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SPOTLIGHT

A second chance at yeast early endosomes

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Post-endocytic recycling in yeast has been posited to transit solely through the Golgi, raising the possibility that yeast lack early endosomes. In this issue, Laidlaw and colleagues (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202109137) describe a yeast endosomal recycling pathway that gives proteins a second chance to return to the plasma membrane.

Proteins transit the secretory pathway to the plasma membrane (PM) where they assist in nutrient and ion uptake, receptor-mediated signaling, and cell–cell interactions. Though functionally critical, PM proteins are endocytosed in response to environmental changes, reshaping the PM proteome. Endocytosed proteins are often sorted through endosomes and degraded in lysosomes, giving them an ephemeral quality. Ubiquitinated endocytic cargos are sorted into intralumenal vesicles (ILVs) by the endosomal sorting complex required for transport (ESCRT; reviewed in 1). ILV-containing late endosomes (LEs) fuse to lysosomes, delivering ILVs into the degradative lumen. However, recycling pathways provide "escape routes" that return internalized proteins to the PM, giving them a second chance to exert PM functions.

In metazoans, endocytic vesicles fuse to early endosomes (EEs) that promote recycling to the PM either directly or indirectly via recycling endosomes or the Golgi. The molecular machinery that selects proteins for endosomal recycling is exquisite and nuanced, involving many protein complexes (reviewed in 1). Though numerous recycling factors are conserved in *Saccharomyces cerevisiae*, the existence of endosomes distinct from LEs has been questioned (2). A study of retrograde recycling proposed that all post-endocytic trafficking routes first to the TGN (2). Yeast is a powerful protein trafficking model that has been instrumental in defining the secretory and endocytic pathways. Nonetheless, these findings highlighted the need for more focused studies on early events in yeast post-endocytic trafficking. Subsequent identification of signaling endosomes (SEs), which are distinct from LEs and the Golgi, expand the endosomal repertoire in yeast (Fig. 1; 3). SEs, marked by the inverse-Bin-Amphiphysin-Rvs protein, Ivy1, contain the pool of TORC1 that regulates autophagy (3, 4). In this issue, elegant studies by Laidlaw and coauthors (5) provide evidence for yeast EEs—distinct from LEs or the Golgi—required for post-endocytic recycling of nutrient transporters (Fig. 1).

Laidlaw and colleagues (5) first establish clear differences in the trafficking itineraries of the constitutively endocytosed synaptobrevins, Snc1 and Snc2, and the nutrient permeases for methionine and uracil (Mup1 and Fur4, respectively), whose endocytosis is substrate induced (Fig. 1). They fuse a deubiquitinating enzyme (DUb) to these cargos, which helps define the impact of ubiquitination on trafficking by removing this signal. As expected for the synaptobrevins, ubiquitination is required for Golgi-to-PM recycling and Snc-DUbs are TGN retained, as defined by colocalization with the Arf guanine nucleotide exchange factor Sec7 (5). In contrast, Mup1- and Fur4-DUbs are PM retained, even in the presence of a substrate that typically triggers endocytosis. In contrast, a short pulse of substrate induces wild-type nutrient permease endocytosis, resulting in Mup1 and Fur4 colocalization with Vps4, an AAA-ATPase and endosomal marker, but not the Sec7-marked TGN (5). These findings highlight trafficking differences between synaptobrevins and nutrient permeases.

What post-endocytic compartments are accessed by nutrient permeases?

Time-lapse imaging of cells in a microfluidics chamber allows tracking of Mup1 in response to environmental change (5). A methionine pulse induces Mup1 endocytosis, and within 2 min Mup1 forms intracellular foci. A chase with methionine-free medium prevents Mup1 trafficking to the vacuole (yeast lysosome equivalent) and results in almost no Mup1 colocalization with the Sec7-marked TGN (5). To ensure PM-localized Mup1 was under study, the authors optimized photoconversion of mEos-tagged Mup1. They coupled Mup1-mEos, photoconverting only a small PM patch, with methionine pulse-chase to demonstrate that Mup1-mEos foci form and resolve with the same kinetics as Mup1-GFP foci (5). They next track endocytosed Mup1 colocalization with Sec7-marked TGN or Vps4-marked endosomes after methionine pulse-chase. Rapid acquisition, 4D confocal Airyscan microscopy over all timescales shows co-localization of post-endocytic Mup1 with endosomal Vps4 and not Sec7 (5). Thus, the Mup1 recycling route differs from that of synaptobrevins, instead

