

# Autophagy regulation through Atg9 traffic

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Rapid membrane expansion is the key to autophagosome formation during nutrient starvation. In this issue, Yamamoto et al. (2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201202061>) now provide a mechanism for vesicle-mediated initiation of autophagosome biogenesis. They show that Atg9 vesicles, produced *de novo* during starvation, are ~30–60 nm in size and contain ~30 molecules of Atg9. These vesicles assemble to form an autophagosome, and subsequently, the Atg9 embedded in the outer membrane is recycled to avoid degradation.

Autophagy is a highly conserved pathway that is essential for cell survival and is implicated in several physiological processes and human pathologies. Autophagy occurs when cytoplasmic components are sequestered and delivered into vacuoles or lysosomes for degradation by double-membrane vesicles called autophagosomes. Autophagosomes are formed by expansion and sealing of a cisterna known as the phagophore or isolation membrane, which leads to the envelopment of cytoplasmic material. Phagophores are very likely generated at a specialized site where autophagy-related (Atg) proteins come together, which has been called the phagophore assembly site or preautophagosomal structure (PAS) in yeast and appears also to be present in mammals (Suzuki et al., 2007; Nakatogawa et al., 2009; Itakura and Mizushima, 2010). In higher eukaryotes, several membrane sources contribute to the autophagosome lipid bilayers, including specialized ER domains, the Golgi complex, plasma membrane, mitochondria, and recently, recycling endosomes (Longatti et al., 2012).

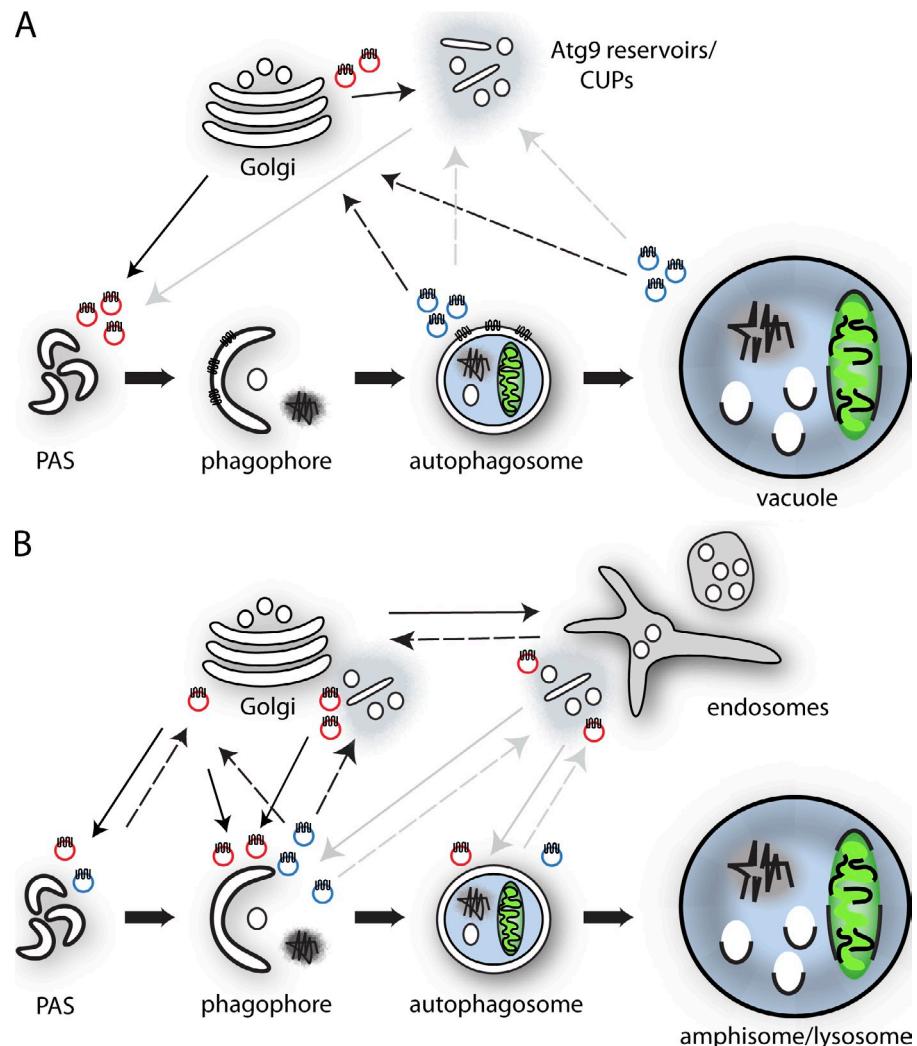
Atg9 is a multispanning membrane protein, essential for autophagy in yeast and mammals (Lang et al., 2000; Noda et al., 2000; Young et al., 2006), highly conserved across species, and ubiquitously expressed in multicellular organisms. This protein has been thought to be involved in the supply of lipid bilayers required for the formation of an autophagosome. However, as a paper in this issue (see Yamamoto et al.) and other recent publications have revealed, Atg9 is in fact a key regulator of autophagy induction. Atg9 provides membranes for the formation of the PAS in yeast (Mari et al., 2010; Yamamoto et al., 2012) and possibly also in mammalian cells during starvation (Orsi et al., 2012) and selective types of autophagy (Kageyama et al., 2011; Itakura et al., 2012).

