[Autophagy 3:3, 285-287; May/June 2007]; ©2007 Landes Bioscience

Addendum

ER-Phagy

Selective Autophagy of the Endoplasmic Reticulum

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Original manuscript submitted: 01/18/07
Manuscript accepted: 02/01/07

Previously published online as an *Autophagy* E-publication:
http://www.landesbioscience.com/journals/autophagy/abstract.php?id=3930

KEY WORDS

unfolded protein response, organelle degradation, endoplasmic reticulum proliferation, ER stress, ER-containing autophagosome, electron microscopy

ACKNOWLEDGEMENTS

This work was supported by an American Heart Predoctoral Fellowship to S.B., by an Ernst Schering Foundation Postdoctoral Fellowship to S.S., and by grants from the National Institutes of Health to P.W. P.W. is an Investigator of the Howard Hughes Medical Institute.

Addendum to:

Autophagy Counterbalances Endoplasmic Reticulum Expansion during the Unfolded Protein Response

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PLoS Biol 2006; 4:e423

ABSTRACT

Throughout their life, cells must maintain homeostasis while facing constantly fluctuating demands on their different organelles. A major mechanism for the homeostatic control of organelle function is the unfolded protein response (UPR), a signaling pathway that triggers a comprehensive remodeling of the endoplasmic reticulum (ER) and the biosynthetic pathway according to need. We discovered that activation of the UPR in yeast also induces a new branch of macroautophagy that selectively targets the ER. We term this process "ER-phagy", in analogy to pexophagy and mitophagy, the two other known forms of organelle-specific marcoautophagy. ER-phagy involves the generation of autophagosomes that selectively include ER membranes and whose delimiting double membranes also derive, at least in part, from the ER. This finding provides direct evidence that the ER can serve as a membrane source for autophagosome formation and indicates that ER-phagy entails engulfment of the ER by itself. ER-phagy could remove damaged or redundant parts of the ER and thus represent an important degradative functionality of the UPR that helps to afford homeostatic control.

Integral membrane and secretory proteins are folded and modified in the endoplasmic reticulum (ER). An elaborate system of homeostatic control ensures that the protein processing capacity in the ER is adjusted to need. At the core of this system is the unfolded protein response (UPR), an ancient ER-to-nucleus signaling pathway that is conserved in all eukaryotic cells.[1-3] When changes in external conditions or cell fate lead to the accumulation of unfolded proteins in the ER-that is, when the protein folding and modification capacity of the ER is exceeded-the UPR activates a massive transcriptional program. In yeast, this program comprises about 5% of all genes, many of which function in the biosynthetic pathway.[4] As a result, the expression of ER enzymes is augmented to enhance protein folding, and the machinery for protein retrotranslocation and degradation is induced to clear folding failures from the ER lumen. Together, the broad scope of these responses enables cells to overcome folding stress and redress the balance between the folding load and capacity of the ER.

To characterize the UPR-induced remodeling of the ER, we carried out an ultrastructural analysis of the ER using electron microscopy.[5] We found that folding stress increased the volume of the yeast ER significantly, which may help to accommodate newly synthesized ER enzymes and inhibit the aggregation of unfolded proteins by reducing their concentration. Surprisingly, in many cells ER expansion was followed by the appearance of large vesicles of 300–700 nm in diameter, which were bounded by double membranes. These vesicles were densely filled with stacked membrane cisternae, and they contained little cytosol and no other organelles, indicating that membranes were packaged into them selectively (Fig. 1). The sequestered membranes were mostly free of ribosomes, but immunogold labeling of an ER marker protein showed that they were derived, at least in part, from the ER. We therefore name these vesicles ERAs, for "ER-containing autophagosomes". The delimiting membranes of ERAs were frequently studded with ribosomes and therefore also originated from the ER. Hence, both the sequestered and the sequestering membranes come from the ER, indicating that the formation of ERAs involves an engulfment of the ER by itself (Fig. 2). How such a complex rearrangement can be achieved presents a fascinating puzzle.

