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Endocytosis and exocytosis in hyphal growth

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ABSTRACT

Two ancient processes, endocytosis and exocytosis, are employed by eukaryotic cells to shape their plasma membrane and interact with their environment. Filamentous fungi have adapted them to roles compatible with their unique ecological niche and morphology. These organisms are optimal systems in which to address questions such as how endocytosis is localized, how endocytosis and exocytosis interact, and how large molecules traverse eukaryotic cell walls. In the tips of filamentous (hyphal) cells, a ring of endocytosis encircles an apical crescent of exocytosis, suggesting that this area is able to support an endocytic recycling route, although both processes can occur in subapical regions as well. Endocytosis and exocytosis underlie growth, but also facilitate disease progression and secretion of industrially relevant compounds in these organisms. Here we highlight recent work on endocytosis and exocytosis in filamentous fungi.

1. Introduction

Filamentous fungi (FF) are a diverse organisms defined by the remarkably polarized growth of their characteristic cell type, the hypha. Underlying this lifestyle is an exquisite spatial control over two ubiquitous cellular processes: endocytosis and exocytosis (Araujo-Bazán *et al.*, 2008; Caballero-Lima *et al.*, 2013; Shaw *et al.*, 2011; Taheri-Talesh *et al.*, 2008; Upadhyay and Shaw, 2008). Endocytosis, or membrane internalization, and exocytosis, or secretion, govern a large portion of the interactions of cells with their environment. Both are highly regulated and complex, with endocytosis in budding yeast involving more than 60 proteins (Brach *et al.*, 2014; Weinberg and Drubin, 2012, 2014) and exocytosis employing conserved tethering complexes, lipids, and the cytoskeleton to overcome the energetic barrier for membrane fusion (Fig. 1A and B) (Finger and Novick, 1998; He and Guo, 2009; Jahn and Südhof, 1999). Exocytosis has long been implicated in membrane expansion of vegetative cells in FF, but a role for endocytosis in maintaining hyphal shape has only recently been appreciated (Caballero-Lima *et al.*, 2013; Hervás-Aguilar and Peñalva, 2010; Lee *et al.*, 2008; Read and Kalkman, 2003; Upadhyay and Shaw, 2008). Much of what is known about these processes comes from studies in the budding yeast *Saccharomyces cerevisiae*, and a large portion of the machinery involved is conserved among eukaryotes. However, FF have some distinctions from yeast. For example, hyphae exhibit an enormous rate of exocytosis (Bartnicki-Garcia *et al.*, 1989; Howard, 1981). Moreover, filamentous ascomycetes and basidiomycetes possess a Spitzenkörper, an organelle that regulates secretion at the tips of growing hyphae (Brunswick, 1924; Dijksterhuis and Molenaar, 2013; Girbardt, 1957; Jones and Sudbery, 2010; Riquelme and Sánchez-León, 2014). Additionally, endocytosis and exocytosis are clearly separated in

Abbreviations: GE, Golgi Equivalent; PM, Plasma Membrane; FF, Filamentous Fungi.

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growing hyphae (Sudbery, 2011; Taheri-Talesh *et al.*, 2008). Here we summarize the most recent work done on endocytosis and exocytosis in FF, with a focus on growth and their importance in microbe-host interactions.

2. Exocytosis: vesicle tethering, secretion, and the Spitzenkörper

The exocytic machinery

Exocytosis is the process by which vesicles associate with the plasma membrane (PM) and empty their contents into the extracellular space (Finger and Novick, 1998; He and Guo, 2009; Jahn and Südhof, 1999). Membrane fusion in living cells involves donor membranes (e.g. secretory vesicles) passing multiple tests for specificity with target membranes. These include at least three components for exocytosis in yeast (and, presumably, FF). The Rab GTPase Sec4p associates with secretory vesicles in its active GTP-bound form and interacts with the membrane fusion machinery (Guo *et al.*, 1999b). Next, a pair of Soluble N-ethylmaleimide-sensitive factor Attachment REceptor (SNARE) proteins, one on the vesicle (v-SNARE) and one on the target membrane (t-SNARE), are required to complete membrane fusion (Ferro-Novick and Jahn, 1994; Söllner *et al.*, 1993). Finally, exocytosis involves an octameric tethering complex, the exocyst, and associated Sec1/Munc18 (SM) proteins (Carr *et al.*, 1999; Novick *et al.*, 1981). Strains with a disrupted exocyst accumulate secretory vesicles in the cytoplasm and are severely compromised in polarized growth, highlighting the importance of this complex in exocytosis and membrane expansion (Guo *et al.*, 1999a). In fungi, the exocyst is composed of eight proteins, corresponding to *S. cerevisiae* Exo70p, Exo84p, Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, and Sec15p (TerBush *et al.*, 1996; Guo *et al.*, 1999a,b). Of these proteins, only Sec3 is dispensable in yeast (Haarer *et al.*, 1996), which also holds true for *Aspergillus niger* and *Candida albicans* (Kwon *et al.*, 2014; Li *et al.*, 2007). In *N. crassa*, however, Sec-3 was essential and Sec-5 was not (Riquelme *et al.*, 2014), and both a *Magnaporthe oryzae* exo70 and a sec5 deletion were viable (Giraldo *et al.*, 2013). These findings indicate that more work needs to be done to understand the assembly and structure of the exocyst in fungi.

