H3K4me3 in genes within specific gene clusters, such as olfactory receptor genes—almost all of which, we note, are controlled by CpG-poor promoters. Clearly, further work is needed to determine how active histone marks around transcription start sites of inactive genes are set, whether they serve to protect these regions against establishment of an "irreversible" silent state, and, if so, whether this phenomenon is unique to vertebrates.

How might transcription be inhibited at a step following Pol II recruitment? Transcriptional pausing, poor processivity, degradation of the transcription machinery, and abortive initiation are all plausible explanations (Saunders et al., 2006). Intriguingly, a number of genes in mouse embryonic stem cells and in human T cells are enriched not only for H3K4me3 but also for H3K27me3 (Bernstein et al., 2006; Barski et al., 2007), a mark associated with the Polycomb group of transcriptional repressor proteins. The presence of this mark and associated Polycomb proteins may antagonize transcription at a postinitiation stage (Dellino et al., 2004). Alternatively, for many genes, the transition to productive elongation may require the activity of additional regulatory proteins. The experiments of Guenther et al. (2007) challenge the view that Pol II recruitment is the predominant rate-limiting event in gene activation for most genes in human cells and highlight the fact that much is still to be learned about the regulation of the postinitiation stages of transcription.

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A Ubiquitin-like Protein Involved in Membrane Fusion

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Atg8 is a ubiquitin-like protein involved in autophagy in yeast that is targeted to membranes through conjugation to the lipid phosphatidylethanolamine (PE). In this issue of *Cell*, Nakatogawa et al. (2007) show that Atg8 conjugated to PE mediates tethering between adjacent membranes and stimulates membrane hemifusion, an event that may mimic expansion of the autophagosomal membrane during autophagy.

In the budding yeast *Saccharomyces cerevisiae*, two ubiquitin-like (UBL) molecules, Atg8 and Atg12, are required for the formation of autophagosomes, the double-membrane vesicles that engulf cytosol and organelles during autophagy (Ichimura et al., 2000). These ubiquitin-like proteins participate in unusual reactions using E1-, E2-, and E3-like enzymes similar to those that conjugate ubiquitin to protein substrates (Kerscher et al., 2006). One protein, Atg12, is conjugated to the protein Atg5, and this conjugate then stimulates linkage of Atg8 to the lipid phosphatidylethanolamine (PE) present in specific intracellular membranes to generate Atg8-PE. Atg8 is rendered competent for conjugation to PE by a proteolytic processing event, catalyzed by the protease Atg4, which cleaves Atg8 near its C terminus (Kirisako et al., 2000). The conjugation reaction is

reversible and Atg4 regulates both the conjugation and the deconjugation of Atg8 to PE. Although yeast Atg8 and its counterparts in plants and mammals (LC3, GABARAP, GATE-16, and Atg8L) are required for autophagosome formation and autophagy, the precise function of Atg8 is unknown.

Nakatogawa et al. (2007) now show that Atg8-PE multimerizes, causing membrane tethering between liposomes. Membrane tethering mediated by Atg8-PE then leads to membrane hemifusion, which is normally a transient intermediate in membrane fusion reactions. Hemifusion involves lipid mixing only between the outer or proximal leaflets of the liposome membranes but not between the inner leaflets (Chernomordik and Kozlov, 2005). This work sheds light on an unanticipated cellular function of a ubiquitin-like protein and describes a unique membrane tethering and fusion reaction that is not mediated by SNAREs or other proteins with fusion domains (Jahn and Scheller, 2006; Langosch et al., 2007). The membrane fusion mediated by Atg8-PE may allow expansion of the autophagosomal membrane or completion of autophagosome formation.

The Ohsumi lab had previously established an experimental system in which Atg8 is conjugated to PE on liposomes in an ATP-dependent reaction catalyzed by Atg7 (an E1 enzyme) and Atg3 (an E2 enzyme) (Ichimura et al., 2004). In their current work, the authors show that liposomes containing Atg8-PE aggregate in an Atg8-PE dose-dependent manner (Nakatogawa et al., 2007). These aggregates are tethered liposomes, whose formation is reversed by Atg4, which deconjugates Atg8-PE.

The authors used liposomes in which a pair of fluorophores coupled to PE in the membrane quenched each other due to their close proximity. They then mixed Atg8-PE-containing, fluorescence-quenched liposomes with unlabeled liposomes and observed membrane fusion by monitoring fluorescence dequenching. In these reactions, only lipids in the outer membrane leaflets were found to mix, providing evidence that Atg-

