Developmental Cell
Previews

Reactive Oxygen Species Regulate Autophagy through Redox-Sensitive Proteases

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DOI 10.1016/j.devcel.2007.03.016

Starvation induces autophagy through a signal transduction pathway that is not fully understood. In a recent issue of *The EMBO Journal*, Scherz-Shouval and colleagues (Scherz-Shouval et al., 2007) show that reactive oxygen species (ROS) occurring during starvation serve as signaling molecules that initiate autophagy.

Autophagy is a major intracellular degradation mechanism for long-lived proteins and organelles. This crucial cellular process operates under stress conditions and can promote survival during starvation or lead to cell death under specific conditions such as the inhibition of apoptosis (Gozuacik and Kimchi, 2007; Yu et al., 2004). Autophagy is initiated by engulfing large sections of cytoplasm by a crescent-shaped phagophore that elongates to a closed double-membrane structure, called an autophagosome. Subsequently, the autophagosome fuses with a lysosome and its contents are degraded by lysosomal hydrolases (Figure 1A). This can lead to recycling of the catabolites, hence its role in the survival of starving cells. It is now appreciated that autophagy has broader importance in regulating growth and maintaining homeostasis in multicellular organisms. Defective autophagy contributes to pathogenesis of a number of diseases, including myopathies, neurodegenerative diseases, and some forms of cancers (Kelekar, 2005).

The vital role of autophagy in cellular physiology has spurred extensive research on genes participating in and regulating autophagy. The process of autophagy is governed by a group of genes, denoted as ATGs, conserved from yeast to humans. Biochemical hallmarks of autophagy are the appearance of Atg5-Atg12 covalent protein complex and Atg8-phosphoethanolamine (PE) conjugates on the autophagosome membrane. Ubiquitin-like reactions involving further ATG gene products generate these conjugates (Figure 1B). The reactions share the same E1-like enzyme Atg7 but have different E2-like enzymes—Atg10 for Atg5-Atg12 and Atg3 for Atg8-PE. The Atg5-Atg12 complex appears on phagophore before Atg8-PE conjugate. The Atg8 protein family includes GATE16, LC3, and GABARAP. The Atg4 family of cysteine proteases cleaves Atg8s near the C terminus after a conserved glycine residue. This cleavage allows the covalent bonding of Atg8 to PE through the exposed glycine. Atg4 is also responsible for recycling Atg8s by cleaving PE from PE-conjugated Atg8s (Mizushima et al., 2003).

Autophagy is inducible by a variety of intracelluar and extracellular stimuli, including starvation, pathogen infection, protein aggregates, and damaged organelles. However, the exact molecular mechanism by which these stimuli provoke autophagy remains largely unknown. The article from Scherz-Shouval and colleagues provides evidence for an interesting mechanism by which hydrogen peroxide generated during starvation serves as a signaling molecule that initiates autophagosome formation: hydrogen peroxide inactivates HsAtg4A by oxidation of a critical cysteine residue, leading to accumulation of Atg8-PE on the phagophore membrane and formation of autophagosomes (Scherz-Shouval et al., 2007).

A reactive oxygen species (ROS) response has been associated with a variety of stimuli, such as tumor necrosis factor (TNF), endoplasmic

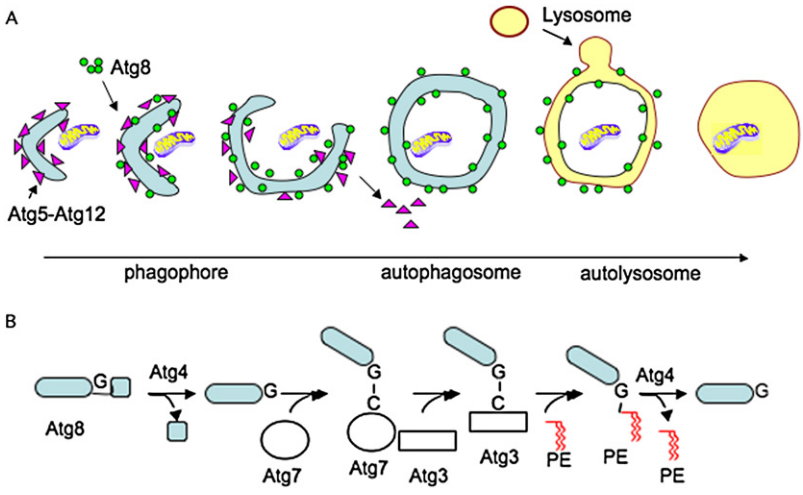

Figure 1. Autophagy Formation and Conjugation Reactions
(A) Diagrammatic illustration of autophagy process. (B) Schematic diagram of Atg8-PE conjugation reactions. A parallel enzymatic system carries out Atg5-Atg12 conjugations with Atg10 at the place of Atg3.

