filamentous fungi	*Neurospora crassa* and 9 other species	[17]
First description of fungal kinesin-3 and its role in opposing dynein in motility of early endosomes	*Ustilago maydis*	[37]
Identification of the apical MT plus end as a ''dynein loading zone'' for binding EEs to the retrograde motor	*Ustilago maydis*	[36]
First report of a role of endocytosis in receptor recycling during fungal pathogenicity	*Ustilago maydis*	[24]
Identification of an apical collar-like region of endocytic uptake and recycling	*Aspergillus nidulans*	[60–62]
Discovery that Ascomycete kinesin-3 utilizes a subset of detyrosinated (less dynamic) microtubules	*Aspergillus nidulans*	[40]
Report on the down-regulation of a plasma membrane transporter by substrate-induced endocytosis	*Aspergillus nidulans*	[95]}
First insight into EE-to-motor attachment by reporting a role of dynactin subunit p25	*Aspergillus nidulans*	[51]
First report of a role of EEs in transporting mRNA	*Ustilago maydis*	[66•]
First report on a role of retrograde motility in early-to-late endosome maturation	*Aspergillus nidulans*	[64•]
First description of an up-regulation of actin-patch dynamics and associated endocytosis in hyphal versus yeast-like growth	*Candida albicans*	[97•]
Discovery of clathrin-independent endocytosis	*Candida albicans*	[94•]
Discovery of a biological role of EE-associated translation	*Ustilago maydis*	[69••]
Identification of a role of bidirectional EE motility in distribution the machinery for protein translation	*Ustilago maydis*	[56••]
Identification of Hook proteins as adapters for EE motors	*A. nidulans*/*U. maydis*	[53••]/[52••]

Note: Reports on eisosomes, which were implied in fungal endocytosis [98], are not included as their suggested role as endocytic portals is a matter of debate [99].

convert into late endosomes and, finally, subapical vacuoles [57]. During this maturation, Rab5 (RabB/A in *A. nidulans*) is replaced by Rab7 (RabS in *A. nidulans*). In temperature-sensitive dynein mutants, retrograde EE motility is blocked and Rab7-positive late endosomes/vacuoles, stained with the blue dye CMAC (7-Amino-4-Chloromethylcoumarin), cluster near the hyphal tip. These results suggest that endosomes mature and fuse to form vacuoles as they move away from the tip [64•]. A role for EE motility in sorting to the vacuole was also reported in *U. maydis* [56••]. When binding of dynein was impaired in *hook* deletion mutants (see above), or when the dynein heavy chain was inactivated in temperature-sensitive mutants, EEs formed clusters at the hyphal tip and delivery of FM4-64 to the vacuoles was blocked [56••]. Surprisingly, FM4-64 concentrated in a 'cloud' of Rab7-positive small vesicles that were not stained with CMAC [56••], while vacuole organization remained largely unaffected. This raises the possibility that EE motility is required to allow efficient fusion of Rab7-positive transport vesicles with the vacuole.