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Several previous studies have looked at the distribution and the dynamics of the movement of endogenous Atg9 by fluorescence microscopy (see for example Mari et al., 2010). Exploiting the technique of tagging Atg9 at its chromosomal locus with multiple copies of GFP (previously used by Monastyrskaya et al., 2008) and combining it with high-sensitivity light microscopy, Yamamoto et al. (2012) visualize Atg9 vesicles as highly motile puncta in the cytosol and provide new information about their dynamic interaction with autophagosomal membranes. Using single-particle tracking, they show the existence of a highly motile population of Atg9 vesicles freely diffusing within the cytosol. They further analyzed immunoisolated vesicles with dynamic light-scattering and electron microscopy techniques, leading to a comprehensive description of these carriers: 30–60 nm in size containing 24–32 Atg9 molecules per vesicle. Observing an increased number of vesicles during starvation, Yamamoto et al. (2012) use photoconversion techniques to monitor the lifetimes of the forming Atg9 vesicles. They show that the number of Atg9 vesicles is up-regulated with rapamycin treatment, a drug that inhibits the TOR (target of rapamycin) kinase and induces autophagy. The authors finally demonstrate that Atg9 vesicles are a source of autophagosomal membranes, in agreement with proposals made in previous studies (Noda et al., 2000; He et al., 2008; Mari et al., 2010). Furthermore, using quantitative fluorescence microscopy, they show a coalescence of Atg9 vesicles at the PAS, leading to the model whereby, on average, three vesicles contributes to the formation of the PAS. In this model, depicted in Fig. 1 A, Atg9 is now inserted into the outer bilayer of the phagophore, where it remains until it is removed from complete autophagosomes or the vacuole membrane. Importantly, their data suggest that Atg9-containing vesicles are among the first components of the PAS but that, after nucleation of the phagophore, these vesicles do not contribute to later stages of autophagosome biogenesis.

Before this publication, yeast Atg9 was known to traffic through the Golgi complex, and it was known that the Atg9-positive membranes observed by fluorescence microscopy could exchange material with the endosomal system (Mari et al., 2010; Ohashi and Munro, 2010). Yamamoto et al. (2012) have confirmed these data and, additionally, show that two proteins in complex

