Unconventional secretion by autophagosome exocytosis

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In this issue, Duran et al. (2010. *J. Cell Biol.* doi: 10.1083/jcb.200911154) and Manjithaya et al. (2010. *J. Cell Biol.* doi: 10.1083/jcb.200911149) use yeast genetics to reveal a role for autophagosome intermediates in the unconventional secretion of an acyl coenzyme A (CoA)–binding protein that lacks an endoplasmic reticulum signal sequence. Medium-chain acyl CoAs are also required and may be important for substrate routing to this pathway.

In eukaryotic cells, most secreted proteins rely on the highly conserved secretory pathway for their release into the extracellular space. Signal sequences target them for cotranslational translocation across the ER membrane, and the proteins fold within the ER lumen. The proteins are then transported to and through the Golgi apparatus, sorted, and delivered to the cell surface. The machinery responsible for the secretory pathway is comprised of proteins that collect cargo, form transport vesicles, and help vesicles recognize and fuse at the correct target membranes. A small number of secreted proteins use secretory pathway-independent routes by a process called unconventional secretion (Nickel and Rabouille, 2009). In this issue, Duran et al. and Manjithaya et al. make powerful use of yeast genetics to provide new mechanistic insight into the previously unknown, unconventional route taken by an acyl CoA–binding protein (ACBP) to reach the extracellular space.

The simplest pathway for unconventional secretion is that taken by the yeast a-factor mating pheromone. This farnesylated and methylated dodecapeptide is exported by the STE6 gene product that encodes an ATP-binding cassette (ABC) family transporter (Kuchler et al., 1989; McGrath and Varshavsky, 1989). Larger proteins, including FGF2, galectins 1 and 3, a subset of interleukins, and the engrailed homeodomain protein are also unconventional secretory cargoes, but their precise routes of export are unknown (Nickel and Rabouille, 2009). During an inflammatory response, interleukin-1β is somehow translocated from the cytosol into secretory lysosomes for release from cells by a still poorly defined mechanism. Caspase-1 may be required for the unconventional secretion of all of these proteins, suggesting that they may use a common route (Keller et al., 2008).

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Unconventional secretion of an ACBP was first reported in *Dictyostelium discoideum*. In this organism, spore formation is activated by release of the 10-kD, ER signal sequence lacking, AcbA protein from prespore cells. Secreted AcbA is proteolyzed extracellularly to produce SDF-2 (spore differentiation factor–2; Anjard et al., 1998). Kinseth et al. (2007) showed that a Golgi-associated protein, GRASP, was required for AcbA release and subsequent SDF-2 production but not for cell growth. Inhibitors of ABC family transporters had no influence on AcbA release. Kinseth et al. (2007) revealed an entirely unexpected role for a Golgi protein in unconventional secretion and a route for AcbA that was distinct from a-factor. A conserved role for GRASP was also later reported for the unconventional secretion of α-integrin at a specific stage of *Drosophila melanogaster* development (Schotman et al., 2008).

Duran et al. (2010) now show that secretion of the *Saccharomyces cerevisiae* AcbA orthologue, Acb1, also requires the corresponding yeast GRASP orthologue, Grh1. As in *D. discoideum*, nitrogen starvation triggered Acb1 secretion in a concerted pulse. Genes known to be essential for conventional secretion (SEC23, SEC7, or SEC1) or a-factor release (STE6) were not needed for Acb1 release, confirming that the protein uses an unconventional pathway. However, the SEC18 gene that encodes the NSF ATPase was needed. This ATPase disassembles SNARE proteins and is required for all cellular membrane fusion events.

The requirement for starvation suggested that an autophagosome intermediate might be involved. Indeed, mutants impaired in various stages of autophagy were all deficient in Acb1 secretion. Fusion of autophagosomes with the vacuole was not required, but the endosomal t-SNARE, Tlg2, and the plasma membrane t-SNARE, Sso1, were required. Finally, convergence of the autophagosomal and the multivesicular endosome pathways was required.