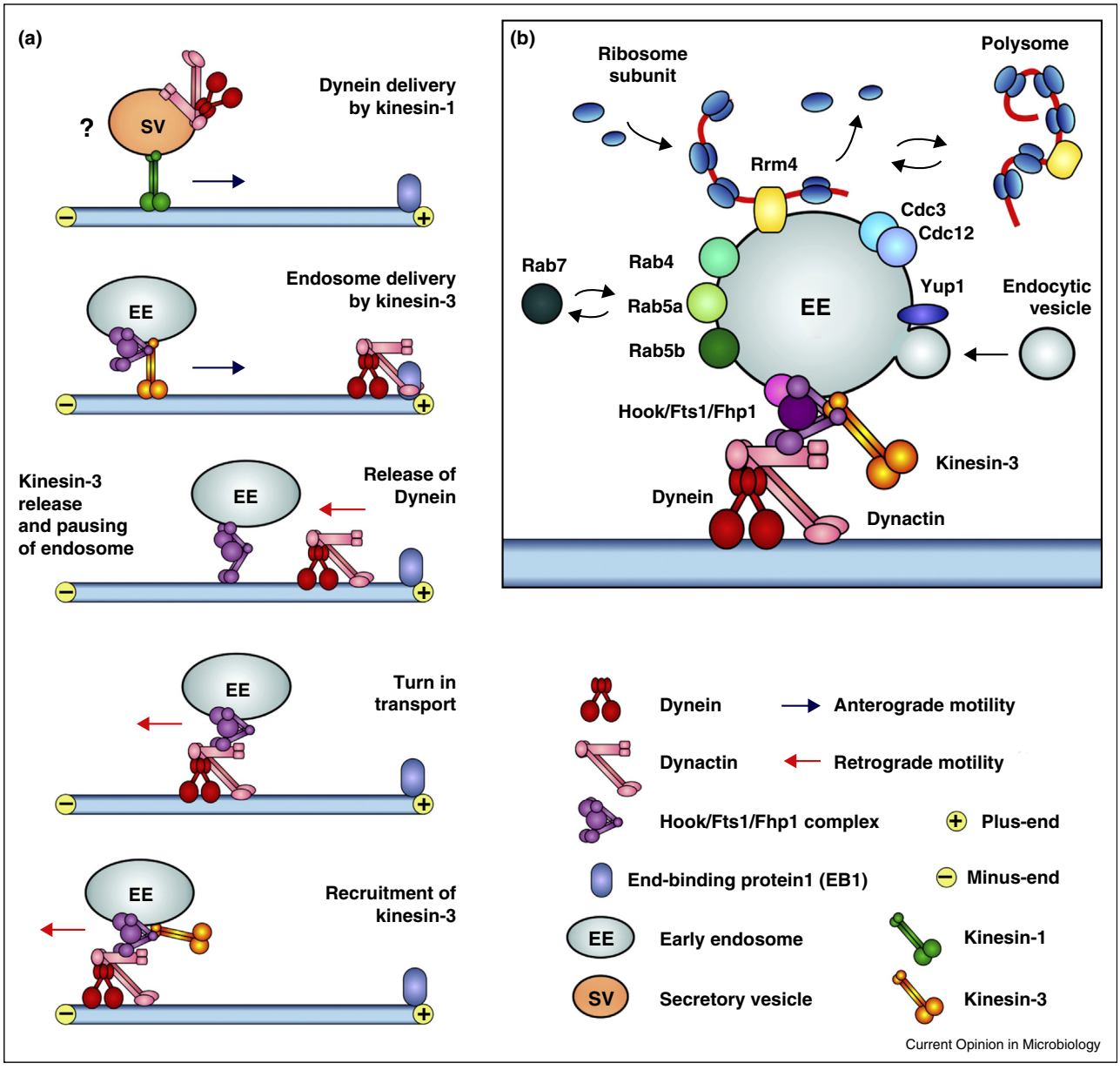
EEs deliver mRNA to the cell poles

An unexpected insight into a cellular role for EE motility in fungi was provided by work on RNA-binding proteins. In *U. maydis*, the putative RNA-binding protein Rrm4 moves along microtubules [65]. This motility requires dynein and kinesin-3 [66•], motors that were shown previously to mediate bi-directional EE motility [36,37]. Indeed, co-localization studies and the use of temperature-sensitive Yup1 mutants revealed that Rrm4 'hitchhikes' on moving EEs [66•]. Rrm4 binds various mRNAs, including those encoding the ubiquitin fusion protein Ubi1 and the small G protein Rho3 [67]. It was therefore suggested that EE motility towards the cell ends supports polar delivery of Rrm4-bound RNA [68]. However, *in vivo* observation of fluorescent Rrm4 particles demonstrated that Rrm4 motility is bi-directional and that extended runs of these particles are rare [67]. These results raised doubt about an exclusive role of EE motility in long-distance transport of mRNA. A recent paper by Feldbrügge [69••] provides an unexpected answer to this conundrum. It shows that the mRNA of the septin *cdc3* and *cdc12* is actively-translated on moving EEs and both proteins bind to EEs. Interestingly, the presence of Cdc3 on EEs requires Cdc12, suggesting that assembly of septin complexes occurs on EEs. This is important for proper septin filament formation and, in turn, hyphal growth.

EEs distribute the machinery for protein translation

A second study in *U. maydis* confirms translation of mRNA on moving EEs, but it came to the conclusion that ribosome binding to mRNA serves to distribute polysomes throughout the cell [56••]. Ribosomal subunits are produced constantly in the nucleus and are thought to

Figure 2



(a) Schematic illustration of the role of the Hook-complex in coordinating motor attachment in *U. maydis*. EE motility depends on three motors, kinesin-1, kinesin-3 and dynein. Kinesin-1 delivers the dynein/dynactin complex to microtubule plus-ends near the hyphal apex. This may involve secretory vesicles, though experimental evidence for this is missing (indicated by '?'). Kinesin-3 binds to EEs via the Hook-complex, consisting of Hok1, and homologues of the human oncogene FTS and FHIP, both shown to interact with Hook in humans [77]. Dynein accumulates at plus-ends via a stochastic traffic jam and a specific interaction of the $p150^{glued}$ subunit of dynactin and EB1 [43]. The motor is released from the microtubule plus-ends and can bind to EEs during its journey towards the minus-end. EEs usually pause prior to rebinding, which coincides with a kinesin-3 release [52••]. Binding of dynein initiates retrograde motility, which persists while kinesin-3 is recruited back onto the EEs. **(b)** The current knowledge of proteins binding to EEs in *U. maydis*. The Hook-complex, consisting of Hok1, Fts1 and Fhp1 serves as an adapter for kinesin-3 and dynein [52••]. Yup1 is a putative endosomal SNARE [16] that mediates fusion of transport vesicles the organelles. This function is crucial for endocytic recycling, and $yup1^{ts}$ mutants are defective in morphology [16] and receptor recycling [24]. The small GTPases Rab5a, Rab5b and Rab4 locate to EEs [24,56••], but their cellular role is not known. Rrm4 is an EE-associate RNA-binding protein [65,66•] that anchors mRNA and associates ribosomes to EEs [56••]. The entire polysome can can 'fall off' or rebind to moving EEs, which evenly-distributes the translation machinery [56••]. In addition, EEs have recently implied in assembly of septin filaments (Cdc3 and Cdc12) [69••].

