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A role for endocytic recycling in hyphal growth

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ABSTRACT

Actin plays multiple complex roles in cell growth and cell shape. Recently it was demonstrated that actin patches, which represent sites of endocytosis, are present in a sub-apical collar at growing tips of hyphae and germ tubes of filamentous fungi. It is now clear that this zone of endocytosis is necessary for filamentous growth to proceed. In this review evidence for the role of these endocytic sites in hyphal growth is examined. One possibility if that the role of the sub-apical collar is associated with endocytic recycling of polarized material at the hyphal tip. The 'Apical Recycling Model' accounts for this role and predicts the need for a balance between endocytosis and exocytosis at the hyphal tip to control growth and cell shape. Other cell differentiation events, including appressorium formation and *Aspergillus* conidiophore development may also be explained by this model.

Introduction

With the first live cell imaging of GFP labeled actin in filamentous fungi came the realization that growing hyphae have a sub-apical collar of endocytic actin patches directly behind the growing apex (Fig 1) (Taheri-Talesh *et al.* 2008; Upadhyay & Shaw 2008). This sub-apical collar is now also demonstrated in *Neurospora crassa* (Berepiki *et al.* 2010; Delgado-Alvarez *et al.* 2010), indicating that it is likely found in most if not all filamentous fungi. These observations, coupled with early demonstrations of endocytic uptake of the lipophilic dye FM4-64 in the hyphal tip (Hoffmann & Mendgen 1998; Fischer-Parton *et al.* 2000; Atkinson *et al.* 2002), have forced a re-examination of our understanding of how the hyphoid shape is created during growth. In this review, experimental evidence for a key role of endocytosis in hyphal growth will be discussed. The 'Apical Recycling Model' accounts for the need of a balance between exocytic and endocytic processes at the tip. This model stresses the importance of maintenance of a zone of exocytosis at the growing apex by the sub-apical collar of endocytosis. Results from recent studies will be used to examine this model.

Hyphal growth

Polarized cell expansion is a developmental mode observed in many biological systems but is perhaps best exemplified by pollen tube formation of plants, neuronal cell development of animals, and hyphal growth of filamentous fungi. In filamentous fungi, conidia (and other spore types) initially establish polarity by producing a germ tube during germination. From this point, all cell growth and expansion is confined to the apex of the hyphae. The Spitzenkörper, an amorphous accumulation of at least two populations of vesicles found at the growing apex of most filamentous fungi (Verdin *et al.* 2009), is thought to direct hyphal growth [reviewed in (Harris *et al.* 2005) and elsewhere in this special issue]. The best

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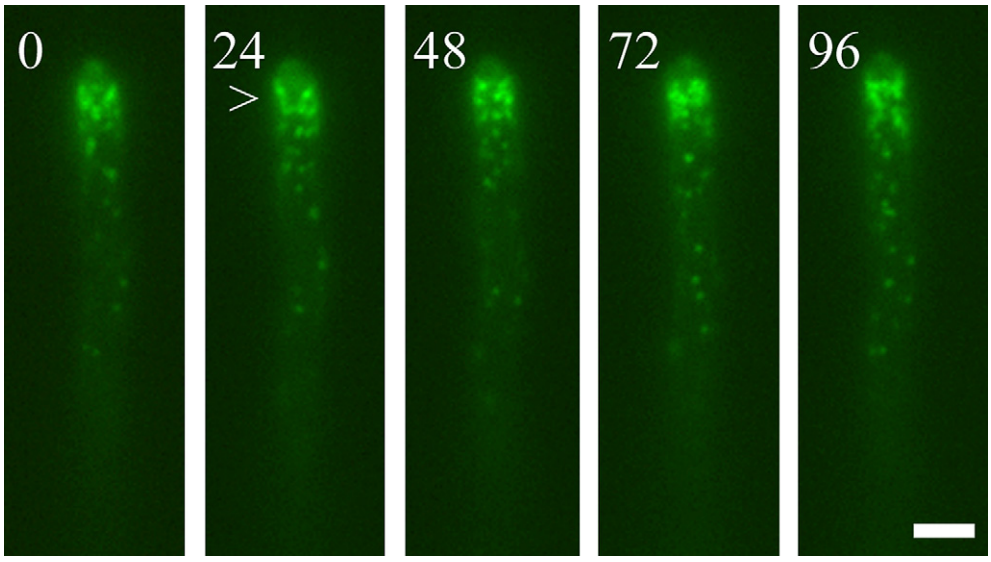

