ELSEVIER

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/fbr

Review

Early endosomes motility in filamentous fungi: How and why they move

Yujiro HIGUCHI[a,*], Gero STEINBERG[b,*]

[a]Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Fukuoka 812-8581, Japan
[b]Biosciences, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

ARTICLE INFO

Article history:
Received 28 September 2014
Received in revised form
19 January 2015
Accepted 10 February 2015

Keywords:
early endosome
effector protein
filamentous fungi
membrane trafficking
microtubule
motor protein
polysome

ABSTRACT

Elongate hyphae of filamentous fungi grow predominantly at their tips, whereas organelles are positioned in the subapical parts of the cell. Organelle positioning and long-distance intracellular communication involves active, energy-dependent transport along microtubules (MTs). This is mediated by specialized molecular motors, named kinesins and dynein, which utilize ATP hydrolysis to "walk" along the tubulin polymers. Work in the basidiomycete *Ustilago maydis* and the ascomycete *Aspergillus nidulans* has shown that early endosomes (EEs) are one of the major cargos of MT-dependent motors in fungi. EEs are part of the early endocytic pathway, and their motility behavior and the underlying transport machinery is well understood. However, the physiological role of constant bi-directional EE motility remains elusive. Recent reports, conducted in the corn smut fungus *U. maydis*, have provided novel insights into the cellular function of EE motility. They show that EE motility is crucial for the distribution of the protein synthesis machinery, and also that EEs transmit signals during plant infection that trigger the production of fungal effector proteins, required for successful invasion into host plants.

1. Introduction

Filamentous fungi are important model systems to study fundamental principles of eukaryotic cell biology, and also of high importance in food production. The genus *Aspergillus* is a good example, as it hosts the fungus *Aspergillus nidulans*, which is a powerful model system to study fundamental aspects of intracellular membrane trafficking (e.g. Egan *et al.*, 2012a; Zhang *et al.*, 2014), while *Aspergillus oryzae* is of high economic importance in Japan, due to its role in traditional fermentation in Japanese food products, such as sake, miso and soy sauce (Machida *et al.*, 2005; Shoji *et al.*, 2014). However, fungi also threaten human, animal and plant health (Fisher *et al.*, 2012). Amongst the Aspergilli, *Aspergillus fumigatus* causes serious mycosis in immunocompromised humans (Nierman *et al.*, 2005), whereas the smut fungus *Ustilago maydis* is a very well-characterized pathogen on maize (Kämper *et al.*, 2006; Brefort *et al.*, 2009; Djamei and Kahmann, 2012). Interestingly, although there is no report yet in *A. fumigatus*, the other fungi share an important intracellular transport process, as they all show rapid and long-range motility of early endosomes (EEs; Wedlich-Söldner *et al.*, 2000; Peñalva, 2005;

* Corresponding authors.
E-mail addresses: y.higuchi@agr.kyushu-u.ac.jp (Y. Higuchi), G.Steinberg@exeter.ac.uk (G. Steinberg).
http://dx.doi.org/10.1016/j.fbr.2015.02.002
1749-4613/

Higuchi *et al.*, 2006; overview in Steinberg, 2014). This review focuses on recent advancements in the understanding of the molecular machinery and the cellular role of EE motility in filamentous fungi.

2. Early endosome as an organelle in the endocytic pathway

Endocytosis is the uptake of material into the cell (Mellman, 1996) and is now widely accepted as a fundamental pathway in fungal cells (Peñalva, 2010). Early studies of this process have been based on the lipophilic dye FM4-64, which was shown to be taken up into various filamentous fungi (work by Steinberg *et al.*, 1998; Fisher-Parton *et al.*, 2000; Peñalva, 2005; Higuchi *et al.*, 2009). Further evidence came from visualizing a fluorescent plasma membrane-located purine transporter AoUapC (Higuchi *et al.*, 2006), which, upon changes in the growth media, is internalized and delivered to vacuoles for degradation. This endocytic pathway involves endocytic transport vesicles at the plasma membrane, which subsequently take their cargo to EEs, where sorting towards the vacuole or back to the plasma membrane occurs (Seaman *et al.*, 2008). Fungal EEs were firstly described as FM4-64 stainable motile organelles, carrying a soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), required for polarized growth of *U. maydis* (Wedlich-Söldner *et al.*, 2000). Subsequent studies showed that these EEs carry the organelle-specific small GTPase Rab5 (Fuchs *et al.*, 2006) and that they colocalized with a fluorescent Phox-domain (Higuchi *et al.*, 2014). This domain is known to bind specifically to the lipid phosphatidylinositol 3-phosphate (Lemmon, 2003), which is enriched in EEs (Gillooly *et al.*, 2000). On their voyage to the degrading vacuole, endocytic cargo passes through several endosomal compartments. In animals and fungi, cargo firstly arrives in EEs, which mature to late endosomes by replacing the small GTPase Rab5 with Rab7, a small GTPase characteristic for late endosomes (Rink *et al.*, 2005; Abenza *et al.*, 2012). In *U. maydis*, Rab5-positive EEs are motile (Wedlich-Söldner *et al.*, 2000; Lenz *et al.*, 2006; Schuster *et al.*, 2011b, c), whereas Rab7-positive endosomes are stationary (Higuchi *et al.*, 2014). In *A. nidulans* and *A. oryzae*, EEs motility was initially observed by FM4-64 and AoUapC-EGFP, respectively (Peñalva, 2005; Higuchi *et al.*, 2006). Motile EEs, carrying the Rab5-homologue YPT52 and being stainable with FM4-64, were also described in *Neurospora crassa* (Seidel *et al.*, 2013).

