Regulation of autophagy in yeast and mammals

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Understanding the process of intracellular protein transport, whereby protein-carrying vesicles move across and between membranes, is of fundamental importance in cell physiology and medicine. Autophagy is a unique membrane trafficking process essential for the degradation and recycling of excess or defective macromolecules and organelles in eukaryotes. This pathway is activated under environmental stress conditions as well as during certain developmental stages and has been linked to programmed cell death, cancer, pathogen infection, and degradation of ubiquitinated protein aggregates formed in many pathological conditions. The main goal of our studies is to understand the mechanism of autophagocytosis and characterize the relationship between this process and the secretory pathway while utilizing mammalian, plant and yeast systems.

Autophagy in living cells

The biogenesis and turnover of autophagosomes in mammalian cells as well as the molecular mechanisms underlying induction of autophagy and trafficking of these vesicles are poorly understood. We have utilized different autophagic markers to determine the involvement of microtubules in the autophagic process. We found that autophagosomes associate with microtubules and concentrate near the microtubule-organizing center (Figure 1). Moreover, we demonstrated that autophagosomes, but not phagophores (the autophagosomes origin), move along these tracks en route for degradation. Disruption of microtubules leads to a significant reduction in the number of mature autophagosomes but does not affect their lifespan or their fusion with lysosomes. We propose that microtubules serve to deliver only mature autophagosomes for degradation, thus providing a spatial barrier between phagophores and lysosomes.

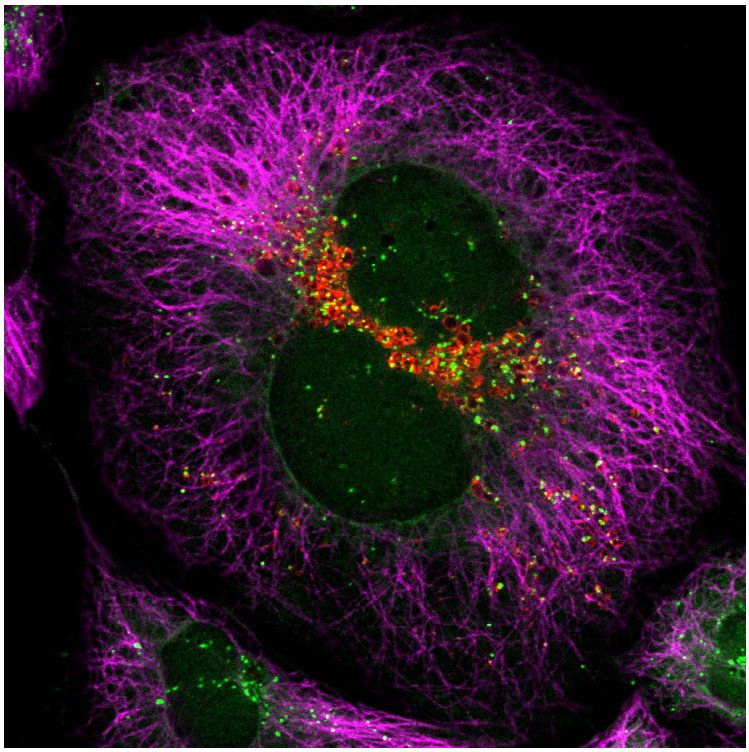

Fig. 1 *Delivery of autophagosomes to lysosomes along microtubules. The image shows autophagosomes labeled with anti-LC3 antibodies (green) and lysosomes/autolysosomes labeled by RFP-LAMP-1 (red), associated with microtubule fibers labeled by anti-tubulin antibodies (magenta) in a dividing CHO cell. The cells were starved of amino acids to induce autophagy and treated with lysosomal inhibitor Bafilomycin A to visualize autolysosomes.*

Experimental methods to monitor autophagy in mammalian cells are limited due to lack of autophagic markers and the current methods to quantify autophagic activity using LC3 are time-consuming, labor-intensive and require much experience for accurate interpretation. We have recently utilized the Fluorescence Activated Cell Sorter (FACS) to develop a novel assay to measure autophagic activity in living mammalian cells.

The Atg8 family and autophagy

A hallmark event in the autophagic process is the reversible conjugation of the Atg8 family of proteins to the autophagosomal membrane. This ubiquitin-like (UBL) protein family, which has been implicated in a variety of cellular processes, includes Atg8p in yeast, and GATE-16 (Golgi-associated ATPase Enhancer of 16 kDa), LC3 (Light Chain 3) and GABARAP (GABA Receptor-Associated Protein) in mammals. In the past, we found that GATE-16 interacts with components of the membrane fusion machinery and characterized these interactions. We have also shown that GATE-16 plays an important role in Golgi re-arrangement during mitosis by interacting with Gos-28, a pivotal Golgi SNARE molecule. Moreover, we determined the three-dimensional structure of GATE-16 at 1.8 Å resolution and used this structure for modeling and identification of two functional sites required for protein-protein interaction in yeast Atg8 and in LC3 (Figure 2). The first site, which includes residues Phe77 and Phe79 in yeast Atg8 and residues Phe80 and Leu82 in LC3, is essential for