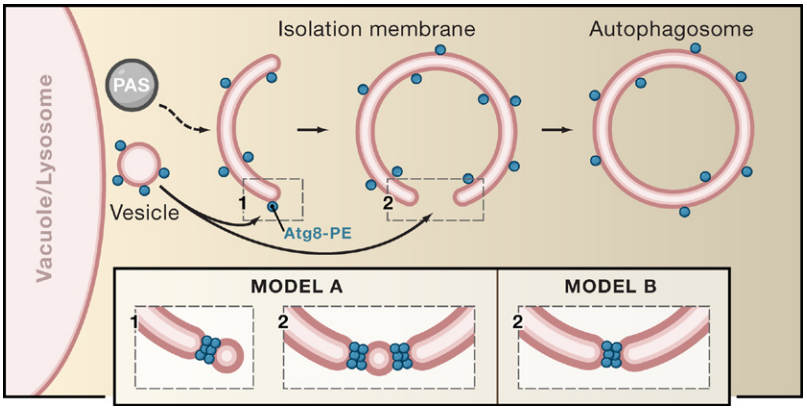

Figure 1. Atg8-PE and the Autophagosome Membrane

In yeast, the isolation membrane that engulfs nonselective cargo during autophagy is believed to form at the preautophagosomal structure (PAS), which is adjacent to the vacuole/lysosome. In the vesicle-dependent (Model A) expansion or closure of the autophagosome membrane, Atg8-containing vesicles of unknown origin (Kirisako et al., 1999) fuse with the isolation membrane, in a reaction involving Atg8-PE on both isolation membranes and vesicles (Boxes 1 and 2). (Box 1) The vesicles allow expansion of the isolation membrane; (Box 2) the vesicles contribute to completion of autophagosome formation. Events depicted in Box 2 seem more likely because expanded isolation membranes and autophagosome-like structures are seen even in cells lacking Atg8, albeit at a lower frequency (Kirisako et al., 1999). Model B shows the vesicle-independent expansion or closure of the isolation membrane during autophagosome formation. The inner (light pink) and outer (dark pink) leaflets of the lipid bilayer are shown. Hemifusion caused by Atg8-PE (blue) would involve mixing of the outer or proximal leaflets.

PE promoted membrane hemifusion and not complete fusion. Interestingly, during such fusion reactions, Atg8-PE, but not Atg8 alone, multimerizes and tethers liposomes—the activities suggested to facilitate fusion.

The authors examined the activity of Atg8 proteins carrying mutations in the ubiquitin-like domain and found autophagy-defective mutants that were not defective in Atg8-PE formation but were impaired in membrane fusion and/or tethering. The mutants fell into three classes (I, II, and III) in which the level of unconjugated Atg8, was similar to, less than, or more than that seen with wild-type Atg8. The class II and III mutations impaired membrane tethering, and several class II mutants multimerized poorly, whereas at least one class I mutation increased tethering. Interestingly, the tethering ability of mutant Atg8 proteins correlated, in most instances, with their ability to stimulate liposome fusion. Yeast cells expressing Atg8 mutants that were partially-functional in membrane tethering and fusion formed smaller than normal autophagosomes, suggesting a role for Atg8-PE in the membrane expansion (or completion) step of autophagosome formation.

These interesting results point to a role of Atg8-PE in multimerization and membrane tethering and fusion, consistent with its suggested role in the expansion of the autophagosome membrane. Several cellular or viral fusion proteins containing transmembrane domains can arrest fusion at the hemifusion stage when they are anchored to only one leaflet of the lipid bilayer (as Atg8-PE is), or when their transmembrane segments are shortened such that they no longer span both leaflets of the membrane bilayer. In this context, the promotion of hemifusion by Atg8-PE may not be entirely surprising (Chernomordik and Kozlov, 2005).

This study raises many new questions. First, over a dozen Atg and Vps proteins required for expansion of the autophagosome membrane (Yorimitsu and Klionsky, 2005) are completely dispensable for the liposome fusion reaction. Are these proteins simply helping to recruit Atg8 from the cytosol to the membrane of the yeast preautophagosomal structure, or do they play a role in regulating fusion mediated by Atg8-PE?

Second, the authors have not yet shown that the hemifusion product is an intermediate that ultimately proceeds to complete fusion, raising the possibility that hemifusion might be a dead-end product or accumulate as an intermediate that cannot proceed to completion because some additional proteins and/or lipids are missing. In this context, it should be noted that particular cone-shaped lipids such as PE, and to a lesser extent cylindrical phosphatidylcholine (PC), favor hemifusion, whereas lipids such as lysophosphatidylcholine with inverted cone shapes favor the hemifusion to fusion step (Chernomordik and Kozlov, 2005). The liposomes used in the study by Nakatogawa et al. (2007) comprised only PE, PC, and some phosphatidylinositol. Third, the authors' estimate that as many as 150 Atg8-PE molecules are required per liposome for fusion to commence appears to be quite high, at least in comparison with the 3-15 SNARE complexes required for vesicle fusion (Jahn and Scheller, 2006). Fourth, it is unclear whether fusion events mediated by Atg8-PE expand the preautophagosomal structure into cup-shaped membranes of greater surface area and/or cause the final closure of these membranes around cargo to form autophagosomes (Figure 1). And finally, how does Atg8-PE promote hemifusion? Does Atg8-PE multimerization concentrate PE locally to promote hemifusion? Alternatively, are domains of the proximal layers of the opposing lipid bilayers destabilized by lateral Atg8-PE interactions in each membrane, such that hemifusion would relieve this local strain? Or, analogous to trans-pairing of SNAREs or the assembly of trans-complexes of the V_0 domain of vacuolar ATPase, do interactions and associated conformational changes between Atg8-PE on the opposing membranes bring the two bilayers in close contact for hemifusion (Langosch et al., 2007)? Stay tuned for further insights into the mechanism of autophagosome formation.

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