restricts or otherwise skews the Wnt transcriptome (Figure 1). If it is the interplay between the two sets of second messengers that forms a gradient of cellular responsiveness, it is important to remember that Hippo signals respond to sharp discontinuities in the level of cell surface receptors on neighboring cells (Halder and Johnson, 2011). Defining how this signature feature of Hippo influences patterns of WNT activity will be important for understanding how zones of stem cells in normal niches are established and how niches of surviving cancer stem cells are created in tumors.

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A Dimer to Bridge Early Autophagosomal Membranes

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The Atg1/ULK complex plays a key role in the early stages of autophagosome assembly. In this issue, Ragusa et al. reveal the molecular basis for some interactions within this complex, finding that the crescent-shaped Atg17 dimer is critical for autophagy, whereas Atg1 may have the ability to cluster membranes.

Atg proteins, the key factors involved in autophagy, can be organized into four functional groups (Mizushima et al., 2011). Three of them—the Atg1/ULK complex, the autophagy-specific phosphatidylinositol 3-kinase complex, and the Atg9 cycling system—have been implicated in the early events of autophagosome biogenesis (Mizushima et al., 2011). In particular, they are critical in regulating and forming the phagophore assembly site (or preautophagosomal structure [PAS]) upon autophagy induction, and they are also likely involved in the generation of the phagophore, a precursor cisterna that through the acquisition of extra lipid bilayers gives rise to an autophagosome. The yeast Atg1/ULK complex comprises Atg1, Atg13, Atg17, and two nonconserved subunits, Atg29 and Atg31, whereas the mammalian ULK1 (or ULK2) associates with mATG13 and FIP200, the counterparts of Atg13 and Atg17, and the nonconserved component ATG101 (Chan et al., 2009). Autophagy induction requires the activation of the Atg1 kinase activity, which is under the direct control of both mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) (Mizushima et al., 2011). The only known substrate of Atg1 is Atg1 itself, and therefore how its kinase activity results in the formation of an autophagosome remains totally obscure.

Ragusa et al. now address the function of the five Atg proteins composing the yeast Atg1/ULK complex (Ragusa et al., 2012). Interactions within this complex had been revealed previously (Kabeya et al., 2005); however, it is not clear how the proteins may cooperate at the PAS and what their ultimate function is. In addition to the Atg1 kinase activity, the predicted coiled-coil protein Atg17 is a critical initiator of autophagy (Suzuki et al., 2007).

The authors took a structural approach and succeeded in solving the structure of Atg17 in complex with fragments of Atg29 and Atg31 from a thermostable yeast (Ragusa et al., 2012). The latter two proteins are critical for nonselective bulk autophagy, though they do not seem to be conserved across species (Kawamata et al., 2008). The Atg17 dimer forms a crescent shape with an extended interface along the C-terminal region that is reminiscent of BAR domain proteins.