distribute by diffusion. However, the visco-elastic properties of the cytoplasm limit passive diffusion of large particles and vesicles [70]. Indeed, the use of photo-activatable ribosomal proteins in *U. maydis* revealed that passive diffusion is too slow to ensure even cellular distribution of the ribosomal subunits [56••]. Mathematical modelling indicated that an active transport component is required to spread ribosomes through the fungal cell. Indeed, ribosome distribution was impaired in kinesin-3 and dynein mutants, but was also involved the RNA-binding protein Rrm4. This suggests that ribosome distribution involves EE motility. Surprisingly, quantitative live-cell imaging of ribosomes demonstrated that entire polysomes assemble on moving EEs [56••]. While travelling, the ribosomes are translationally active, thereby binding Rrm4-anchored mRNA to be spread within the cell. This is achieved by random release, but also rebinding, of entire polysomes from the EEs. Thus bi-directional EE motility constantly ''stirs'' the translation machinery in the cell, thereby adding an active transport component to the passive diffusion of ribosomes in the cytoplasm. Null mutants in *rrm4* and the kinesin-3 gene *kin3* exhibit a very similar phenotype [56••], suggesting that distributing of the translation machinery is a major role of EE motility in *U. maydis*.

Conclusion

The existence of endocytosis and EEs in fungi was a matter of debate until relatively recently. Today, it is well-established that endocytic recycling supports fungal morphology and that EEs rapidly move within the fungal cell. Intensive research in *U. maydis* and *A. nidulans* has elucidated the molecular machinery underlying EE motility and has provided insight into fundamental principles of motor co-operation in membrane trafficking [71]. Most recently, genetic screening led to the identification of novel kinesin-3 adapters and this discovery will most likely stimulate future research in mammalian systems. We are also beginning to understand why EEs move. While a role for retrograde EE motility in sorting to the vacuole is expected, its function in distributing mRNA, supporting protein translation 'on the move' and spreading the translation machinery is surprising, yet potentially of great significance for growth and function of fungal hyphae. New biological roles for EEs in fungi will inevitably be discovered. Indeed, indirect evidence suggests aflatoxin biosynthesis in *Aspergillus parasiticus* involves EEs [72] (J.E. Linz, pers. communication). Thus, fungal EEs may provide a 'platform' to integrate several cellular pathways and their respective functions (Figure 2b), a concept first suggested in animal systems [7]. Highly complementary work on EE function and motility in the model systems *U. maydis* and *A. nidulans* has turned this pioneering field into a fast-moving research area in fungal cell biology (Table 1). Future discoveries in this area promise exciting and unexpected insight into how fungal cells function. Furthermore, work in fungal systems will almost certainly help unveil fundamental principles of membrane trafficking in eukaryotic cells.

Acknowledgement

The author wishes to thank Drs MA Penalva and JE Linz for helpful discussion, Dr S Gurr for constructive comments on the manuscript and the BBSRC for continued funding.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Seaman MN: **Endosome protein sorting: motifs and machinery**. *Cell Mol Life Sci* 2008, **65**:2842-2858.
2. Nielsen E, Severin F, Backer JM, Hyman AA, Zerial M: **Rab5 regulates motility of early endosomes on microtubules**. *Nat Cell Biol* 1999, **1**:376-382.
3. Zeigerer A, Gilleron J, Bogorad RL, Marsico G, Nonaka H, Seifert S, Epstein-Barash H, Kuchimanchi S, Peng CG, Ruda VM, Del Conte-Zerial P *et al.*: **Rab5 is necessary for the biogenesis of the endolysosomal system *in vivo***. *Nature* 2012, **485**:465-470.
4. Pal A, Severin F, Lommer B, Shevchenko A, Zerial M: **Huntingtin–HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease**. *J Cell Biol* 2006, **172**:605-618.
5. Zerial M, McBride H: **Rab proteins as membrane organizers**. *Nat Rev Mol Cell Biol* 2001, **2**:107-117.
6. Miaczynska M, Pelkmans L, Zerial M: **Not just a sink: endosomes in control of signal transduction**. *Curr Opin Cell Biol* 2004, **16**:400-406.
7. Gould GW, Lippincott-Schwartz J: **New roles for endosomes: from vesicular carriers to multi-purpose platforms**. *Nat Rev Mol Cell Biol* 2009, **10**:287-292.
8. Jenness DD, Spatrick P: **Down regulation of the alpha-factor pheromone receptor in *S. cerevisiae***. *Cell* 1986, **46**:345-353.
9. Raths S, Rohrer J, Crausaz F, Riezman H: **End3 and end4: two mutants defective in receptor-mediated and fluid-phase endocytosis in *Saccharomyces cerevisiae***. *J Cell Biol* 1993, **120**:55-65.
10. Fernandez N, Puente P, Leal F: **Fluid-phase endocytosis in yeasts other than *Saccharomyces cerevisiae***. *FEMS Microbiol Lett* 1990, **57**:7-11.
11. Proszynski TJ, Klemm R, Bagnat M, Gaus K, Simons K: **Plasma membrane polarization during mating in yeast cells**. *J Cell Biol* 2006, **173**:861-866.
12. Valdez-Taubas J, Pelham HR: **Slow diffusion of proteins in the yeast plasma membrane allows polarity to be maintained by endocytic cycling**. *Curr Biol* 2003, **13**:1636-1640.
13. Weinberg J, Drubin DG: **Clathrin-mediated endocytosis in budding yeast**. *Trends Cell Biol* 2012, **22**:1-13.
14. Penalva MA: **Endocytosis in filamentous fungi: Cinderella gets her reward**. *Curr Opin Microbiol* 2010, **13**:684-692.
15. Shaw BD, Chung DW, Wang CL, Quintanilla LA, Upadhyay S: **A role for endocytic recycling in hyphal growth**. *Fungal Biol* 2011, **115**:541-546.
16. Wedlich-Söldner R, Bolker M, Kahmann R, Steinberg G: **A putative endosomal t-SNARE links exo- and endocytosis in the phytopathogenic fungus *Ustilago maydis***. *EMBO J* 2000, **19**:1974-1986.
17. Fischer-Parton S, Parton RM, Hickey PC, Dijksterhuis J, Atkinson HA, Read ND: **Confocal microscopy of FM4-64 as a tool for analysing endocytosis and vesicle trafficking in living fungal hyphae**. *J Microsc* 2000, **198**:246-259.