The discovery of ERAs led us to explore the role autophagy might have in the UPR. We found that folding stress induced the expression of *ATG8*, the yeast homolog of the mammalian *LC3*. Deletion of *ATG8* prevented ERAs formation and impaired the ability of the cells to survive strong folding stress. Therefore, the general autophagy machinery is used to generate ERAs, and it is required for cells to withstand folding stress. Consistent

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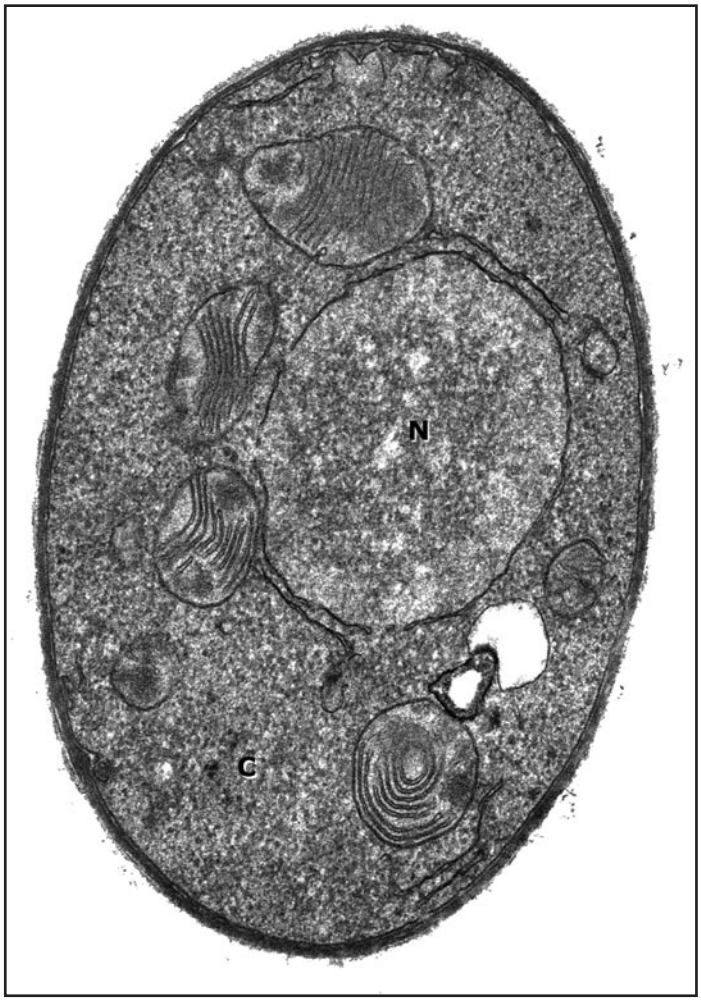

Figure 1. ER-containing autophagosomes (ERAs). Electron micrograph image of a representative UPR-induced wild-type cell that contains ERAs. Nuclei and cytoplasm are indicated as N and C, respectively.

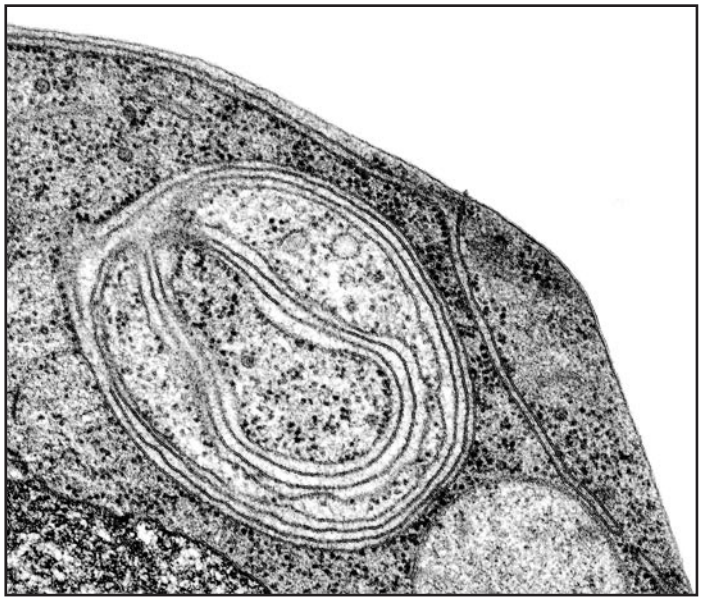

Figure 2. Visualization of ERA membranes. High-pressure freezing/freeze substitution image of an ERA to reveal the membrane content. Note that both content and delimiting membranes contain bound ribosomes, and therefore are at least partially derived from the ER.