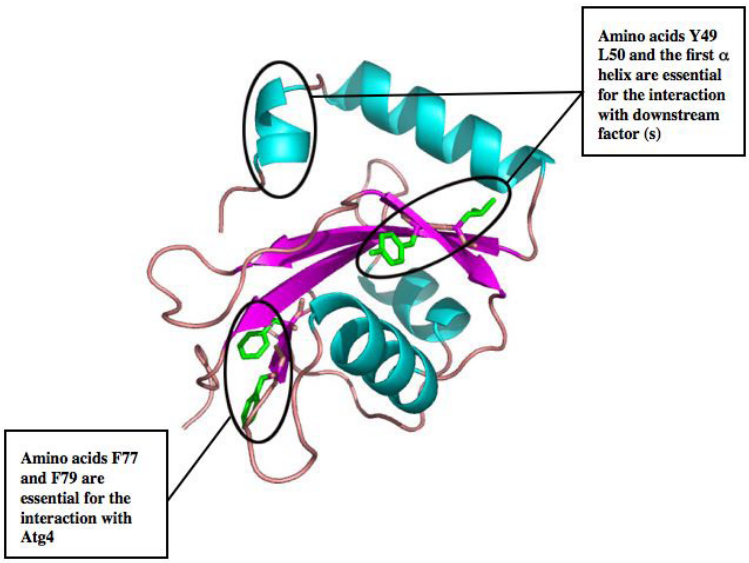

Fig. 2 *Essential sites in Atg8 prediction structure are crucial for the autophagic activity. Atg8 is an ubiquitin like protein decorated by two a-helices at its N-terminus. Amino acids F77 and F79 in the ubiquitin core of the protein were found to be essential for its interaction with the cystein protease Atg4, whereas amino acids Y49, L50 at the ubiquitin core together with the first a-helix are essential for the interaction with downstream factors.*

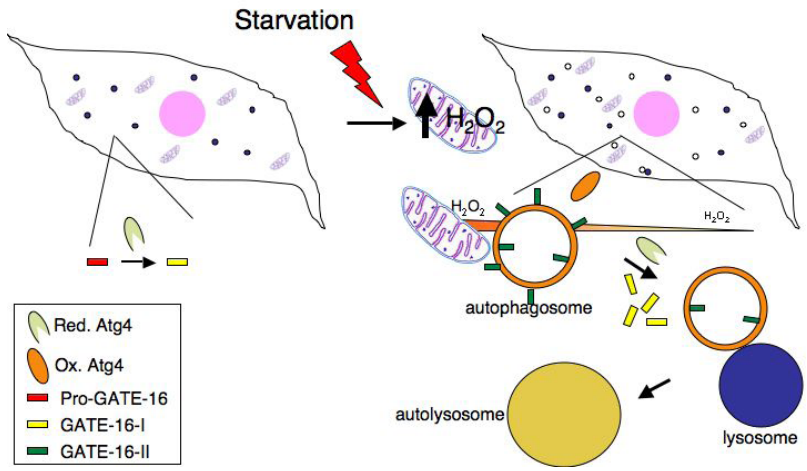

Fig. 3 *A model for the redox regulation of Atg4 during starvation. Amino acid starvation induces accumulation of H_2O_2 near the mitochondria. The oxidative environment inhibits the activity of Atg4, and allows autophagosome formation. Further away from mitochondria a reducing environment is maintained, where Atg4 can cleave Atg8 from autophagosomes before fusion with lysosomes.*

C-terminal cleavage of these proteins by the Atg4 protease. The other site includes residues Tyr49 and Leu50 in Atg8 and Phe52 and Leu53 in LC3, is essential for the interaction of these proteins with downstream targets (see bellow).

We also found that LC3 N-terminal region is essential for its interaction with p62/SQSTM1 (hereafter termed p62), a protein known to interact with polyubiqitinated aggregates that cannot be processed by the proteasome system. This interaction is dependent on LC3 first 10 amino acids and on specific residues located within the ubiquitin core. Our findings support the proposal that LC3 is responsible for recruiting p62 together with polyubiqitinated aggregates into autophagosomes en route to lysosomal degradation.

Regulation of autophagy by Reactive Oxygen Species (ROS)

We have recently discovered a novel involvement of ROS in regulation of starvation-induced autophagy (figure 3). We showed that nutrient starvation leads, partially through class III PI3K, to accumulation of H2O2 in the mitochondria, which is essential for the induction of autophagy. The oxidative signal in this experimental setup appeared minutes after induction of starvation and did not cause cell death. Furthermore, Atg4, an essential protease in the autophagic pathway, has been identified as a direct target for oxidation by H_2O_2. This protease cleaves the c-terminus of the Atg8 family of ubiquitin-like proteins, as a prerequisite for their conjugation to phosphatidylethanolamine (PE) on the autophagosomal membrane. This ubiquitin-like conjugation, mediated by Atg7 and Atg3 as E1 and E2 modifiers, respectively, is essential for autophagosome maturation. Conjugated Atg8 serves as another substrate for Atg4, cleaving and removing it from the mature autophagosome for recycling.