18. Hoffmann J, Mendgen K: **Endocytosis and membrane turnover in the germ tube of *Uromyces fabae***. *Fungal Genet Biol* 1998, **24**:77-85.

19. Penalva MA: **Tracing the endocytic pathway of *Aspergillus nidulans* with FM4-64**. *Fungal Genet Biol* 2005, **42**:963-975.

20. Steinberg G, Schliwa M, Lehmler C, Bölker M, Kahmann R, McIntosh JR: **Kinesin from the plant pathogenic fungus *Ustilago maydis* is involved in vacuole formation and cytoplasmic migration**. *J Cell Sci* 1998, **111**:2235-2246.

21. Vida TA, Emr SD: **A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast**. *J Cell Biol* 1995, **128**:779-792.

22. Lemmon MA: **Phosphoinositide recognition domains**. *Traffic* 2003, **4**:201-213.

23. Chavrier P, Parton RG, Hauri HP, Simons K, Zerial M: **Localization of low molecular weight gtp binding proteins to exocytic and endocytic compartments**. *Cell* 1990, **62**:317-329.

24. Fuchs U, Hause G, Schuchardt I, Steinberg G: **Endocytosis is essential for pathogenic development in the corn smut fungus *Ustilago maydis***. *Plant Cell* 2006, **18**:2066-2081.

25. Van Der Sluijs P, Hull M, Zahraoui A, Tavitian A, Goud B, Mellman I: **The small GTP-binding protein Rab4 is associated with early endosomes**. *Proc Natl Acad Sci U S A* 1991, **88**:6313-6317.

26. Abenza JF, Pantazopoulou A, Rodriguez JM, Galindo A, Penalva MA: **Long-distance movement of *Aspergillus nidulans* early endosomes on microtubule tracks**. *Traffic* 2009, **10**:57-75.

27. Seidel C, Moreno-Velasquez SD, Riquelme M, Fischer R: ***Neurospora crassa* nKin2, a kinesin-3 motor, transports early endosomes and is required for polarized growth**. *Eukaryot Cell* 2013, **12**:1020-1032.

28. Bartnicki-Garcia S: *Hyphal Tip Growth: Outstanding Questions*. New York, NY: Marcel Dekker; 2002, 29-58.

29. Steinberg G: **Hyphal growth: a tale of motors, lipids, and the Spitzenkörper**. *Eukaryot Cell* 2007, **6**:351-360.

30. Steinberg G, Schliwa M: **The *Neurospora* organelle motor: a distant relative of conventional kinesin with unconventional properties**. *Mol Biol Cell* 1995, **6**:1605-1618.

31. Wu Q, Sandrock TM, Turgeon BG, Yoder OC, Wirsel SG, Aist JR: **A fungal kinesin required for organelle motility, hyphal growth, and morphogenesis**. *Mol Biol Cell* 1998, **9**:89-101.

32. Steinberg G: **A kinesin-like mechanoenzyme from the zygomycete *Syncephalastrum racemosum* shares biochemical similarities with conventional kinesin from *Neurospora crassa***. *Eur J Cell Biol* 1997, **73**:124-131.

33. Lehmler C, Steinberg G, Snetselaar KM, Schliwa M, Kahmann R, Boelker M: **Identification of a motor protein required for filamentous growth in *Ustilago maydis***. *EMBO J* 1997, **16**:3464-3473.