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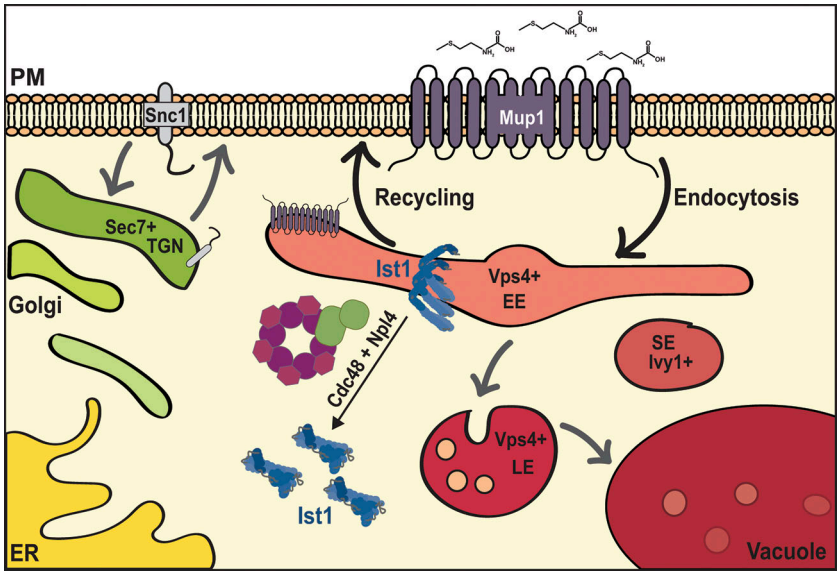

Figure 1. **Ist1 regulates recycling from EEs in yeast.** Methionine induces Mup1 endocytosis, but rapid removal of this signal captures Mup1 in Vps4-positive EEs. Ist1 is required for Mup1 recycling from EEs to the PM. Snc1 recycles through a different route, using the Sec7-positive TGN. Cdc48-Npl4 are needed for Ist1-mediated recycling and may control Ist1 depolymerization. Vps4-positive LEs, Ivy1-positive SEs, and Sec7-positive TGN are all distinct from the Ist1-marked EEs.

defining an endosomal recycling pathway (Fig. 1; 2, 5).

What controls this new endocytic recycling route?

An unbiased screen to identify recycling factors identified 88 candidates (6), of which only Ist1 could bind Vps4. Ist1 interacts with other ESCRT-III proteins, including CHMP1B, and functions in metazoan protein recycling (7). Ist1-CHMP1B forms a polymeric coat on membrane tubules (8), but unlike other ESCRT-mediated scission, it severs membranes that bud toward the cytosol (8, 9). Ist1-CHMP1B promotes friction-driven tubule scission in vitro, suggesting an analogous activity may exist in vivo (8, 9). Laidlaw and colleagues (5) find that Ist1 operates in protein recycling independently of ESCRT-mediated ILV formation. Further, loss of Ist1 impairs post-endocytic recycling of G protein coupled receptors and nutrient permeases (5). Like Mup1, Ist1 co-localizes with dynamic, Vps4-positive compartments. Cells lacking Ist1 retain Mup1 in Vps4-positive EEs. During methionine pulse-chase, Ist1 and Mup1 colocalize in punctae that transit toward the PM (5). These data demonstrate that Ist1 is needed for Mup1 recycling through EEs. Given its structure, perhaps Ist1 promotes tubule scission from endosomes to drive nutrient permease recycling.

If Ist1 forms an EE coat, what stimulates its disassembly?

Again, a functional link came from the previous screen for recycling factors (6); Npl4, a ubiquitin binding receptor that recruits the AAA-ATPase Cdc48 (p97 ortholog) to targets, is needed for protein recycling (5, 6). While known for its role in ER-associated degradation, Npl4-Cdc48 also participates in membrane remodeling and trafficking. Epistasis analysis with mutated Npl4 and Cdc48 suggests they could operate in the Ist1 recycling pathway (5). Ist1 ubiquitination is required for recycling, as Ist1-DUb fusions are nonfunctional, raising the possibility that ubiquitinated Ist1 recruits Npl4-Cdc48 (5). Further studies are required to demonstrate direct activity of Npl4-Cdc48 in recycling and Ist1 depolymerization. Ist1-DUb impairs recycling and does not colocalize with the dynamic, EE pool of Vps4; instead, it co-localizes with a static Vps4 compartment, likely LEs (5). Importantly, lattice light sheet microscopy similarly defines both dynamic and static Vps4 pools that could represent unique endosomal compartments (10).

These findings, coupled with other recent fast-acquisition, high-resolution, and multi-color fluorescent imaging (3–5, 10), provide strong evidence for distinct endosomes in yeast, including Ist1- and Vps4-marked EEs, Vps4-marked LEs, and Ivy1-marked SEs, each of which are distinct from Sec7-marked TGN (3–5). Use of additional molecular signposts in combination with these markers will be important for further evaluating the yeast early endocytic pathway.

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