COMMUNICATIVE & INTEGRATIVE BIOLOGY
2016, VOL. 9, NO. 2, e1141843 (3 pages)
http://dx.doi.org/10.1080/19420889.2016.1141843

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COMMENTARY

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The flippase DnfB is cargo of fimbrin-associated endocytosis in *Aspergillus nidulans*, and likely recycles through the late Golgi

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ARTICLE HISTORY Received 21 December 2015; Revised 6 January 2016; Accepted 8 January 2016

KEYWORDS apical recycling; endocytosis; flippase; fungi; Spitzenkörper

Filamentous fungi exhibit rapid, polarized growth governed by precise segregation of endocytosis and exocytosis at the site of cell expansion. Endocytosis, in particular, is concentrated at a zone termed the endocytic collar, surrounding the cell tip, where secretory vesicle fusion occurs. How the separation of these processes is established and maintained is still unclear, but one possible purpose for this organization is the ability of endocytosis to remove and recycle proteins that diffuse away from the site of growth. The phospholipid flippases DnfA and DnfB localize to sites of growth and endocytosis in the fungus *Aspergillus nidulans*, and may be involved in secretion. DnfA is endocytosed in part through an NPFxD motif, but whether DnfB is endocytosed or recycled is unknown. Here, we demonstrate that DnfB is also endocytosed, and that recycling through the late Golgi maintains its organization in the growing tip.

Results

Endocytosis and endocytic recycling are conserved processes that provide cells the chance to interact with their environment and regulate plasma membrane dynamics, among other things. Inhibiting endocytosis has many effects on filamentous fungi, including disrupting their ability to maintain polarity, an almost complete abolishment of growth, and causing aberrant cell shape.[1-6] The direct reason for these phenotypes has not been demonstrated. Most mutations or treatments that target endocytosis may also target the actin cytoskeleton, which has several ancillary roles in growth and cell shape including intracellular membrane trafficking.[3,7] Some hypotheses for the purpose of the endocytosis in growth and shape have been proposed, including the apical recycling model, which suggests that endocytosis acts to counteract to the passive diffusion of proteins from the site of growth. In this system, proteins that are necessary for certain processes at the cell tip are internalized at the endocytic collar as they are misplaced during growth. Additionally, some of these proteins could potentially be recycled back to the site of growth through a variety of endomembrane trafficking pathways.[1,5,8,9]

To understand the function of endocytosis in fungi, as well as the results that come from mutations that block endocytosis, it is helpful to characterize proteins that are cargo of the endocytic collar. To this date, however, only a few proteins have been shown to be endocytosed in the tips of filamentous fungi,[10,11] One of these proteins is the *Aspergillus nidulans* phospholipid flippase DnfA.[12] Phospholipid flippases control lipid asymmetry, primarily in the plasma membrane and throughout the secretory pathway.[13-15] DnfA has an NPFxD motif, which is known to associate with the endocytic adaptor Sla1p in yeast.[16-18] Upon mutation of this motif, DnfA relocalized from the hyphal tip to being present throughout the plasma membrane in an apolar manner, suggesting it is endocytosed in part through this motif. DnfA-GFP was, moreover, diffuse throughout the cytoplasm in a *vftD* deletion mutant. VftD is part of the Golgi Associated Retrograde Protein (GARP) complex, a 4-subunit tethering complex required for fusion of vesicles traveling from early and late endosomes to the late Golgi, and thus for endocytic recycling of proteins through the late Golgi.[12,19] A *vftD* deletion confers a strong growth

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Commentary to: Schultzhaus Z, Yan H, Shaw B. *Aspergillus nidulans* flippase DnfA is cargo of the endocytic collar, and plays complementary roles in growth and phosphatidylserine asymmetry with another flippase, DnfB. Mol Microbiol 2015; 97:18-32; PMID:25846564; http://dx.doi.org/10.1111/mmi.13019

