Autophagy plays a WASHing game

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Beclin 1 is a crucial regulator of autophagy. It forms a complex with ATG14L, VPS34 or the class III phosphatidylinositol 3-kinase AMBRA1 to control autophagosome formation. A study in this issue of *The EMBO Journal* (Xia *et al*, 2013) reports that WASH, a protein known to regulate endosomal sorting, hampers autophagy by inhibiting the AMBRA1-dependent polyubiquitylation of Beclin 1. This modification is required to promote VPS34 activity and to initiate autophagy during starvation.

Macroautophagy (hereafter called autophagy) is a lysosomal degradation pathway for cytoplasmic components. Autophagy is initiated by the formation of a double-membrane bound vacuole, the autophagosome that sequesters the autophagic cargo and eventually fuses with the

lysosomal compartment, leading to cargo degradation. Autophagy plays a major role in cytoplasm homeostasis by removing protein aggregates and controlling organelle quality, and it is stimulated to promote cell survival during stressful situations, such as nutrient starvation and microbial infection. Autophagosome formation is dependent on evolutionarily conserved Atg (Autophagy-related) proteins initially identified in yeast (Mizushima *et al*, 2011). They function in complexes or functional modules on a membrane known as the phagophore that matures into the autophagosome via several stages (initiation, elongation and sealing). Phosphatidylinositol 3-phosphate kinase complex I (PI3K complex I) plays a key role in the initiation step. In this complex, Beclin 1 (the mammalian homologue of yeast Atg6)

Figure 1 Role of WASH in autophagosome formation. WASH interacts with Beclin 1 via a sequence (121–221) that is not involved in endosomal sorting. In the absence of WASH, when autophagy is stimulated by nutrient deprivation, Beclin 1 interacts with VPS34 (and its adaptor VPS15), ATG14L and AMBRA1. In this complex, Beclin 1 is polyubiquitylated by AMBRA1, which acts as an E3 ligase. The activation of VPS34 produces PI3P at the phagophore to recruit WIPI proteins, and then triggers the machinery to elongate and seal the membrane, thus forming an autophagosome. The VAC domain of WASH involved in endosomal sorting is not required for autophagy regulation.

interacts with ATG14L, AMBRA1, and class III PI3K or VPS34 (Cecconi and Levine, 2008; Figure 1). The activity of this complex, which produces the lipid phosphatidylinositol 3-phosphate (PI3P) to recruit Atg18 homologues (WIPIs), has to be tightly regulated to keep autophagy under control.

In this issue of *The EMBO Journal*, Xia *et al* (2013) demonstrate that WASH (Wiskott-Aldrich syndrome protein (WASP) and SCAR homologue) regulates autophagosome formation in response to nutrient starvation by influencing the ubiquitylation of Beclin 1. WASH forms a complex with four other proteins and plays an essential role in endosomal sorting by promoting actin polymerization to facilitate segregation of endosomal proteins (Seaman *et al*, 2013). Using WASH^{−/−} mice, Xia *et al* (2013) observe that WASH deficiency causes embryonic lethality (E7.5–E9.5) with massive apoptosis-independent cell death and the accumulation of autophagic structures. However, whether autophagy is instrumental in the cell death observed in WASH^{−/−} embryos remains to be investigated. From a series of classical readouts, the authors conclude that WASH downregulates starvation-induced autophagy. Interestingly, WASH is detected on the autophagosomal membrane before and after closure of the autophagosome, but not on the autolysosomes (organelles formed when the autophagosome fuses with the lysosome). The authors first show that WASH has distinct roles in autophagy and in endosomal sorting, because deletion of the VCA domain of WASH that is required for its endosomal function and the knockdown of FAM21 (a protein that directs WASH to the endosomal membrane) do not influence autophagy. They then show that WASH interacts with the coil-coiled domain of Beclin 1. WASH depletion induces more VPS34 to interact with Beclin 1, leading to augmented formation of PtdIns3P and increased recruitment of WIPI-1 to the autophagosomal membrane. However, WASH does not influence the stability of Beclin 1, although it does regulate its K63-linked polyubiquitylation at position K437 during starvation (K63-linked polyubiquitylation suggests a regulatory role, whereas K48-linked polyubiquitylation is frequently associated with protein degradation). Overexpression of WASH reduces the ubiquitylation of Beclin 1, and the K437R Beclin 1 mutant that cannot be ubiquitylated has a low level of interaction with VPS34, which prevents the stimulation of autophagy by starvation (Figure 1). Recently,

Beclin 1 has been shown to be ubiquitylated at position K117 during TLR4-induced autophagy (Shi and Kehrl, 2010). Interestingly, Xia *et al* (2013) show that the K117R Beclin 1 mutant is still ubiquitylated in response to nutrient starvation. Finally, the authors identify the substrate-specific E3 ubiquitin ligase for Beclin 1 in this context. They exclude TRAF6 and NEDD4, two E3 ligases reported to ubiquitylate Beclin 1 (Kuang *et al*, 2013). AMBRA1, which regulates autophagy in the PI3K complex 1, is also known as DCAF3 and interacts with the Cullin 4–DDB1 E3 ligase complex (Jin *et al*, 2006). Following *in vitro* E3 ligase assays, Xia *et al* (2013) conclude that AMBRA1 is the substrate receptor for Beclin 1 ubiquitylation at position K437, adding Cullin4/DDB1 and AMBRA1 to the list of E3 ligases involved in autophagy (Kuang *et al*, 2013). The finding that WASH and AMBRA1 compete to regulate starvation-induced autophagy raises several questions: how do WASH and AMBRA1 influence one another's binding to Beclin 1? WASH is evolutionarily conserved, although it is not present in yeast (Linardopoulou *et al*, 2007)—does it also reduce starvation-induced autophagy in non-mammalian cells? WASH has recently been reported to be required for the lysosomal digestion of autophagy cargo in Dictyostelium during starvation (King *et al*, 2013). Does this mean that the function of WASH in autophagy has evolved from controlling cargo degradation to forming autophagosomes? Xia *et al* (2013) do not report any association between WASH and the lysosomal compartment during autophagy; the role of WASH in autophagosome maturation calls for further investigation. Finally, a recent report demonstrates that AMBRA1 interacts with the E3 ligase TRAF6 to ubiquitylate ULK1 (the mammalian orthologue of the yeast Atg1) and to ensure its subsequent stabilization and function (Nazio *et al*, 2013). ULK1 is part of a complex that acts with PI3K complex 1 to regulate the initiation of autophagy (Mizushima *et al*, 2011). How are these two AMBRA1-dependent ubiquitylation processes coordinated to control autophagy? In conclusion, both the study reported here (Xia *et al*, 2013) and the recent study of Nazio *et al* (2013) clearly demonstrate that AMBRA1 plays a central role in regulating the initiation of autophagy.

Conflict of interest

The authors declare that they have no conflict of interest.

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