34. Schuster M, Treitschke S, Kilaru S, Molloy J, Harmer NJ, Steinberg G: **Myosin-5, kinesin-1 and myosin-17 cooperate in secretion of fungal chitin synthase**. *EMBO J* 2012, **31**:214-227.

35. Higuchi Y, Nakahama T, Shoji JY, Arioka M, Kitamoto K: **Visualization of the endocytic pathway in the filamentous fungus *Aspergillus oryzae* using an eGFP-fused plasma membrane protein**. *Biochem Biophys Res Commun* 2006, **340**:784-791.

36. Lenz JH, Schuchardt I, Straube A, Steinberg G: **A dynein loading zone for retrograde endosome motility at microtubule plus-ends**. *EMBO J* 2006, **25**:2275-2286.

37. Wedlich-Söldner R, Straube A, Friedrich MW, Steinberg G: **A balance of KIF1a-like kinesin and dynein organizes early endosomes in the fungus *Ustilago maydis***. *EMBO J* 2002, **21**:2946-2957.

38. Zhang J, Zhuang L, Lee Y, Abenza JF, Penalva MA, Xiang X: **The microtubule plus-end localization of *Aspergillus* dynein is important for dynein-early-endosome interaction but not for dynein ATPase activation**. *J Cell Sci* 2010, **123**:3596-3604.

39. • Egan MJ, Tan K, Reck-Peterson SL: **Lis1 is an initiation factor for dynein-driven organelle transport**. *J Cell Biol* 2012, **197**:971-982.

In this interesting paper, the authors describe a role of the dynein-assoociated protein Lis-1/NudF in cargo transport in *A. nidulans*. Peroxisomes and Rab5-positive EEs do move in a bidirectional fashion, which is driven by dynein and the kinesin-3 UncA. In *nudf* mutants, frequency of organelle retrograde transport is reduced, while velocity is not affected. Unlike dynein/dynactin, Lis1 does not move together with retrograde dynein cargo, including EEs. This observation confirmed previous reports on Lis1 in EE in *U. maydis* [36]. The authors conclude that Lis1 is required to initate the loading of EEs to dynein at microtubule plus-ends.

40. Zekert N, Fischer R: **The *Aspergillus nidulans* kinesin-3 UncA motor moves vesicles along a subpopulation of microtubules**. *Mol Biol Cell* 2009, **20**:673-684.

41. Han G, Liu B, Zhang J, Zuo W, Morris NR, Xiang X: **The *Aspergillus* cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics**. *Curr Biol* 2001, **11**:719-724.

42. Zhang J, Li S, Fischer R, Xiang X: **Accumulation of cytoplasmic dynein and dynactin at microtubule plus ends in *Aspergillus nidulans* is kinesin dependent**. *Mol Biol Cell* 2003, **14**:1479-1488.

43. Schuster M, Kilaru S, Ashwin P, Lin C, Severs NJ, Steinberg G: **Controlled and stochastic retention concentrates dynein at microtubule ends to keep endosomes on track**. *EMBO J* 2011, **30**:652-664.

44. Schuster M, Lipowsky R, Assmann MA, Lenz P, Steinberg G: **Transient binding of dynein controls bidirectional long-range motility of early endosomes**. *Proc Natl Acad Sci U S A* 2011, **108**:3618-3623.

45. Xiang X, Osmani AH, Osmani SA, Xin M, Morris NR: **NudF, a nuclear migration gene in *Aspergillus nidulans*, is similar to the human LIS-1 gene required for neuronal migration**. *Mol Biol Cell* 1995, **6**:297-310.

46. Miki H, Setou M, Kaneshiro K, Hirokawa N: **All kinesin superfamily protein, KIF, genes in mouse and human**. *Proc Natl Acad Sci U S A* 2001, **98**:7004-7011.

47. Schuchardt I, Assmann D, Thines E, Schuberth C, Steinberg G: **Myosin-v, kinesin-1, and kinesin-3 cooperate in hyphal growth of the fungus *Ustilago maydis***. *Mol Biol Cell* 2005, **16**:5191-5201.

48. Jolly AL, Gelfand VI: **Bidirectional intracellular transport: utility and mechanism**. *Biochem Soc Trans* 2011, **39**:1126-1130.

49. Schuster M, Kilaru S, Fink G, Collemare J, Roger Y, Steinberg G: **Kinesin-3 and dynein cooperate in long-range retrograde endosome motility along a nonuniform microtubule array**. *Mol Biol Cell* 2011, **22**:3645-3657.

50. Akhmanova A, Hammer JA III: **Linking molecular motors to membrane cargo**. *Curr Opin Cell Biol* 2010, **22**:479-487.

51. Zhang J, Yao X, Fischer L, Abenza JF, Penalva MA, Xiang X: **The p25 subunit of the dynactin complex is required for dynein–early endosome interaction**. *J Cell Biol* 2011, **193**:1245-1255.