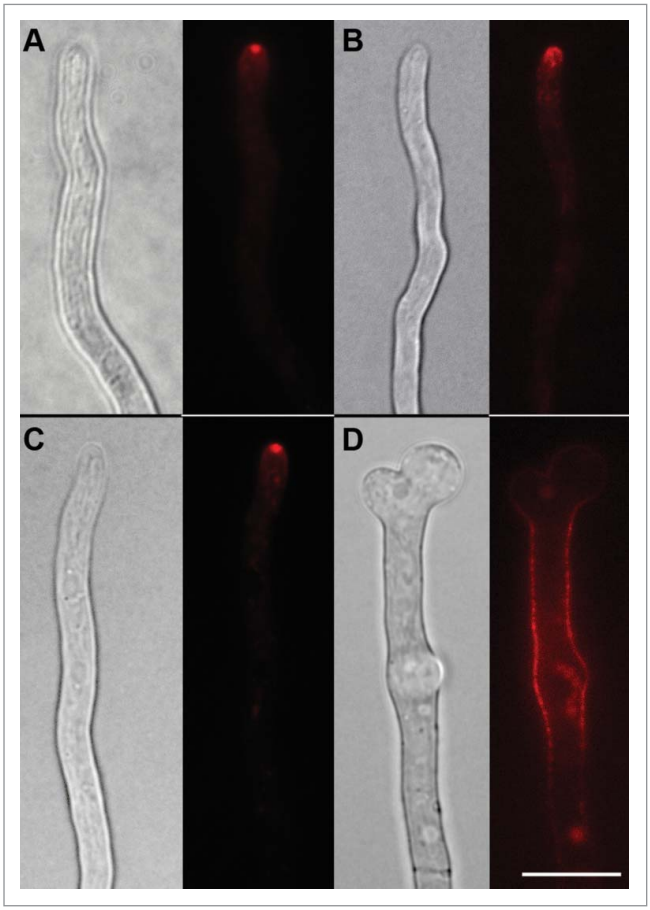

Figure 1. DnfB is endocytosed and possibly recycles through the late Golgi. DnfB-mCherry is present in the Spitzenkörper and the apical plasma membrane in wild type hyphae (A), while this organization is lost when *vftD* is deleted (B). When endocytosis is depleted through regulation of *fimA* by the ammonium-repressible *niiA* promoter, DnfB-mCherry relocalizes from the Spitzenkörper (C, grown with NO_3) to the plasma membrane (D, grown with NH_4). Scale bar = 5μm.

phenotype, which suggests that GARP function is abolished in this mutant, and, further, that endocytic recycling through the late Golgi could act to polarize tip components in *A. nidulans*.

Another flippase, DnfB, is also primarily localized to the hyphal tip and the plasma membrane under normal conditions (Fig. 1A).[12] Particularly, it localizes to the Spitzenkörper: a spherical, fungal organelle just behind the growing cell tip, composed primarily of secretory vesicles. When endocytosis is blocked by downregulating the endocytic gene *fimA* (fimbrin)[6] with the nitrate-expressing, ammonium-repressible *niiA* promoter,[3,20,21] DnfB-mCherry depolarizes and moves from the tip (Fig. 1C) to being present throughout the plasma membrane (Fig. 1D). Interestingly, although DnfB trails off of the plasma membrane before DnfA in wild type cells,[12] it does not possess an NPFxD motif, and its mechanism for being recognized by the endocytic machinery is not yet known.

DnfB is also present at the late Golgi in wild type.[12] To see if it also traffics to the late Golgi after being endocytosed, we viewed DnfB-mCherry in a *vftD* deletion mutant. As seen in Figure 1B, although DnfB-mCherry is still polarized, its apical accumulation is less organized, suggesting that the presence of DnfB-mcherry at the Spitzenkörper, and possibly Spitzenkörper structure, is dependent upon traffic between endosomes and the late Golgi. Whether DnfB can be polarized through a secondary recycling pathway is unknown. The homolog in yeast, Drs2p, has been shown to act in concert with Rcy1p to support endocytic recycling,[22] but the importance of the *A. nidulans* Rcy1p homolog, RcyA, in recycling is unclear,[23] and could be resolved in the future. What is clear is that one effect of abolished endocytosis is the likely loss of plasma membrane lipid asymmetry, the consequences of which is a currently unexplored topic in fungal cell biology.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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