Fig 1 – The sub-apical collar of actin patches in a growing hyphae of *A. nidulans*. Actin::GFP is imaged by widefield fluorescence microscopy in a growing hypha. The sub-apical collar or cortical actin patches is marked with an arrow head in the second panel. Time from start is shown in seconds. Scale bar = 5 μm.

explanation to date for the Spitzenkörper is that it serves as a Vesicle Supply Center (Bartnicki-Garcia *et al.* 1995; Gierz & Bartnicki-Garcia 2001; Bartnicki Garcia 2002) for exocytic vesicles that fuse with the growing surface of the hyphal apex. Data now show that a sub-apical collar of endocytosis just behind the Spitzenköper is also required for hyphal growth (Araujo-Bazan *et al.* 2008; Taheri-Talesh *et al.* 2008; Upadhyay & Shaw 2008; Berepiki *et al.* 2010; Delgado-Alvarez *et al.* 2010). This compels the rethinking of the dynamic mechanism of membrane trafficking at the hyphal tip.

Endocytic recycling

An understanding of why an organism would associate an area of intense exocytic activity, at sites of active growth is not a difficult concept to accept. New growth materials including membrane, membrane bound proteins, cell wall constituents, and enzymes needed to construct the wall must be delivered to the site of growth. Why the organism requires endocytosis just behind this growth site is initially not as intuitive. Exocytic vesicles arrive at the growing apex and merge into the plasma membrane at the site of growth. This membrane and any proteins and other molecules associated with the membrane will then be part of the polarized site of growth. When one considers that the cell apex continues to advance so that membrane that was once the site of growth will in time be distal to the active growth site, it becomes clear that mechanism must exist to maintain the polarized growth machinery at the apex. One plausible mechanism to explain this maintenance of the polarized growth machinery is through endocytic recycling of the apical membrane and of proteins and lipids associated with this polarization site. Examples of this recycling are found in the literature. For example, in *Saccharomyces cerevisiae*, polarization of the exocytic SNARE protein Snc1 is maintained at bud sites and shmoo tips through endocytic recycling (Valdez-Taubas & Pelham 2003). Endocytic uptake of pheromone receptors (Davis *et al.* 1993), and recycling of the chitin synthases Chs1p and Chs3p (Ziman *et al.* 1996) and the cell wall integrity sensors Wsc1p and Wsc2p (Wilk *et al.* 2010) have also been demonstrated in yeast. Endocytic recycling mechanisms appear to be at play in plants as well, where polarization of auxin transport machinery relies on endocytic recycling (Kleine-Vehn & Friml 2008). Here we will consider the growing evidence for the requirement of endocytic recycling for hyphal growth in the filamentous fungi. First a brief synopsis of the molecular mechanism for clathrin-mediated endocytic vesicle formation and uptake will be considered.

Molecular mechanism for endocytosis

The molecular mechanism of endocytic vesicle formation has been extensively examined in *Saccharomyces cerevisiae* and has been reviewed recently (Kaksonen *et al.* 2006; Toret & Drubin 2006; Galletta & Cooper 2009). Here only some of the more important proteins and those proteins with orthologs that have been investigated in filamentous fungi are described (Fig 2). In brief, the heavy and light chains of the coat protein, clathrin, and the adaptor protein, Ede1, are among the first proteins to arrive at the endocytic site (Fig 2A) (Newpher *et al.* 2006; Toshima *et al.* 2006). The Arp2/3 complex activator, Las17, arrives next (Fig 2B). The Arp2/3 complex mediates assembly of the actin meshwork (Kaksonen *et al.* 2003). As Ede1 dissipates, Sla2, an additional coat protein, accumulates at the incipient endocytic site (Fig 2B) (Kaksonen *et al.* 2003). Next the motor proteins, Myo3/5, is recruited to the assembling actin patch (Sun *et al.* 2006). A connection between the assembling coat and the actin meshwork may be formed by Sla2 (Kaksonen *et al.* 2003; Newpher *et al.* 2006). The actin binding proteins Abp1 and Sac6 (fimbrin) are important for the actin