52. •• Bielska E, Schuster M, Roger Y, Berepiki A, Soanes DM, Talbot N, Steinberg G: **Hook is an adapter that coordinates kinesin-3 and dynein cargo-attachment on early endosomes**. *J Cell Biol* 2014, **204**:989-1007.

This study, together with [53••], describes the identification of Hook proteins as novel adapters for motors on EEs. Hok1, the Hook protein in *U. maydis*, binds to EEs via its C-terminus, which alone localizes to the organelles, while the N-terminal half is still able to bind to dynein. The N-terminal region is not required for EE association, but a highly conserved stretch in the N-terminal coiled-coil region Hok1 is required for EE-binding of dynein, but also kinesin-3. Both motors are released from the organelles in *hok1* mutants, which abolishes bi-directional EE motility. In wildtype cells, anterograde-to-retrograde turning is initiated by the release of kinesin-3 from the EEs, which allows dynein binding and turning of transport direction. Thus, Hook is most likely coordinating the opposing activity of both motors. Hook and kinesin-3 form a functional pair in various animals and filamentous fungi. In addition, the paper shows that human and fungal Hook bind the same associated proteins and that chimaera of human hook3 and *U. maydis* Hok1 is functional. This suggests that the role as a kinesin-3/dynein coordinating adapter is conserved.

53. •• Zhang J, Qiu1 R, Arst HNJ, Peñalva MA, Xiang X: **HookA is a novel dynein-early endosome linker critical for cargo movement *in vivo***. *J Cell Biol* 2014, **204**:1009-1026.
This study, together with [52••], describes the identification of Hook proteins as novel adapters for motors on EEs. HookA, the Hook protein in *A. nidulans*, binds to EEs via its C-terminus, which alone localizes to the organelles. The paper provides solid biochemical evidence that the N-terminal part of HookA binds to dynein/dynactin and dynein-associated Lis1/NUDF. This interaction requires the p25 protein of the dynactin complex. This activity is crucial for EE motility and mutants show almost complete absence of retrograde EE motions. Interestingly, HookA is also required for peroxisome distribution, although a physical interaction with peroxisomal proteins was not found.

54. Krämer H, Phistry M: **Mutations in the drosophila hook gene inhibit endocytosis of the boss transmembrane ligand into multivesicular bodies**. *J Cell Biol* 1996, **133**:1205-1215.

55. Walenta JH, Didier AJ, Liu X, Krämer H: **The Golgi-associated hook3 protein is a member of a novel family of microtubule-binding proteins**. *J Cell Biol* 2001, **152**:923-934.

56. •• Higuchi Y, Ashwin P, Roger Y, Steinberg G: **Early endosome motility spatially organizes polysome distribution**. *J Cell Biol* 2014, **204**:343-357.
Using mathematical modelling and live-cell imaging, this paper shows that bi-directional, motor-driven motility of early endosomes support the passive diffusion of ribosomes. Ribosomes attach to the organelles by binding to EE-associated mRNA, where they actively translate the message into proteins while the organelles move (see also [69••]). Random 'off-loading' of entire polysomes from the moving EEs is required to spread the machinery for protein translation. This paper proviudes both, a molecular mechabnism of ribosome attachment to EEs and an new mechanism by which ribosomes get distributed in a eukaryotic cell.

57. Abenza JF, Galindo A, Pantazopoulou A, Gil C, de los Rios V, Penalva MA: **Aspergillus RabB Rab5 integrates acquisition of degradative identity with the long distance movement of early endosomes**. *Mol Biol Cell* 2010, **21**:2756-2769.

58. Echauri-Espinosa RO, Callejas-Negrete OA, Roberson RW, Bartnicki-Garcia S, Mourino-Perez RR: **Coronin is a component of the endocytic collar of hyphae of *Neurospora crassa* and is necessary for normal growth and morphogenesis**. *PLoS ONE* 2012, **7**:e38237.

59. Higuchi Y, Shoji JY, Arioka M, Kitamoto K: **Endocytosis is crucial for cell polarity and apical membrane recycling in the filamentous fungus *Aspergillus oryzae***. *Eukaryot Cell* 2009, **8**:37-46.

60. Taheri-Talesh N, Horio T, Araujo-Bazan L, Dou X, Espeso EA, Penalva MA, Osmani SA, Oakley BR: **The tip growth apparatus of *Aspergillus nidulans***. *Mol Biol Cell* 2008, **19**:1439-1449.

61. Upadhyay S, Shaw BD: **The role of actin, fimbrin and endocytosis in growth of hyphae in *Aspergillus nidulans***. *Mol Microbiol* 2008, **68**:690-705.

62. Araujo-Bazan L, Penalva MA, Espeso EA: **Preferential localization of the endocytic internalization machinery to hyphal tips underlies polarization of the actin cytoskeleton in *Aspergillus nidulans***. *Mol Microbiol* 2008, **67**:891-905.

63. Jorde S, Walther A, Wendland J: **The *Ashbya gossypii* fimbrin SAC6 is required for fast polarized hyphal tip growth and endocytosis**. *Microbiol Res* 2011, **166**:137-145.