The finding that this protease is redox-regulated signifies a novel signal transduction pathway in which ROS function as signaling molecules to trigger autophagy as a survival mechanism. H_2O_2, the signaling ROS in this system, is an attractive candidate for signaling, since it is relatively stable and long lived compared to other ROS, and its neutral ionic state allows it to exit the mitochondria easily. Indeed, H_2O_2 was implicated in various signal transduction pathways as a modifier of thiol-containing proteins. But is Atg4 the

only target of H_2O_2 in this pathway? And more importantly, considering that the basic autophagic machinery appears to be conserved regardless of the specific inducer and outcome, is the inhibition of Atg4 unique to starvation-induced autophagy or will it turn out to be a general characteristic of autophagy? These questions remain to be solved.

Selected publications

Legesse-Miller, A., Sagiv, Y., Glozman, R. and Elazar, Z. Aut7p, a soluble autophagic factor, participates in multiple membrane trafficking processes. J Biol Chem 2000; 275:32966-73.

Paz, Y., Elazar, Z. and Fass, D. Structure of GATE-16, membrane transport modulator and mammalian ortholog of autophagocytosis factor Aut7p. J Biol Chem 2000; 275:25445-50.

Porat, A., Sagiv, Y. and Elazar, Z. A 56-kDa selenium-binding protein participates in intra-Golgi protein transport. J Biol Chem 2000; 275:14457-65.

Porat, A. and Elazar, Z. Regulation of intra-Golgi membrane transport by calcium. J Biol Chem 2000; 275:29233-7.

Sagiv, Y., Legesse-Miller, A., Porat, A. and Elazar, Z. GATE-16, a membrane transport modulator, interacts with NSF and the Golgi v-SNARE GOS-28. Embo J 2000; 19:1494-504.

Muller, J. M., Shorter, J., Newman, R., Deinhardt, K., Sagiv, Y., Elazar, Z., Warren, G. and Shima, D. T. Sequential SNARE disassembly and GATE-16-GOS-28 complex assembly mediated by distinct NSF activities drives Golgi membrane fusion. J Cell Biol 2002; 157:1161-73.

Scherz-Shouval, R., Sagiv, Y., Shorer, H. and Elazar, Z. The COOH terminus of GATE-16, an intra-Golgi transport modulator, is cleaved by the human cysteine protease HsApg4A. J Biol Chem 2003; 278:14053-8.

Stuven, E., Porat, A., Shimron, F., Fass, E., Kaloyanova, D., Brugger, B., Wieland, F. T., Elazar, Z. and Helms, J. B. Intra-Golgi protein transport depends on a cholesterol balance in the lipid membrane. J Biol Chem 2003; 278:53112-22.

Shorer, H., Amar, N., Meerson, A. and Elazar, Z. Modulation of N-ethylmaleimide-sensitive factor activity upon amino acid deprivation. J Biol Chem 2005; 280:16219-26.

Slavikova, S., Shy, G., Yao, Y., Glozman, R., Levanony, H., Pietrokovski, S., Elazar, Z. and Galili, G. The autophagy-associated Atg8 gene family operates both under favourable growth conditions and under starvation stresses in Arabidopsis plants. J Exp Bot 2005; 56:2839-49.

Amar, N., Lustig, G., Ichimura, Y., Ohsumi, Y. and Elazar, Z. Two newly identified sites in the ubiquitin-like protein Atg8 are essential for autophagy. EMBO Rep 2006; 7:635-42.

Fass, E., Shvets, E., Degani, I., Hirschberg, K. and Elazar, Z. Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. J Biol Chem 2006; 281:36303-16.

Fass, E., Amar, N. and Elazar, Z. Identification of essential residues for the C-terminal cleavage of the mammalian LC3: a lesson from yeast Atg8. Autophagy 2007; 3:48-50.

Scherz-Shouval, R., Shvets, E., Fass, E., Shorer, H., Gil, L. and Elazar, Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. Embo J 2007; 26:1749-60.

Scherz-Shouval, R., Shvets, E. and Elazar, Z. Oxidation as a Post-Translational Modification that Regulates Autophagy. Autophagy 2007; 3:371-3.

Scherz-Shouval, R. and Elazar, Z. ROS, mitochondria and the regulation of autophagy. Trends Cell Biol 2007; 17:422-7.

Shvets, E., Fass, E. and Elazar, Z. Utilizing flow cytometry to monitor autophagy in living mammalian cells. Autophagy, 2008; in press

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