Self-eating from an ER-associated cup

Anne Simonsen and Harald Stenmark

Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, and Department of Biochemistry, Institute for Cancer Research, Norwegian Radium Hospital, Rikshospitalet University Hospital, Montebello, 0310 Oslo, Norway

Since the first morphological description of autophagosomes in the early 1960s, two critical questions have been a matter of intense investigation and debate: what is the origin of the autophagosomal membrane and how is it formed? A study by Axe et al. (E.L. Axe, S.A. Walker, M. Manifava, P. Chandra, H.L. Roderick, A. Habermann, G. Griffiths, and N.T. Ktistakis. 2008. *J. Cell Biol.* 182:685–701) provides evidence that cup-shaped protrusions from the endoplasmic reticulum, named omegasomes, serve as platforms for autophagosome biogenesis in mammalian cells.

Normal cell growth and proliferation require intricate coordination between stimulatory signals from nutrients and growth factors, and inhibitory signals from intracellular and extracellular stress. One of the key outputs for such signaling is protein metabolism, and macroautophagy ("self-eating") is important in this context. This catabolic pathway involves sequestration of cytoplasmic material within double-membrane–enclosed vesicles (autophagosomes), which eventually fuse with lysosomes, where the encapsulated material is degraded (Mizushima et al., 2008). "Housekeeping" levels of autophagy probably occur in most cells to remove cellular garbage such as misfolded aggregate-prone proteins and defective organelles. In addition, certain environmental cues, including starvation, low oxygen, hormonal stimulation, microbial invasion, and intracellular stress can activate signaling pathways that trigger autophagy.

The origin of the sequestration membrane, called phagophore or isolation membrane, has been a topic of lively debate over the past four decades, and two general models, maturation versus assembly, have been proposed (Juhasz and Neufeld, 2006). The maturation model proposes that the ER is the origin of the autophagosomal membrane, whereas the assembly model implies that autophagosomal membranes form de novo from localized lipid synthesis. In yeast, the autophagosome assembles at a membrane-free preautophagosomal structure found in close proximity to the lysosome-like vacuole, and the molecular mechanisms underlying this process have been elucidated using genetic screens. Several autophagy-related (Atg) and vacuolar protein sorting (Vps) gene products are important for the initial sequestration process. Among these is the phosphatidylinositol 3-kinase Vps34, which phosphorylates phosphatidylinositol to generate phosphatidylinositol 3-phosphate (PI3P) (Kihara et al., 2001). However, the specific functions of PI3P in autophagy have not been clarified so far.

Axe et al. (see p. 685) find that PI3P-enriched structures, named omegasomes by the authors because of their Ω-like shape, form in close proximity to ER membranes and Vps34-positive endosomes in mammalian cells subject to amino acid starvation. The omegasomes colocalize with the autophagy-specific proteins Atg8 and Atg5, and the authors demonstrate in a series of elegant live-imaging experiments that newly formed autophagosomes appear to emanate from these ER-associated PI3P-enriched structures. The authors suggest that the PI3P-enriched omegasome acts as a cradle for recruiting autophagic proteins and formation of the curved phagophore by membrane invagination at the center of the omegasome. An autophagic structure seems to exit the omegasome, either smoothly or by some sort of zippering mechanism (Fig. 1).

One essential question is whether the starvation-induced omegasomes are part of the ER membrane. Live-cell imaging suggests that the omegasomes form at the rim of ER elements. Moreover, their expansion and collapse are found to coincide with changes in the underlying ER membrane, and the omegasome seems to extend around and enclose an Atg8-positive autophagic structure. After exit from this structure, the omegasome appears to collapse back onto the ER. The most convincing argument for omegasomes being formed from ER membranes is the finding that ER-targeted PI3P-binding proteins, even transmembrane proteins, translocate to omegasomes in a PI3P-dependent manner upon starvation. The PI3P-binding protein double FYVE domain–containing protein 1 (DFCP1), which normally localizes to ER and Golgi membranes, translocates to the omegasomes, whereas a mutant DFCP1 lacking the ability to bind PI3P is retained in the ER in response to starvation. However, DFCP1 itself is not required for autophagosome formation at the omegasome. Rather, DFCP1 overexpression inhibits autophagy, probably by sequestering PI3P and thereby recruitment of autophagic PI3P effector proteins.

How then is PI3P generated at the ER membrane? Of the two Vps34 complexes that exist in yeast, one complex (containing

Correspondence to Harald Stenmark: stenmark@ulrik.uio.no

Abbreviations used in this paper: Atg, autophagy-related; DFCP1, double FYVE domain–containing protein 1; PI3P, phosphatidylinositol 3-phosphate; Vps, vacuolar protein sorting.