64. • Abenza JF, Galindo A, Pinar M, Pantazopoulou A, de los Rios V, Penalva MA: **Endosomal maturation by Rab conversion in *Aspergillus nidulans* is coupled to dynein-mediated basipetal movement**. *Mol Biol Cell* 2012, **23**:1889-1901.
This is an interesting report on a role of EE motility in vacuole organization in fungi. The paper demonstrates that endosomes mature by a replacement of the small GTPase Rab5 GTP, characteristic for EEs, with the late endosomal GTPase Rab7. In a dynein mutant, retrograde EE motility is impaired and small Rab7-positive late endosomes and vacuoles accumulate in the tip region. This demonstrates that endosome maturation, and therefore localization of vacuoles in sub-apical regions of the hyphal cell, is linked to retrograde dynein motility.

65. Becht P, König J, Feldbrügge M: **The RNA-binding protein Rrm4 is essential for polarity in *Ustilago maydis* and shuttles along microtubules**. *J Cell Sci* 2006, **119**:4964-4973.

66. • Baumann S, Pohlmann T, Jungbluth M, Brachmann A, Feldbrügge M: **Kinesin-3 and dynein mediate microtubule-dependent co-transport of mRNPs and endosomes**. *J Cell Sci* 2012, **125**:2740-2752.
The authors found that in the Basidiomycete *U. maydis* the RNA-binding protein Rrm4 is located on rapidly moving early endosomes. The motility is driven by kinesin-3 and dynein, which is consistent with previous reports on the molecular transport machinery for these organelles in this fungus [36].

67. König J, Baumann S, Koepke J, Pohlmann T, Zarnack K, Feldbrügge M: **The fungal RNA-binding protein Rrm4 mediates long-distance transport of ubi1 and rho3 mRNAs**. *EMBO J* 2009, **28**:1855-1866.

68. Vollmeister E, Schipper K, Feldbrügge M: **Microtubule-dependent mRNA transport in the model microorganism *Ustilago maydis***. *RNA Biol* 2012, **93**:261-268.

69. •• Baumann S, König J, Koepke J, Feldbrügge M: **Endosomal transport of septin mRNA and protein indicates local translation on endosomes and is required for correct septin filamentation**. *EMBO Rep* 2014, **15**:94-102.
This paper shows that the septin mRNA *cdc3* associates with EEs via the mRNA-binding protein Rrm4. Interestingly, the mRNA is translated by ribosomes into septin proteins, while EEs are moving along microtubules (see also [56••]). Another septin filament component, Cdc12, is also translated on moving endosomes. EE attachment of Cdc3 protein requires Cdc12, suggesting that EE-based translation supports the assembly of septins into short septin filaments on the organelles. The attachment of *cdc3* and *cdc12* mRNA to EEs is essential for septin filament formation at growth poles and required for hyphal growth. This paper shows that EE-associated translation supports septin filament formation and, as such, is of high importance for growth of hyphal cells.

70. Luby-Phelps K: **Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area**. *Int Rev Cytol* 2000, **192**:189-221.

71. Steinberg G: **Motors in fungal morphogenesis: cooperation versus competition**. *Curr Opin Microbiol* 2011, **14**:660-667.

72. Chanda A, Roze LV, Kang S, Artymovich KA, Hicks GR, Raikhel NV, Calvo AM, Linz JE: **A key role for vesicles in fungal secondary metabolism**. *Proc Natl Acad Sci U S A* 2009, **106**:19533-19538.

73. Kaksonen M, Sun Y, Drubin DG: **A pathway for association of receptors, adaptors, and actin during endocytic internalization**. *Cell* 2003, **115**:475-487.

74. Grant BD, Donaldson JG: **Pathways and mechanisms of endocytic recycling**. *Nat Rev Mol Cell Biol* 2009, **10**:597-608.

75. Jedd G, Mulholland J, Segev N: **Two new Ypt GTPases are required for exit from the yeast trans-Golgi compartment**. *J Cell Biol* 1997, **137**:563-580.

76. Rink J, Ghigo E, Kalaidzidis Y, Zerial M: **Rab conversion as a mechanism of progression from early to late endosomes**. *Cell* 2005, **122**:735-749.

77. Xu L, Sowa ME, Chen J, Li X, Gygi SP, Harper JW: **An FTS/hook/p107(FHIP) complex interacts with and promotes endosomal clustering by the homotypic vacuolar protein sorting complex**. *Mol Biol Cell* 2008, **19**:5059-5071.

78. Crampin H, Finley K, Gerami-Nejad M, Court H, Gale C, Berman J, Sudbery P: ***Candida albicans* hyphae have a Spitzenkörper that is distinct from the polarisome found in yeast and pseudohyphae**. *J Cell Sci* 2005, **118**:2935-2947.

79. Girbardt M: **Der Spitzenkörper von *Polystictus versicolor***. *Planta* 1957, **50**:47-59.

80. Grove SN, Bracker CE: **Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper**. *J Bacteriol* 1970, **104**:989-1009.

