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SPOTLIGHT

ATG9 raises the BAR for PI4P in autophagy

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ATG9 vesicles are crucial for autophagy, yet the role of ATG9 remains unclear. In this issue, Judith et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201901115) implicate the BAR protein Arfaptin2 in the loading of PI4-kinase IIIβ onto ATG9 vesicles for recruitment of ATG13 to the site of autophagosome biogenesis.

Autophagy targets cytosolic contents for lysosomal degradation by sequestration in double-membrane autophagosomes. Upon starvation, the ULK1 kinase activates a class III PI3-kinase complex 1 (PI3K3-C1) at a phagophore assembly site (PAS). The resulting local PI3P production leads to nucleation of an isolation membrane and recruitment of the PI3P effector WIPI2, which recruits the machinery to conjugate the ubiquitin-like Atg8 proteins LC3 and GABARAP to phosphatidylethanolamine. This results in membrane expansion into a cup-shaped phagophore, sealing into an autophagosome, and lysosomal fusion (1). The identity of the compartments that serve as a platform for PAS and as a source of lipids for phagophore expansion is debated. Vesicles carrying the transmembrane protein ATG9 are derived from RAB11-positive recycling endosomes (REs) and the TGN and shuttle rapidly between a starvation-enhanced peripheral reservoir and the PAS, hinting at RE or TGN as sources of lipids (2). Several imaging approaches identified the ER-associated specialized omega-shape domain (omegasome) as the PAS, which is supported by local enrichment and direct functional involvement of ER-resident integral membrane proteins in autophagy from omegasome formation up to autophagosome maturation (3). More recently, REs were named as PASs; however, the link to RE-derived ATG9 vesicles remained unclear (4). In contrast, ATG9 vesicles had been shown to mark a ULK1 landing site on the ER (3). The accurate role of ATG9 is therefore key to understanding autophagy.

In this issue, Judith et al. (5) examine the role of ATG9 by SILAC mass spectrometry of immuno-isolated ATG9 vesicles to identify starvation-enriched proteins as candidate contributors to autophagy. While Golgi proteins were largely excluded, in line with the well-recognized peripheral redistribution of ATG9 upon starvation, specific Golgi proteins were enriched, including the N-BAR protein Arfaptin2 (ARFIP2), which is recruited to the Golgi by ARF-like 1 (Arl1). Loss of ARFIP2 or its capacity to bind PI4P at the Golgi led to an exaggerated peripheral localization of ATG9, coupled with defects in PAS recruitment of ULK1, WIPI2, and LC3, as well as reduced LC3 lipidation. Further mass spectrometry of ATG9 vesicles from starved ARFIP2-depleted cells suggested that ARFIP2 promotes enrichment of enzymes of PI4P metabolism on ATG9 vesicles. Indeed, ATG9 colocalized and interacted in a detergent-resistant manner with PI4-kinase IIIβ (PI4KIIIβ), which, in fed conditions, promotes secretion at the Golgi. This further hinted at a functional relevance of PI4P in general and PI4KIIIβ in particular for ATG9 trafficking, as described for the yeast Arl1 and PI4KIIIβ homologue Pik1 (6, 7).

In fed cells, the Golgi localization of PI4KIIIβ and proper distribution of PI4P were both dependent on ARFIP2. Starvation maintained ARFIP2 at the Golgi while dispersing PI4KIIIβ independently of ARFIP2. Strikingly, dispersal of PI4KIIIβ and PI4P were both impaired upon loss of ATG9; reciprocally, peripheral redistribution of ATG9 was arrested upon loss of PI4KIIIβ, accompanied by impairment in PAS recruitment of WIPI2 and LC3 and in LC3 lipidation. It therefore appears that Golgi-resident ARFIP2 marries PI4KIIIβ with ATG9 and loads them onto dedicated vesicles on a joint honeymoon at the PAS. Remarkably, PI4KIIIβ can directly interact with RAB11 and recruit it to the Golgi, which may account for its cooperation with ATG9 at vesicle derivation from a RAB11-positive RE or TGN membrane. ATG9 may thus physically coordinate cellular PI4P homeostasis with autophagy during starvation. Importantly, PI4KIIα was also identified here as another ATG9 vesicle cargo but was not pursued further due to its prior implication in autophagosome maturation (8).

Does PI4KIIIβ promote autophagy in situ beyond its role in ATG9 trafficking? Judith et al. (5) not only show that PI4KIIIβ is required for PAS recruitment of the ULK1 complex subunit ATG13, but also visualize PI4P in association with omegasomes, phagophores, and autophagosomes, thereby physically implicating PI4-kinase activity throughout autophagy. As depletion of PI4KIIα has been shown to block only at a late stage (8), PI4P associated with earlier stages is likely formed by ATG9-delivered PI4KIIIβ. Intriguingly, the active form of PI4KIIIβ was coenriched in a pull-down of membranes positive for ATG13 but not for LC3, and ATG13 interacted directly with PI4KIIIβ in a detergent-resistant manner. Furthermore, both the enrichment of PI4KIIIβ in ATG13 membranes and the colocalization of PI4P and PI4KIIIβ with ATG13 were enhanced by arrest of autophagy at an early stage by specific inhibition of PI3P formation. This implies that the primary role of PI4KIIIβ and PI4P in early autophagy is to act in concert with ATG13 downstream of ATG9 arrival at the PAS but upstream of PI3KC3-C1 activity. Delivery of specific proteins to the PAS through ATG9 vesicles therefore promotes omegasome formation, phagophore nucleation, or both.