Manjithaya et al. (2010) monitored release of Acb1 from the yeast *Pichia pastoris* by assaying the generation of an SDF-2–like activity that would trigger sporulation in *D. discoideum*. As in *S. cerevisiae*, release from *P. pastoris* required the GRASP homologue Grh1 and numerous autophagy gene products, in particular, Atg11, which is required for receptor-dependent autophagy (Xie and Klionsky, 2007). Similar to

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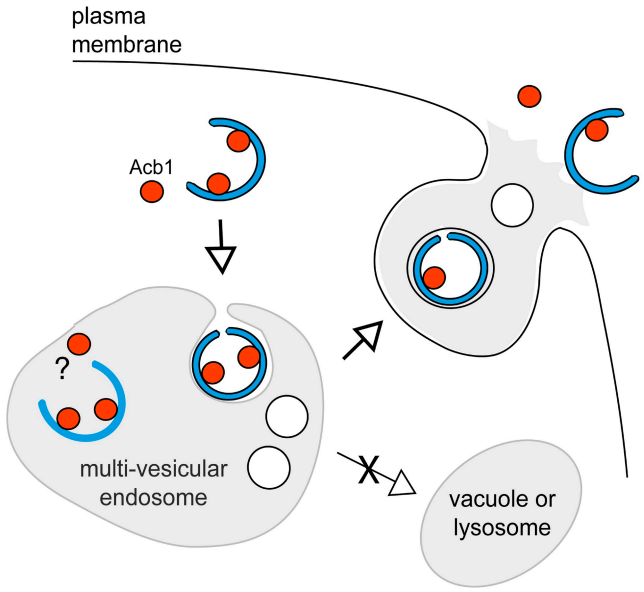

Figure 1. **A model for unconventional secretion of Acb1.** Selective autophagy involves cargo collection on the surface of a phagophore membrane (blue). These are engulfed by a multivesicular endosome that fuses with the plasma membrane to release its content. Whether the phagophore is released from an endosomal, lumenal vesicle by lipase action before exocytosis (?) is not known. Duran et al. (2010) and Manjithaya et al. (2010) show that the t-SNARE Sso1 is needed for exocytosis, and fusion with the vacuole is not required.

the baker's yeast findings, a plasma membrane t-SNARE was also implicated.

Production of medium chain fatty acyl CoAs was needed for Acb1 secretion from *P. pastoris*. Manjithaya et al. (2010) propose that Acb1 secretion may require that Acb1 bind its medium-chain acyl CoA substrate. Alternatively, the acyl CoA could be needed to acylate a protein (or proteins) that participates in autophagosomal incorporation of Acb1 protein. Lipid modification and/or binding seem to be a recurring theme for unconventional secretion cargoes (Nickel and Rabouille, 2009) and may contribute to incorporation into nascent autophagosomal structures.

These experiments suggest that Acb1 is targeted for selective autophagy, a process that begins with recruitment to a so-called phagophore assembly site (Fig. 1). Phagophores are engulfed by multivesicular endosomes that normally deliver their contents to the yeast vacuole (or lysosomes). In some cases, a subset of multivesicular endosomes fuses with the plasma membrane and releases their contents (Simons and Raposo, 2009; Théry et al., 2009). In these studies, fusion of phagophores with multivesicular endosomes and subsequent fusion of these compartments with the plasma membrane appear to represent the major route of unconventional secretion of ACBPs. The use of specific mutant yeast strains has provided key insight into the specific pathways taken by unusual secretory cargoes. These studies also implicate specific SNARE proteins in the poorly understood, multivesicular endosome release process.

What conserved role does GRASP play? A connection between autophagy and the Golgi complex was recently reported by Itoh et al. (2008), who showed a direct link between the autophagy protein Atg16L1 and the Golgi Rab GTPase Rab33b. We do not yet know the precise origins of the phagophore membrane that participates in unconventional secretion, but roles for GRASP and Rab33b suggest that the Golgi is clearly important for this process. Does GRASP help segregate membrane components needed to form a nascent phagophore? How do ACBPs and other unconventionally secreted substrates actually engage the autophagy machinery? ACBP release involves nitrogen starvation; therefore, is stress important for unconventional secretion, and do other stress signals trigger an autophagic response? Important areas for future research include the identification of such signals, the elucidation of the mechanisms by which these signals are translated into cargo sequestration, and determination of the breadth and diversity of proteins that make use of this unconventional secretory pathway.

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