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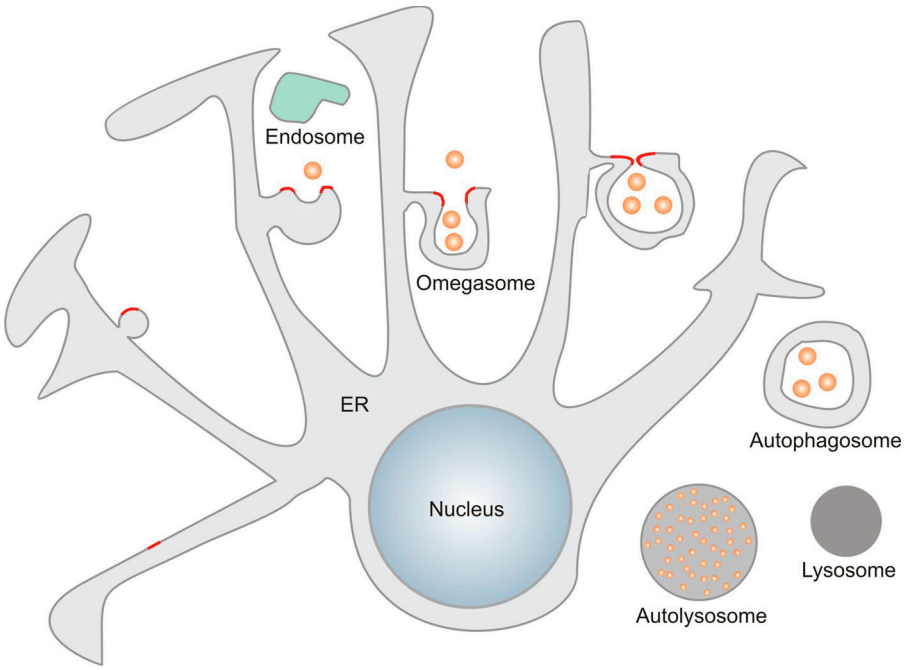

Figure 1. **Proposed model for autophagosome formation from the ER.** PI3P (red), generated by Vps34, marks the site for omegasome formation on the ER membrane by recruiting autophagic effectors. A cisterna expands and invaginates to form an omegasome, into which autophagic cargo is sequestered. Sequestered cargo is degraded when the resulting autophagosome fuses with a lysosome to form an autolysosome. Note that the nature of the cargo and the continuity of the omegasome with the ER membrane still remain to be established.

Vps34, Vps15, Atg6, and Atg14) is specifically involved in autophagy (Kihara et al., 2001). Although no mammalian equivalent of the Atg14 subunit has been identified so far, an autophagy-specific Vps34-containing complex is very likely to also exist in mammalian cells. The ER contains little PI3P under normal conditions (Gillooly et al., 2000), although Axe et al. (2008) found Vps34-positive vesicles in close proximity to ER membranes even in nonstarved cells. Thus, starvation may induce Vps34-positive vesicles to fuse with the ER, or Vps34 might act in trans to generate PI3P on ER membranes. The tumor suppressor beclin-1, a homologue of yeast Atg6, is essential for omegasome formation, indicating that a Vps34–beclin-1–containing complex is involved. Axe et al. (2008) show evidence that the Vps34-positive vesicles found in close contact with omegasomes correspond to endosomes or lysosomes. The endosomal GTPase Rab5 regulates ER structure in *Caenorhabditis elegans*, and depletion of Rab5 inhibits formation of peripheral ER tubules (Audhya et al., 2007). Together with the finding that Rab5 forms a complex with Vps34 and beclin-1 and is involved in autophagosome formation (Ravikumar et al., 2008), this raises the possibility that Rab5 activation may also promote omegasome formation.

It is not clear which PI3P effector protein is involved in autophagosome formation at the omegasome. A possible candidate protein is WIPI-1 (Proikas-Cezanne et al., 2004), whose yeast homologue, Atg18, is required for autophagy and is recruited to PI3P-containing autophagic membranes in a PI3P-dependent manner (Obara et al., 2008). Recruitment of WIPI-1/Atg18 or another autophagic PI3P effector protein might lead to engagement of the autophagic machinery required for autophagosomal membrane formation.

Biogenesis of an autophagosome from the omegasome necessarily involves input of new membrane within the omegasome. This might involve de novo lipid synthesis or fusion of small vesicles. Phosphatidylethanolamine (PE)-conjugated Atg8 mediates membrane tethering and hemifusion in vitro, which suggests that PE-Atg8 could contribute to autophagosomal membrane formation and expansion in vivo (Nakatogawa et al., 2007).

Although further work is needed to elucidate the signals and molecular mechanisms underlying autophagy, the study by Axe et al. (2008) lends supports to the maturation model and provides an interesting clue to understand the membrane source of autophagosome formation.

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