**Figure 1. Trafficking routes of Atg9.** Red carriers indicate vesicles delivering Atg9 to autophagosomal intermediates (anterograde transport; continuous arrows). The vesicles involved in the retrieval of Atg9 from the autophagosomal membranes are shown in blue (retrograde transport; dashed arrows). Note that for clarity, the Atg9 present in the Golgi complex, the Atg9 reservoirs, or the endosomes is not depicted in either A or B. (A) In yeast, Atg9 is transported from the Golgi to the PAS and/or early autophagosomal precursors in small, highly motile vesicles described by Yamamoto et al. (2012) and then retrieved from complete autophagosomes and/or vacuole membranes (black arrows). Gray arrows indicate possible transport routes of Atg9 from and to the Atg9 reservoirs and/or the CUPs (involved in unconventional secretion of Acb1; Bruns et al., 2011). (B) In mammals, mAtg9 trafficked between the Golgi and endosomes in the presence of nutrients. mAtg9 is also found in reservoir-like structures close to Golgi and endosomes. Upon autophagy induction, mAtg9 distributes to endosomal compartments, although the pool at the Golgi (continuous black arrows) or in reservoir-like structures (continuous gray arrows) appears to play a more significant part in autophagosome biogenesis. Contributions from endosomes, however, cannot be excluded. mAtg9 dynamically associates with autophagosomal precursors and undergoes continuous cycling, but it remains unclear whether it is retrieved back to the Golgi (dashed black arrows) or endosomes (dashed gray arrows). Because mAtg9 is not detected on the autophagosome, amphosome, and lysosome surface, mAtg9 removal from the autophagosomal membrane is probably completed before the autophagosome is closed or completed.



with Atg9, Atg23, and Atg27 (Legakis et al., 2007) are required for Atg9 sorting from the Golgi.

There are two issues to consider when placing these important new findings into the existing data. The first is the relationship between Atg9 vesicles and Atg9 reservoirs. The latter consist of a population of Atg9 found on distinct membrane structures in both yeast and mammalian cells (Mari et al., 2011; Orsi et al., 2012) and may relate to the 15–20% immobile population of Atg9-positive membranes detected by Yamamoto et al. (2012). These immobile Atg9-positive structures may also correspond to the compartments for unconventional protein secretion (CUPs), which possess the same characteristics as the Atg9 reservoirs (e.g., positive for Atg9 and Atg8 and negative for other organelle markers; Bruns et al., 2011).

The second issue is how do the Atg9 vesicles contribute to the PAS? Do they fuse with themselves and/or other membranes at this site during the formation of an autophagosome? Previous work has implicated the small Rab GTPase Ypt1 in autophagy and shown that the TRAPP III tethering complex, directed to the PAS by its unique subunit Trs85, acts as an autophagy-specific guanine nucleotide exchange factor for Ypt1 (Lynch-Day et al., 2010). Furthermore, Ypt1 was found on Atg9-positive structures. Lipatova et al. (2012) have now found that Atg11, which

binds and directs Atg9 to the PAS (He et al., 2006), is a TRAPP III effector of Ypt1 and could act as a classical coiled-coil membrane tether to target Atg9-containing membranes to the PAS. A Rab/tether-mediated membrane fusion event during the early stages of the formation of an autophagosome is supported by the data that SNAREs are implicated both in fusion and Atg9 trafficking (Nair et al., 2011).

How do these findings relate to Atg9 trafficking and its contribution to autophagy in mammalian cells? Mammalian Atg9 (mAtg9) mainly resides in the Golgi complex (Young et al., 2006) but, after nutrient starvation, can be found on a compartment that shows partial colocalization with protein markers of early endosomes and recycling endosomes (Fig. 1 B), which could represent an early intermediate of the phagophore formed de novo (Longatti et al., 2012; Orsi et al., 2012). Importantly, mAtg9 does not completely colocalize with other Atg proteins at the site where autophagosomes are generated, appears to only transiently interact with the phagophore, and is not significantly incorporated into complete autophagosomes (Orsi et al., 2012). Trafficking of mAtg9 is controlled by the same genes identified in yeast. Atg1, Atg2, Atg18, and Atg17 are required for the retrieval of Atg9-formed autophagosomal membranes (Reggiori et al., 2004; Cheong et al., 2005). However, in mammalian cells,

the effect of the orthologues on Atg9 trafficking remains less clear, as the loss of the Atg1 orthologue ULK1/2 causes mAtg9 to remain in the Golgi complex (Young et al., 2006), whereas loss of the Atg17 counterpart FIP200, mammalian Atg2A/B, and the Atg18 counterpart WIPI2 affects its retrograde trafficking (Itakura et al., 2012; Orsi et al., 2012; Velikkakath et al., 2012). In starvation, mAtg9 has also been found in recycling endosomes, where it colocalizes with ULK1 but does not contribute to the forming phagophore via the ULK1-transferrin receptor-positive vesicles (Longatti et al., 2012). Thus, as in yeast, it is likely that the mAtg9 vesicles trafficking to the PAS come from Golgi-derived compartments, which morphologically resemble the Atg9 reservoirs. Although the work of Yamamoto et al. (2012) indisputably provides new crucial information about the dynamic association of Atg9 with autophagosomal membranes, future studies will need to address the relationship between the different subpopulations of Atg9 and the function of this protein to solve one of the mysteries that surround the fascinating life of an autophagosome.