81. Reinhardt MO: **Das Wachstum von Pilzhyphen**. *Jahrb Wiss Bot* 1892, **23**:479-566.

82. Verdin J, Bartnicki-Garcia S, Riquelme M: **Functional stratification of the Spitzenkörper of *Neurospora crassa***. *Mol Microbiol* 2009, **74**:1044-1053.

83. Atkinson HA, Daniels A, Read ND: **Live-cell imaging of endocytosis during conidial germination in the rice blast fungus, *Magnaporthe grisea***. *Fungal Genet Biol* 2002, **37**:233-244.

84. Cole L, Hyde GJ, Ashford AE: **Uptake and compartmentalisation of fluorescent probes by *Pisolithus tinctorius* hyphae: evidence for an anion transport mechanism at the tonoplast but not for fluid-phase endocytosis**. *Protoplasma* 1997, **199**:18-29.

85. Torralba S, Heath IB: **Analysis of three separate probes suggests the absence of endocytosis in *Neurospora crassa* hyphae**. *Fungal Genet Biol* 2002, **37**:221-232.

86. Read ND, Kalkman ER: **Does endocytosis occur in fungal hyphae?** *Fungal Genet Biol* 2003, **39**:199-203.

87. Basu R, Munteanu EL, Chang F: **Role of turgor pressure in endocytosis in fission yeast**. *Mol Biol Cell* 2014, **25**:679-687.

88. Fuchs U, Steinberg G: **Endocytosis in the plant-pathogenic fungus *Ustilago maydis***. *Protoplasma* 2005, **226**:75-80.

89. Hervas-Aguilar A, Penalva MA: **Endocytic machinery protein Slab is dispensable for polarity establishment but necessary for polarity maintenance in hyphal tip cells of *Aspergillus nidulans***. *Eukaryot Cell* 2010, **9**:1504-1518.

90. Matsuo K, Higuchi Y, Kikuma T, Arioka M, Kitamoto K: **Functional analysis of Abp1p-interacting proteins involved in endocytosis of the MCC component in *Aspergillus oryzae***. *Fungal Genet Biol* 2013, **56**:125-134.

91. Martin R, Hellwig D, Schaub Y, Bauer J, Walther A, Wendland J: **Functional analysis of *Candida albicans* genes whose *Saccharomyces cerevisiae* homologues are involved in endocytosis**. *Yeast* 2007, **24**:511-522.

92. Walther A, Wendland J: **Polarized hyphal growth in *Candida albicans* requires the Wiskott-Aldrich syndrome protein homolog Wal1p**. *Eukaryot Cell* 2004, **3**:471-482.

93. Walther A, Wendland J: **Apical localization of actin patches and vacuolar dynamics in *Ashbya gossypii* depend on the WASP homolog Wal1p**. *J Cell Sci* 2004, **117**:4947-4958.

94. • Epp E, Nazarova E, Regan H, Douglas LM, Konopka JB, Vogel J, Whiteway M: **Clathrin- and Arp2/3-independent endocytosis in the fungal pathogen *Candida albicans***. *MBio* 2013, **4** e00476-00413.

In the yeast *S. cerevisiae*, endocytosis is restricted to a clathrin-dependent pathway [13], whereas animal cells use additional, clathrin independent endocytosis mechanisms. This paper describes a clathrin-independent pathway in a fungus. It shows that Arp2/3 is required for clathrin-dependent uptake in *C. albicans*. Surprisingly, Arp2/3 mutants were viable and showed fluid phase endocytosis. This was dependent on Arp2/3-independent actin structures, such as actin cables.

95. Gournas C, Amillis S, Vlanti A, Diallinas G: **Transport-dependent endocytosis and turnover of a uric acid-xanthine permease**. *Mol Microbiol* 2010, **75**:246-260.

96. Chapa YLB, Allwood EG, de R II, Snape ML, Ayscough KR: **Yeast endocytic adaptor AP-2 binds the stress sensor Mid2 and functions in polarised cell responses**. *Traffic* 2014, **15**:546-557.

97. • Zeng G, Wang YM, Wang Y: **Cdc28-cln3 phosphorylation of Sla1 regulates actin patch dynamics in different modes of fungal growth**. *Mol Biol Cell* 2012, **23**:3485-3497.

This paper demonstrates that *C. albicans* Sla1, a key component in regulating actin patches, is phosphoregulated by cyclin-dependent kinase CDK and the actin-regulating kinase Prk1. Upon hyphal growth induction, Sla1 is dephosphorylated. Using *sla1* mutant alleles the authors show that dephosphorylation increases actin patch dynamics. Thus, Sla1 dephosphorylation increases endocytosis, which is crucial for hyphal growth.

98. Walther TC, Brickner JH, Aguilar PS, Bernales S, Pantoja C, Walter P: **Eisosomes mark static sites of endocytosis**. *Nature* 2006, **439**:998-1003.

99. Brach T, Specht T, Kaksonen M: **Reassessment of the role of plasma membrane domains in the regulation of vesicular traffic in yeast**. *J Cell Sci* 2011, **124**:328-337.