reticulum stress (ER stress), starvation, and inhibition of mitochondrial function. In most cases, ROS are considered to be an adverse event for cells by triggering a stress response that may involve dire consequences for the cell—protein or organelle damage and possible cell death. Kamata et al. showed previously that hydrogen peroxide induced by TNF inhibits c-Jun N-terminal kinase (JNK)-inactivating phosphatases by converting their catalytic cysteine to sulfenic acid, resulting in sustained JNK activation (Kamata et al., 2005). Here, Scherz-Shouval et al. elucidate another compelling example of a redox-sensitive cysteine regulating enzyme activity and triggering a specific cellular process.

Scherz-Shouval et al. (2007) report that ROS inactivate HsAtg4A which causes the accumulation of Atg8-PE. They found that ROS were synthesized very early during starvation and ROS, at least at the levels observed, did not result in cell death. They found that ROS colocalized with mitochondria and Atg5-Atg12-positive phagophores. They further showed that ROS were necessary for autophagy because a general antioxidant was able to inhibit the formation of autophagosomes. This led to examination of the cysteine protease HsAtg4A whose activity is sensitive to oxidation. HsAtg4A belongs to the Atg4 family and mainly cleaves GATE-16. They were able to pinpoint a critical cysteine, C^{81}, which is four amino acids away from the active site. Replacement of C^{81} with a serine resulted in an HsAtg4A variant less sensitive to redox regulation. Expression of $HsATG4A^{C81S}$ significantly impaired the formation of GATE16-positive autophagosomes in starved cells, compared to control cells expressing $HsATG4A^{WT}$. Finally, they extended their findings to HsATG4B, which is the processing enzyme for LC3 by showing that the counterpart of C^{81} of HsATG4B was also subject to redox regulation. Scherz-Shouval and colleagues focused on the effect of ROS on enzymatic activity of HsAtg4. However, other enzymes involved in the initiation and elongation stages of autophagosome formation, such as the conjugation enzymes Atg3, Atg7, and Atg10, have cysteines as their catalytic residues. Because Atg3, Atg7, and Atg10 need to stay active onsite at the phagophore to catalyze the conjugation of Atg5-Atg12 and Atg8-PE, it will be interesting to determine how ROS inactivate Atg4s without affecting Atg3, Atg7, and Atg10. In any case, at least one critical step in the autophagy pathway appeared to be controlled by ROS.

The work of Scherz-Shouval and colleagues provides a molecular understanding of how starvation might induce autophagy. This mechanism could have broad implications beyond the genesis of autophagosomes during starvation. Autophagy is important in maintaining intracellular homeostasis and keeping the cell healthy. For example, autophagy is essential in maintaining organelle homeostasis during cell growth and damage control during cellular aging. Defective mitochondria and peroxisomes produce more hydrogen peroxide than normal ones (DiMauro et al., 2002; Legakis et al., 2002). It is of great importance for cells, especially long-lived postmitotic cells, to selectively degrade dysfunctional organelles. Peroxisomes are selectively degraded by a specific type of autophagosomes (Dunn et al., 2005). It will be interesting to know whether the ROS produced by an old or damaged peroxisome plays any role in triggering the formation of peroxisome-specific autophagosomes. Similarly, mitochondria are actively turned over in cells and can be swallowed by autophagosomes. It is possible that defective mitochondria locally generate autophagosomes due to increased ROS production by the damaged respiratory chain and therefore have a higher tendency to be autophagocytosed. ROS-induced autophagy may illuminate an important mechanism of quality control: selective degradation of damaged mitochondria and peroxisomes. Thus, the connection between oxygen radical physiology and autophagy promises to yield a variety of fruitful insights.

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