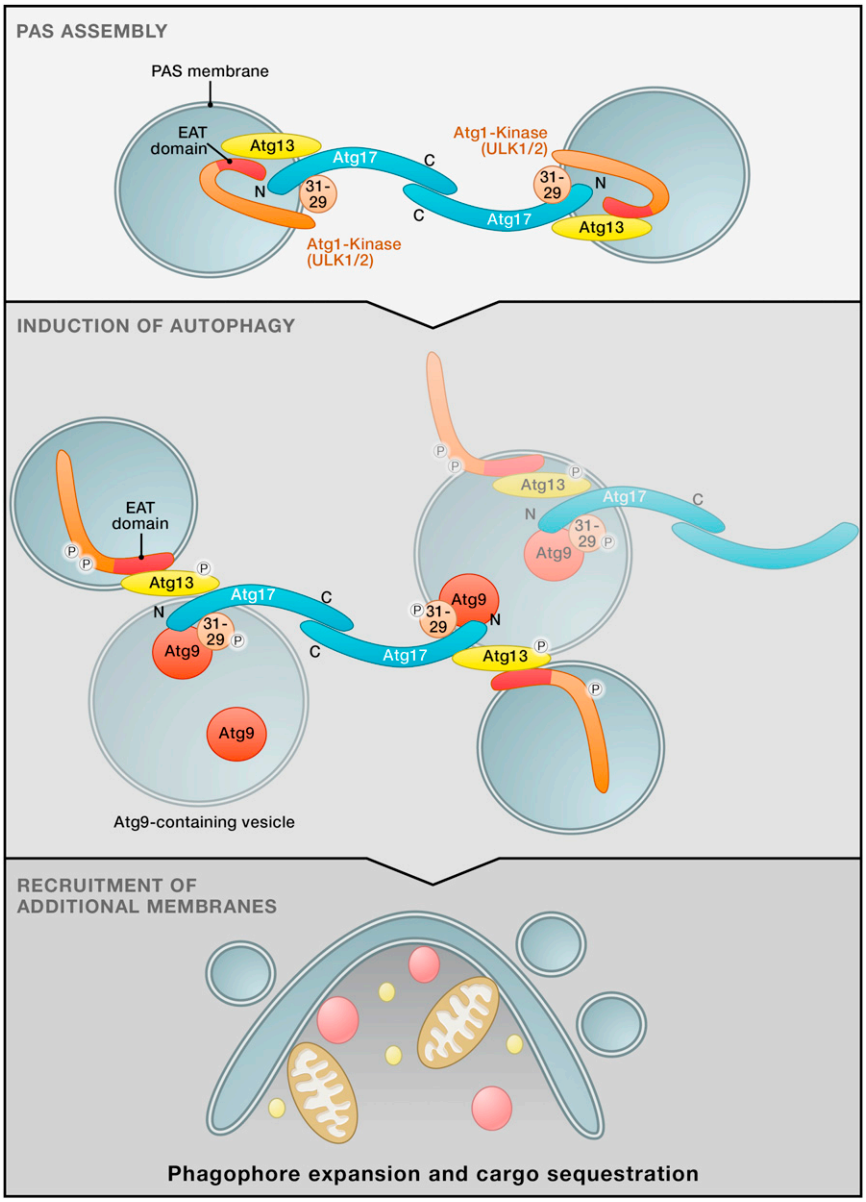

Figure 1. Model for Atg1/ULK-Complex-Mediated Fusion Events at the Phagophore Assembly Site

The Atg1/ULK complex, consisting of Atg1, Atg13, and a dimer of Atg17-Atg31-Atg29, associates with membranes at the phagophore assembly site (PAS). The potential EAT-domain-mediated dimerization of Atg1, which may contribute to the early clustering of membranes at the PAS as proposed by Ragusa et al., has not been included explicitly in this model. Induction of autophagy activates the Atg1 kinase activity, which promotes the ability of the Atg1/ULK complex via Atg17 to bind Atg9-positive vesicles and possibly other cargo adaptors. These steps lead to clustering and fusion of vesicles, which is followed by phagophore expansion and cargo sequestration, likely promoted at least in part by SNAREs, tethers, and the Rab Ypt1 (Mizushima et al., 2011). The Atg31-29 complex appears as 31-29.

Each Atg17 protomer binds to Atg31, which in turn interacts with Atg29 (of which only a small helical segment was resolved in the structure) in agreement with previous interaction studies (Kabeya et al., 2009). The authors used biochemical assays to determine the importance of the Atg17 dimer interface and to show that the dimer is likely the biologically active form.

As Atg17 also binds the transmembrane Atg9 protein, which is delivered on vesicles to the PAS (Mizushima et al., 2011; Mari and Reggiori, 2010; Yamamoto et al., 2012), the authors speculate that the Atg17-Atg31-Atg29 trimer may be able to tether the Atg9 membranes prior to their fusion. Such a tethering event would be predicted either for the biogenesis of the phagophore and/or its expansion into an autophagosome (Figure 1). The authors, however, did not observe any association of the trimer with liposomes. They thus asked whether the membrane-binding ability may be encoded in the other two other subunits of the Atg1/ULK complex, Atg1 and Atg13. Indeed, previous work had indicated that the C-terminal segment of Atg1, now termed the early autophagy targeting/tethering (EAT) domain, is important for Atg13 binding and targeting to membranes (Chan et al., 2009). Ragusa et al. show that this domain binds small liposomes and triggers their clustering (Ragusa et al., 2012). This result predicts that either each EAT domain has two membrane-binding sites or it has a single membrane interaction surface but also the ability to dimerize. Indeed, the latter could be confirmed by the authors. Importantly, the Atg1 EAT domain could be incorporated into a stable complex with part of Atg13 (residues 350–550) and the Atg17-Atg31-Atg29 subcomplex. This well-behaving minipentamer, however, lost the ability to tether (or cluster) membranes (Ragusa et al., 2012), suggesting that the EAT domain may be kept inactive in this complex or requires regulation in vivo.

It is unlikely that Atg1 membrane tethering is regulated by association/dissociation with the Atg17-Atg29-Atg31 subcomplex because the entire Atg1 complex is present regardless of whether autophagy is inhibited or induced (Kraft et al., 2012). One alternative possibility is that the phosphorylation of the Atg1/ULK complex by signaling pathways (suggested in Figure 1) and/or the association with other factors modulates this activity. However, it cannot be excluded that, in the context of full-length Atg1 and the Atg1/ULK complex, the EAT domain is just able to bind membranes without tethering them.

The proposed change in the Atg1 EAT domain to allow it to tether liposomes in combination with the shape of the Atg17 dimer led Hurley and his coauthors to a model in which the entire complex may be responsible for a sequential tethering reaction that would sequester Atg9 positive vesicles at the PAS (Figure 1; Ragusa et al., 2012). The authors are nevertheless cautious in their interpretation of the possible scenarios at this site, and it is indeed challenging to derive a clear model based on the characterized structures and interactions. Even though Atg17 mirrors BAR domain proteins in its shape, the observed lack of association with lipid bilayers suggests that it may bridge membranes via interactions with Atg9 rather than recognize membrane shape coupled with subsequent deformation of the bilayers.

Even with this new insight into Atg17 and Atg1 association, the protein and membrane dynamics leading to autophagosome formation remain an open question. Given the requirement for other proteins to localize and traffic Atg9-positive membranes and that multiple membrane sources contribute to the formation and expansion of the phagophore, other tethering factors such as Ypt1 and TRAPPIII may be involved (Mizushima et al., 2011). The mechanistic scenarios that emerge from this study are rather speculative, and though reasonable, other possibilities remain. The current work highlights the necessity for future studies aimed at understanding the dynamics of the first steps in autophagosome biogenesis.

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