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## References

Bruns, C., J.M. McCaffery, A.J. Curwin, J.M. Duran, and V. Malhotra. 2011. Biogenesis of a novel compartment for autophagosome-mediated unconventional protein secretion. *J. Cell Biol.* 195:979–992. <http://dx.doi.org/10.1083/jcb.201106098>

Cheong, H., T. Yorimitsu, F. Reggiori, J.E. Legakis, C.-W. Wang, and D.J. Klionsky. 2005. Atg17 regulates the magnitude of the autophagic response. *Mol. Biol. Cell.* 16:3438–3453. <http://dx.doi.org/10.1091/mbc.E04-10-0894>

He, C., H. Song, T. Yorimitsu, I. Monastryska, W.-L. Yen, J.E. Legakis, and D.J. Klionsky. 2006. Recruitment of Atg9 to the preautophagosomal structure by Atg11 is essential for selective autophagy in budding yeast. *J. Cell Biol.* 175:925–935. <http://dx.doi.org/10.1083/jcb.200606084>

He, C., M. Baba, Y. Cao, and D.J. Klionsky. 2008. Self-interaction is critical for Atg9 transport and function at the phagophore assembly site during autophagy. *Mol. Biol. Cell.* 19:5506–5516. <http://dx.doi.org/10.1091/mbc.E08-05-0544>

Itakura, E., and N. Mizushima. 2010. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy*. 6:764–776. <http://dx.doi.org/10.4161/auto.6.6.12709>

Itakura, E., C. Kishi-Itakura, I. Koyama-Honda, and N. Mizushima. 2012. Structures containing Atg9A and the ULK1 complex independently target depolarized mitochondria at initial stages of Parkin-mediated mitophagy. *J. Cell Sci.* 125:1488–1499. <http://dx.doi.org/10.1242/jcs.094110>

Kageyama, S., H. Omori, T. Saitoh, T. Sone, J.L. Guan, S. Akira, F. Imamoto, T. Noda, and T. Yoshimori. 2011. The LC3 recruitment mechanism is separate from Atg9L1-dependent membrane formation in the autophagic response against *Salmonella*. *Mol. Biol. Cell.* 22:2290–2300. <http://dx.doi.org/10.1091/mbc.E10-11-0893>

Lang, T., S. Reiche, M. Straub, M. Bredschneider, and M. Thumm. 2000. Autophagy and the cvt pathway both depend on AUT9. *J. Bacteriol.* 182:2125–2133. <http://dx.doi.org/10.1128/JB.182.8.2125-2133.2000>

Legakis, J.E., W.-L. Yen, and D.J. Klionsky. 2007. A cycling protein complex required for selective autophagy. *Autophagy*. 3:422–432.

Lipatova, Z., N. Belogortseva, X.Q. Zhang, J. Kim, D. Taussig, and N. Segev. 2012. Regulation of selective autophagy onset by a Ypt/Rab GTPase module. *Proc. Natl. Acad. Sci. USA*. 109:6981–6986. <http://dx.doi.org/10.1073/pnas.1121299109>

Longatti, A., C.A. Lamb, M. Razi, S. Yoshimura, F.A. Barr, and S.A. Tooze. 2012. TBC1D14 regulates autophagosome formation via Rab11- and ULK1-positive recycling endosomes. *J. Cell Biol.* 197:659–675. <http://dx.doi.org/10.1083/jcb.201111079>

