

SPOTLIGHT

# SNAREing an ARP requires a LIR

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The fusion of autophagosomes with lysosomes is an obligatory step in the self-eating process of autophagy. In this issue, Kumar et al. (2018. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201708039>) identify a protein complex, the autophagosome recognition particle (ARP), that chaperones a key SNARE, syntaxin 17, to the autophagosome membrane. Intriguingly, this protein complex coordinates both delivery and membrane insertion as a prelude to fusion.

Vesicles are like factory dispatchers: they organize and process both incoming and outgoing deliveries and remove waste from the factory floor. For the factory (the cell) to survive, the workers (the vesicles) must operate with accuracy and efficiency. Surprisingly, in our cell factory, waste removal and the workers that do this are not very well understood or known. This problem is addressed by Kumar et al., who reveal the way the waste container, the autophagosome, is prepared for its job to remove waste from the cell.

High-fidelity formation, delivery, and fusion of vesicles with their target membranes is an essential function for cells. For example, the cell needs to fuse vesicles containing bioactive signaling molecules with the cell surface, the plasma membrane, to release them to the outside of the cell. Vesicle fusion is also needed for the transport of proteins and lipids (both on the surface of and inside the vesicles) within the cell and between intracellular compartments as well as to bring material into the cell. Our understanding of how membrane fusion occurs is largely founded upon the discovery and work on the function of SNARE proteins (Söllner et al., 1993) and the SNARE complex (Sutton et al., 1998). SNAREs are membrane-bound coiled-coil domain-containing proteins that are usually but not always found on the vesicle (v- or R-SNAREs) or the target membrane (t- or Q-SNAREs). Fusion of the vesicle membrane with the target organelle membrane occurs when a SNARE complex comes close enough to assemble into a four-helix bundle containing three coiled-coil SNARE domains from the Q-SNARE family and one SNARE domain from the R-SNARE (McNew et al., 2000).

Underlying this conceptually simple mechanism are >60 SNAREs in mammalian cells along with essential layers of regulation including molecular switches (GTP-binding proteins), coiled-coil tethers, and multisubunit tethering complexes (Bröcker et al., 2010). These layers have evolved to ensure accurate formation and timely delivery between intracellular organelles and the plasma membrane, which, coupled with retrieval

mechanisms, ensure the homeostasis of the cell factory. Incorporation of SNAREs into forming vesicles coupled with activation of GTP-binding proteins and high-fidelity tethering and targeting are the basis of cell homeostasis.

Equally essential is the process of selecting, recycling, or destroying waste. Cellular waste consists of damaged or aggregated proteins, organelles such as mitochondria, and intracellular compartments such as the ER. Autophagosomes surround and capture the waste, completely sealing it inside a double membrane. They then mature, becoming competent to deliver the waste to lysosomes by fusion (Reggiori and Ungermann, 2017). Lysosomes, responsible for waste digestion, contain digestive enzymes that can degrade proteins and lipids made by the cell found in organelles and cytoplasm or those delivered from outside the cell.

Given that delivery to the lysosomes is thought to be a one-way process, the selection and identification of waste for the lysosome is crucial. Mistakes could be fatal for the cell. The cell cytoplasm (the factory floor) contains solutes, proteins, lipids, and organelles such as the ER, Golgi, and mitochondria. When conditions are normal, the cell continuously surveys its cytoplasmic material for any newly occurring damage or errors in production. This damaged material is tagged for removal and targeted to autophagosomes in a process known as selective self-eating, or selective autophagy. Autophagy can also be activated when the cell is subjected to nutrient starvation, leading to cytoplasmic material being engulfed by the autophagosome in a process called macroautophagy (which is always referred to as autophagy). In either instance, autophagosomes mature and undergo fusion with the lysosome, forming an autolysosome. Note that although autophagosomes have been referred to in this spotlight as vesicles, they are not “vesicle size,” but rather autophagosomes can be as large as 2  $\mu$ m in cells such as hepatocytes, and they have two membranes: an inner membrane and an outer membrane (Fig. 1).

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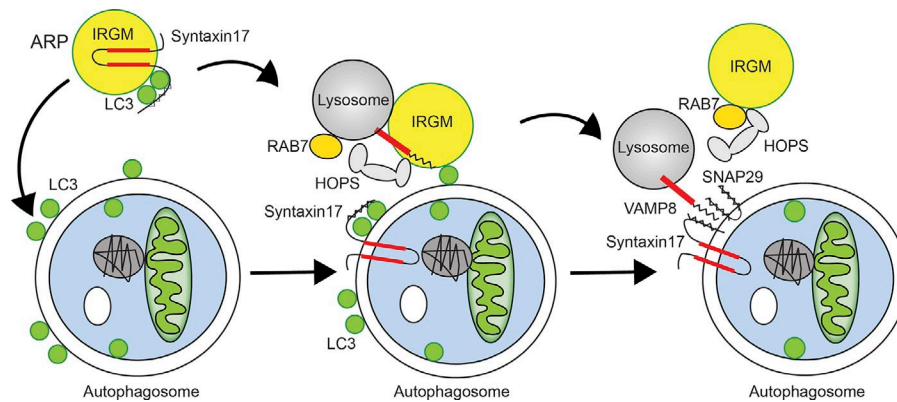


Figure 1. **Kumar et al. (2018)** discovered that **ARP** plays a key function in the delivery of **Stx17** to the autophagosome membrane. ARP contains IRGM, Stx17, and LC3 (or other ATG8s). LC3 is bound to the Stx17 SNARE domain (zig-zagged line) through two LIR motifs, whereas IRGM is bound to ATG8s through a LIR-independent interaction. Importantly, IRGM interacts with Stx17 via the hairpin formed by the two transmembrane domains (shown as red boxes). ARP recruitment to the autophagosome engages the SNARE machinery, including SNAP29 and VAMP8 (in association with IRGM), and the HOPS complex. This allows formation of a SNARE complex, leading to fusion with the lysosome.