with this notion, various other mutants of known autophagy genes, which are viable under normal growth conditions, died upon UPR activation. A mutant lacking functional vacuolar proteases, however, survived. This finding indicates that the primary benefit of ERAs formation in the face of severe folding stress is the selective sequestration rather than the degradation of parts of the ER.

Thus, our study revealed an important link between the UPR and autophagy and has identified a new branch of autophagy, which we term ER-phagy. This discovery extends the group of organelle-selective modes of macroautophagy: pexophagy for engulfing peroxisomes, mitophagy for engulfing mitochondria, and ER-phagy for engulfing ER membranes. Physiologically, the role of ER-phagy may be two-fold: (1) the formation of ERAs during folding stress may serve to sequester parts of the ER that are damaged or contain protein aggregates that cannot be disposed of in other ways, and (2) ER-phagy may reduce the size of the ER back to normal once the folding stress subsides. ER-phagy could therefore represent an important degradative functionality of the UPR and be an integral player in achieving homeostatic control. In this regard, ER-phagy may be analogous to the regulation of peroxisome abundance, whose biogenesis is balanced by pexophagy, and to the regulation of mitochondrial abundance, whose biogenesis is thought to be balanced by mitophagy.[6,7]

Our conclusions are in agreement with a series of recent studies, both in yeast and in mammalian cells, that have revealed different aspects of the connection between the UPR and autophagy described above. In yeast, work from Ohsumi's group first suggested that ER components can be delivered to the vacuole by starvation-induced autophagy.[8] Two studies using overexpression of proteins that are prone to aggregation in the ER then discovered that autophagy is involved in the clearance of misfolded proteins,[9,10] and another report showed that UPR activation induces autophagy.[11] In mammalian cells, autophagy also seems to be needed to rid the ER of protein aggregates,[12] and ER stress triggers LC3 lipidation, suggesting that autophagy is activated.[13] Finally, two further studies showed that UPR activation induces the accumulation of autophagosomes.[14,15] Intriguingly, autophagosomes elicited by folding stress "often contained multi-lamellar structures" that were not observed in starvation-induced autophagosomes.[14] This finding raises the possibility that ER stress causes the generation of ERAs also in mammalian cells.

ER-phagy promises to shed new light on general questions concerning the mechanisms of autophagy. First, the origin of the limiting double membrane of autophagosomes has been a long-standing open question.[16] The ER has been suspected to serve as a membrane source, but evidence for this proposal has not been conclusive.[17,18] Our study now shows that the ER membrane can indeed by used to build autophagosomes. Second, the events that occur between the completion of an autophagosome and its fusion with the degradative compartment, the vacuole in yeast and lysosomes in mammalian cells, are poorly understood. In mammalian cells, autophagosomes may fuse with endosomes before they reach lysosomes.[19] No intermediate steps during the delivery of completed autophagosomes have so far been identified in yeast. However, ERAs apparently remain in the cytosol under UPR-inducing conditions, suggesting that fusion with the vacuole can be regulated (or at least temporarily be suspended). Third, the identification of the molecules involved in ER-phagy, which already include many of the known

Atg proteins, promises new insights into the general autophagy machinery and how it can selectively target an organelle. Finally, ERAs formation itself is an extraordinary process. To generate ERAs, those parts of the ER that become limiting membranes need to be reshaped, ribosomes need to be stripped from those parts that are to be packaged into the lumen of ERAs, these membranes then need to be folded up, membrane severing needs to occur to remove ERAs from the remaining ER, and ERAs finally need to be sealed by homotypic fusion of the delimiting membranes. ER-phagy may therefore tell us a great deal about the mechanisms cells use to achieve such amazing membrane remodeling and packing "acrobatics" as the self-eating of an organelle.

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