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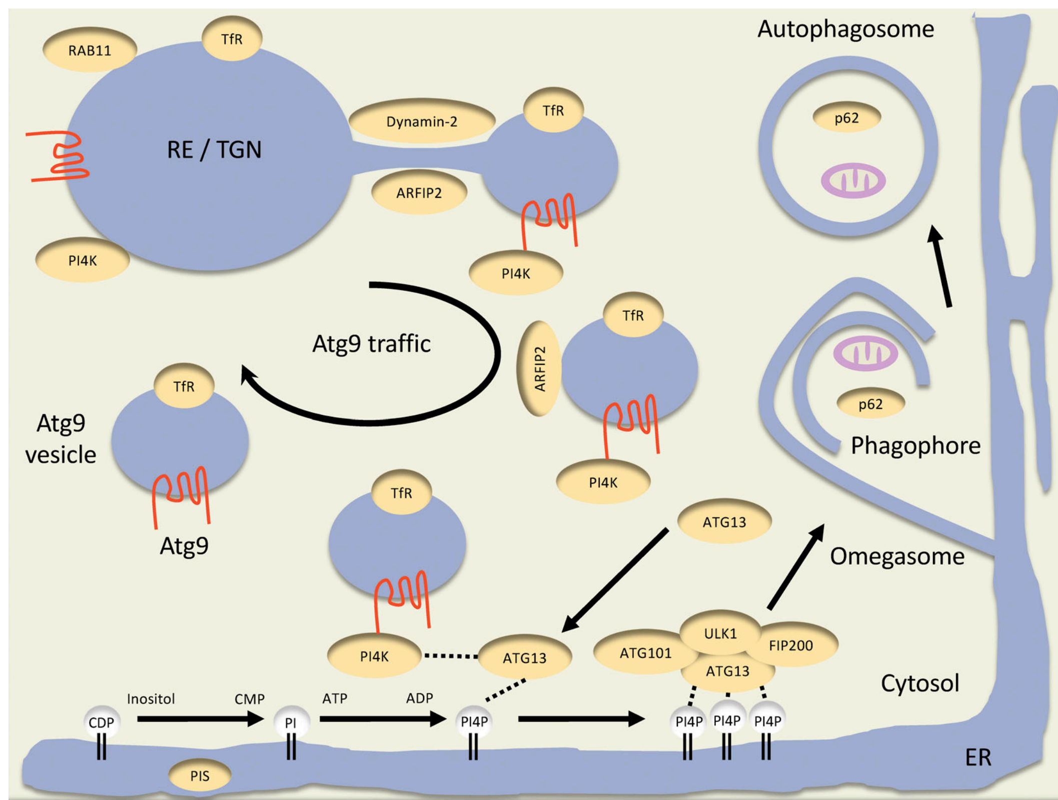

Figure 1. **BAR proteins (and Dynamin-2) act on RAB11 membranes to derive ATG9 vesicles loaded with PI4-kinase enzymes and possibly other PAS-acting proteins and lipids.** PI4P generation at the PAS by different PI4-kinases promotes autophagy by recruitment of ATG13 before phagophore nucleation. CMP, cytidin monophosphate; CDP, cytidin diphosphate; PIS, phosphatidylinositol synthase; TfR, transferrin receptor.

Overall, this study demonstrates the vesicular redistribution of metabolic activity from the Golgi to the PAS following a physical coordination between the metabolic enzyme and the vesicle carrier. This may mark the peak of a timely paradigm shift from vesicles in general and ATG9 vesicles in particular as generic carriers of lipids in autophagy toward context-specific contribution. Indeed, a proteomic study of yeast Atg9 vesicle cargo identified the TRAPPIII subunit Trs85 as an early PAS-acting factor (9). The BAR domain proteins Bif-1, identified here as an ATG9 cargo, and SNX18 each cooperate with Dynamin-2 to derive ATG9 vesicles from RAB11 membranes (10), while the contribution of Dynamin-2 to the loading of PI4KIIIβ onto ATG9 vesicles by ARFIP2 remains to be seen. Future studies should elucidate the distinct biophysical determinants for the engagement of different BAR domain proteins and other membrane remodeling factors with membranes of varying lipid and protein compositions throughout the ATG9 itinerary and the concomitant sorting of cargo.

The ability of ATG9 and ATG13 to each interact physically with PI4KIIIβ is particularly intriguing. A handover of active PI4KIIIβ from ATG9 to ATG13, coupled by PI4P binding by ATG13 (11), may facilitate positive feedback for stabilization of ATG13 at the PAS downstream of ATG9. While the recently discovered enrichment of PI synthase at the PAS was considered in the context of PI3P, which is prominent in early autophagy (12), it is tempting to believe that the earliest prominent modified PI at the PAS may turn out to be PI4P (Fig. 1). Finally, whereas interaction with GABARAP was necessary but not sufficient for in situ activity at autophagosome maturation of a palmitoylation-deficient PI4KIIα (8), the residence of PI4KIIα in ATG9 vesicles in close association with GABARAP may be the missing piece for the membrane-integral context that supports autophagic recruitment of PI4KIIα.

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