Tethering complexes are important throughout eukaryotes for vesicle fusion with various cell destinations such as the PM, the Golgi, and the vacuole. The yeast homotypic fusion and vacuole protein sorting (HOPS) complex can increase lumenal mixing of SNARE-bearing proteoliposomes by 100-fold (Zick and Wickner, 2014). The role that the exocyst plays in fusion is not completely clear, but it appears to be able to associate with the Rab proteins on secretory vesicles, as well as promote binding between SNARE proteins on vesicles and the PM (Guo *et al.*, 1999b; He and Guo, 2009; TerBush *et al.*, 1996). Sso1/2p and Snc1/2p are the exocytic t- and v-SNARE proteins in *S. cerevisiae*, respectively (Valdez-Taubas and Pelham, 2003). Intriguingly, the Sso1p homologue is localized throughout the entire PM in nearly all eukaryotes, including FF (Guo *et al.*, 2000; Taheri-Talesh *et al.*, 2008; Treitschke *et al.*, 2010; Valkonen *et al.*, 2007). In general, however, the Snc1/2p homologues are only found at areas of growth (Hervás-Aguilar and Peñalva, 2010; Taheri-Talesh *et al.*, 2008). Many t-SNAREs can interact with multiple v-SNAREs (Banfield *et al.*, 1995; Götte and Fischer von Mollard, 1998), but whether this occurs for Sso1/2p is not currently known. Currently, therefore, exocytosis in hyphae can be thought to be delimited by the location of the exocyst.

The earliest study of exocyst localization in hyphae was in *Aspergillus nidulans*, where SecC/Sec3p was localized to a small cap at hyphal tips (Taheri-Talesh *et al.*, 2008). Next, *Ashbya gossypii* Exo70p, Sec3p, and Sec5p were observed, depending on growth rate, to a crescent at the apex (slow growth), or the Spitzenkörper (fast growth) (Köhli *et al.*, 2008). The exocyst of *C. albicans* hyphae, on the contrary, forms a stable apical crescent even when the cytoskeleton is disrupted (Jones and Sudbery, 2010). More recently, Riquelme *et al.* (2014) observed that EXO-70 localizes to the periphery of the Spitzenkörper in an actin and microtubule-dependent manner in *N. crassa*, and these subunits may be responsible for tethering this organelle to the rest of the complex. This would be opposed to the situation in *S. cerevisiae* (He and Guo, 2009) and *Schizosaccharomyces pombe* (Bendezu *et al.*, 2012) where Exo70p and Sec3p bind to the PM and recruits the rest of the complex. It would, however, generally agree with the notion that the exocyst exists as two multimeric subunits, one of which travels on vesicles, that assemble at the PM in a reaction that promotes vesicle tethering (Guo *et al.*, 1999a,b; He *et al.*, 2007).

The Spitzenkörper and exocytosis

The Spitzenkörper is commonly viewed as a "vesicle supply center" that provides the membrane for tip growth in some fungi (Gierz and Bartnicki-Garcia, 2001; Riquelme and Sánchez-León, 2014). Exocytic traffic from fungal Golgi equivalents (GEs) awaiting fusion with the PM provides a large part of the membrane for this organelle (Pantazopoulou *et al.*, 2014), which suggests that membrane fusion in the tip is limiting. This limitation may also influence the structure and size of the Spitzenkörper. Having exocyst proteins delivered to the tip on secretory vesicles could provide some of this regulation, if the fusion of subunits on vesicles with subunits on the PM was required for vesicle tethering, and PM-localized subunits were limited in quantify, for example. However, vesicles in the Spitzenkörper are also organized into different layers, with large macrovesicles in the outer layer (termed by the authors the "Spitzenring") and smaller, microvesicles in the "core" (Howard, 1981; Riquelme and Sánchez-León, 2014; Verdín *et al.*, 2009). In *N. crassa* these different layers are known to have different cargo, with the 1,3 β-glucan synthase GS-1 located in the outer layer and the chitin synthase Chs-1 present in the core (Verdín *et al.*, 2009). *S. cerevisiae* also has distinct populations of high and low density vesicles that each have cargo destined for different locations (Harsay and Bretscher, 1995). It is possible that behavior among the different groups of vesicles could be different. Additionally, no studies to date in fungi have differentiated between exocytosis that results in full membrane fusion and the transient "kiss-and-run" exocytosis seen in animal and plant cells (Aravanis *et al.*, 2003; Weise *et al.*, 2000). Analyzing these two populations of vesicles, as well as how they interact with the plasma membrane, will be key to gaining more insight into the nature of the Spitzenkörper and hyphal growth.