3. The molecular mechanism of early endosome motility

Research on fungal EEs began with the discovery of an endosomal SNARE, Yup1, in a genetic screen for morphological mutants in *U. maydis* (Wedlich-Söldner *et al.*, 2000). A fusion protein of GFP and Yup1 localizes on vesicular structures that are moving along microtubules (MTs). These structures were stained with the endocytic marker dye FM4-64, suggesting that they are part of the endocytic pathway. Sequence analysis predicted that Yup1 is a t-SNARE on endosomes, mediating fusion of endocytic transport vesicles with the organelles. Indeed, temperature-sensitive mutants showed defects in delivering FM4-64 down the endocytic pathway into the vacuole. Later studies showed that Yup1-positive organelles carry Rab5-and Rab4-homologues (Fuchs *et al.*, 2006; Higuchi *et al.*, 2014). These small GTPases are characteristic for EEs (Chavrier *et al.*, 1990; Van Der Sluijs *et al.*, 1991; Zerial and McBride 2001), confirming that the rapidly moving Yup1-carrying organelles are an early endocytic compartment. Subsequent studies in the ascomycete *A. nidulans*, *A. oryzae and N. crassa* used FM4-64 (Peñalva, 2005) and AoUapC-EGFP (Higuchi *et al.*, 2006) and fluorescent Rab5 (Abenza *et al.*, 2009, 2010; Seidel *et al.*, 2013) to show that rapid EE motility along MTs is a general feature of filamentous fungi.

Fungal EEs move at a rate of ~1–3 μm/s, which reflects the velocity of motor-dependent transport. Studies on *U. maydis* revealed that kinesin-3 transports EEs to plus-ends of the MTs, whereas counteracting dynein carries the organelles towards MT minus-ends (Wedlich-Söldner *et al.*, 2002; Lenz *et al.*, 2006). Interestingly, molecular and cellular analyses exploiting state-of-the-art microscopy techniques, such as fluorescence recovery after photobleaching (FRAP) and photoactivation, revealed that 3–6 kinesin-3 molecules are constantly bound to the bi-directionally moving EEs, whereas dynein concentrates at the MT plus-ends, from where it leaves to "pick-up" an arriving EE to turn its transport direction around (Schuster *et al.*, 2011b). Mathematical modeling combined with experimental results suggest that the accumulation of dynein at the plus-ends of MTs increases the probability of the kinesin-3-delivered organelles to be "loaded" onto a minus-end directed dynein, thereby ensuring that the organelles do not fall off when reaching the MT end (Schuster *et al.*, 2011a). The dynein comet at the apical MT ends can therefore be considered a "dynein loading zone" (Lenz *et al.*, 2006). Such concept is also found in *A. nidulans* (Zhang *et al.*, 2003; Abenza *et al.*, 2009; Zhang *et al.*, 2010), where dynein and kinesin-3 also provide the transport machinery for EEs (Egan *et al.*, 2012b).

In *A. nidulans* and *U. maydis*, EEs are constantly moving and, at a given time, are equally distributed in the cell. The question is, how are kinesin-3 and dynein controlled to allow balanced bi-directional EE motility in the hypha? Two simultaneous recent studies provided insight into a regulatory mechanism that is based on hook proteins. Hook proteins were initially identified in fruit flies (Krämer and Phistry, 1996) and were found to bind organelles and MTs in humans and flies (Sunio *et al.*, 1999; Walenta *et al.*, 2001; Ge *et al.*, 2010; Baron Gaillard *et al.*, 2011; Maldonado-Báez *et al.*, 2013). This suggested an anchorage role of these proteins. However, recent studies in *U. maydis* and *A. nidulans* unraveled that hook proteins are adapters for dynein, thereby controlling retrograde motility of EEs (Bielska *et al.*, 2014a; Zhang *et al.*, 2014). Interestingly, it was shown in *U. maydis* that hook also controls the attachment of kinesin-3, and that this function is conserved between human and fungi (Bielska *et al.*, 2014a). How hook is mediating binding of both dynein and kinesin-3 is not understood, but the current model suggests that controlled release of kinesin-3 allows dynein to win in a "tug-of-war", leading to a change in transport direction. The molecular detail of this mechanism is far from being