Lynch-Day, M.A., D. Bhandari, S. Menon, J. Huang, H. Cai, C.R. Bartholomew, J.H. Brumell, S. Ferro-Novick, and D.J. Klionsky. 2010. Trs85 directs a Ypt1 GEF, TRAPP III, to the phagophore to promote autophagy. *Proc. Natl. Acad. Sci. USA*. 107:7811–7816. <http://dx.doi.org/10.1073/pnas.1000063107>

Mari, M., J. Griffith, E. Rieter, L. Krishnappa, D.J. Klionsky, and F. Reggiori. 2010. An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. *J. Cell Biol.* 190:1005–1022. <http://dx.doi.org/10.1083/jcb.200912089>

Mari, M., S.A. Tooze, and F. Reggiori. 2011. The puzzling origin of the autophagosomal membrane. *F1000 Biol. Rep.* 3:25. <http://dx.doi.org/10.3410/B3-25>

Monastryska, I., C. He, J. Geng, A.D. Hoppe, Z. Li, and D.J. Klionsky. 2008. Arp2 links autophagic machinery with the actin cytoskeleton. *Mol. Biol. Cell.* 19:1962–1975. <http://dx.doi.org/10.1091/mbc.E07-09-0892>

Nair, U., A. Jotwani, J. Geng, N. Gammoh, D. Richerson, W.-L. Yen, J. Griffith, S. Nag, K. Wang, T. Moss, et al. 2011. SNARE proteins are required for macroautophagy. *Cell.* 146:290–302. <http://dx.doi.org/10.1016/j.cell.2011.06.022>

Nakatogawa, H., K. Suzuki, Y. Kamada, and Y. Ohsumi. 2009. Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* 10:458–467. <http://dx.doi.org/10.1038/nrm2708>

Noda, T., J. Kim, W.-P. Huang, M. Baba, C. Tokunaga, Y. Ohsumi, and D.J. Klionsky. 2000. Apg9p/Cvt7p is an integral membrane protein required for transport vesicle formation in the Cvt and autophagy pathways. *J. Cell Biol.* 148:465–480. <http://dx.doi.org/10.1083/jcb.148.3.465>

Ohashi, Y., and S. Munro. 2010. Membrane delivery to the yeast autophagosome from the Golgi-endosomal system. *Mol. Biol. Cell.* 21:3998–4008. <http://dx.doi.org/10.1091/mbc.E10-05-0457>

Orsi, A., M. Razi, H.C. Dooley, D. Robinson, A.E. Weston, L.M. Collinson, and S.A. Tooze. 2012. Dynamic and transient interactions of Atg9 with autophagosomes, but not membrane integration, are required for autophagy. *Mol. Biol. Cell.* 23:1860–1873. <http://dx.doi.org/10.1091/mbc.E11-09-0746>

Reggiori, F., K.A. Tucker, P.E. Stromhaug, and D.J. Klionsky. 2004. The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. *Dev. Cell.* 6:79–90. [http://dx.doi.org/10.1016/S1534-5807\(03\)00402-7](http://dx.doi.org/10.1016/S1534-5807(03)00402-7)

Suzuki, K., Y. Kubota, T. Sekito, and Y. Ohsumi. 2007. Hierarchy of Atg proteins in pre-autophagosomal structure organization. *Genes Cells.* 12:209–218. <http://dx.doi.org/10.1111/j.1365-2443.2007.01050.x>

Velikkakath, A.K.G., T. Nishimura, E. Oita, N. Ishihara, and N. Mizushima. 2012. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. *Mol. Biol. Cell.* 23:896–909. <http://dx.doi.org/10.1091/mbc.E11-09-0785>

Yamamoto, H., S. Kakuta, T.M. Watanabe, A. Kitamura, T. Sekito, C. Kondo-Kakuta, R. Ichikawa, M. Kinjo, and Y. Ohsumi. 2012. Atg9 vesicles are an important membrane source during early steps of autophagosome formation. *J. Cell Biol.* 198:219–233.

Young, A.R.J., E.Y.W. Chan, X.W. Hu, R. Köchl, S.G. Crawshaw, S. High, D.W. Hailey, J. Lippincott-Schwartz, and S.A. Tooze. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell Sci.* 119:3888–3900. <http://dx.doi.org/10.1242/jcs.03172>