The formation of an autophagosome is a unique process that requires autophagy-related gene (ATG) proteins (Yu et al., 2017). In mammalian cells, autophagosomes form from phagophores, which are double-membrane cup-shaped structures derived from domains of the ER. These double membranes contain receptors that are part of the ATG8 family of proteins, which includes the LC3 and GABARAP subfamilies. ATG8 proteins are small ubiquitin-like proteins that associate with membranes upon activation of autophagy. Importantly, these proteins are widely used to identify autophagosomes. Initially, ATG8s cover both the outside and inside of the phagophore, but once the autophagosome starts to fuse with the lysosome, the outer coating of LC3 proteins fall off, whereas ones on the inside are degraded.

ATG8 proteins have a pocket on their surface called the LC3-interacting region (LIR)-docking site (LDS), which binds to LIR motifs (Birgisdottir et al., 2013). LIR motifs are found on proteins that are either directly recruited to autophagosomes and degraded or on proteins (called autophagy adapters) that bind LC3 and GABARAPs and simultaneously to other proteins that may have, for example, a ubiquitin-binding domain. These adapters may also be degraded. Finally, a third class of LIR motif-containing proteins bind LC3 but escape degradation because, for example, the LC3 they bind falls off the surface before fusion with the lysosome.

Kumar et al. (2018) address the key question of how the large double-membrane autophagosome gets prepared to undergo fusion with the lysosome. In yeast, a canonical SNARE machinery is involved in this fusion step, which consists of three Q-SNAREs ( $Q_a$ ,  $Q_b$ , and  $Q_c$ ) and one R-SNARE. These SNAREs are also involved in endosome-vacuole (yeast lysosome) fusion (Reggiori and Ungermann, 2017). In mammalian cells, multiple SNARE complexes have been implicated in autophagy. This may be because autophagosome formation requires many cellular compartments and multiple SNARE complexes. However, for fusion with the lysosome, the  $Q_a$ -SNARE syntaxin 17 (Stx17), SNAP29 ( $Q_{bc}$ ), and VAMP8 (R-SNARE) are believed to be key players, although there are likely to be others (Itakura et al., 2012). Note that in this SNARE complex, there are only two Q-SNAREs because Stx17 (the  $Q_a$ -SNARE) contains one coiled-coil domain, whereas SNAP29 (the  $Q_{bc}$ -SNARE) contains two coiled-coil domains.

Like many other SNAREs, Stx17 is found on several membranes, including membranes involved in autophagosome formation. Stx17 is an unusual Q-SNARE as it contains two transmembrane

domains that may form a hairpin, allowing it to be inserted into membranes from the cytosol. However, it was not known how and when it is inserted into the autophagosome to mediate fusion.

Using superresolution images, Kumar et al. (2018) saw that Stx17 colocalized unusually closely with the ATG8 family member LC3B on the formed autophagosome. This result led them to test and show that the Stx17 SNARE directly binds to ATG8s via LIR motifs. These LIR motifs are found in the SNARE domain of Stx17, which participates in the formation of the four-helix bundle driving membrane fusion. Intriguingly, they also found a colocalization of the immunity-related GTPase M protein (IRGM), which has been implicated in the regulation of autophagy (Singh et al., 2006), with Stx17. IRGM can also interact with ATG8s, but this does not depend on LIRs or the LDS. This led to the identification of a complex containing IRGM, Stx17, and ATG8. Importantly, IRGM binds Stx17 in the transmembrane domain (the hairpin-type tail-anchored type), the region required for insertion into the autophagosome membrane.

The configuration of the members of the complex of IRGM, Stx17, and the ATG8s, termed the “autophagosome recruitment particle” (ARP), turns out to be the key step leading up to subsequent events such as fusion of the autophagosome with lysosomes previously described in detail by Itakura et al. (2012). ARP coordinates delivery of Stx17 to the autophagosome by allowing Stx17 to be exchanged between ATG8s bound to IRGMs in the ARP and ATG8s on the surface of the autophagosome. It's simple but effective (Fig. 1). IRGM binding to Stx17 does not interfere with the LIR-LDS interaction of Stx17-ATG8. Interestingly, IRGM complexes also contain the R-SNARE VAMP8, and components of the multisubunit tether the homotypic fusion and protein sorting (HOPS) complex. The HOPS tether has been shown to be required for Stx17 SNARE complex formation (Jiang et al., 2014). Potential regulation of the ARP complex may occur when autophagy is activated and IRGM-HOPS interaction increases, possibly displacing the ATG8s from the LIRs on Stx17. When complexed with the tether HOPS, this may release Stx17 from the ARP, allowing targeting of Stx17 to ATG8s on the surface of the autophagosome and further exposing Stx17's SNARE domain to form a trans-SNARE complex leading to fusion. Proximity of VAMP8 bound to IRGM may also favor the formation of a SNARE complex between the autophagosome and lysosome and drive fusion.

What we have learned from the work of Kumar et al. (2018) is that taking out waste is a highly coordinated process that is

designed to ensure that workers do not make irreparable mistakes. Without the ARP, the SNARE might not find its way, and autophagosomes may fuse with the lysosome in an uncontrolled manner, leading to waste products being incompletely selected or sorted before disposal. The simple control of cellular functions achieved by using protein–protein interactions is the basis for the prevention of disease in many tissues, highlighted in this spotlight by the accurate removal of toxic substances, which cause diseases including neurodegeneration, cancer, and infection (Choi et al., 2013).

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