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Degradation of the endoplasmic reticulum by autophagy in plants

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Eukaryotic cells have developed sophisticated strategies to contend with environmental stresses faced in their lifetime. Endoplasmic reticulum (ER) stress occurs when the accumulation of unfolded proteins within the ER exceeds the folding capacity of ER chaperones. ER stress responses have been well characterized in animals and yeast, and autophagy has been suggested to play an important role in recovery from ER stress. In plants, the unfolded protein response signaling pathways have been studied, but changes in ER morphology and ER homeostasis during ER stress have not been analyzed previously. Autophagy has been reported to function in tolerance of several stress conditions in plants, including nutrient deprivation, salt and drought stresses, oxidative stress, and pathogen infection. However, whether autophagy also functions during ER stress has not been investigated. The goal of our study was to elucidate the role and regulation of autophagy during ER stress in *Arabidopsis thaliana*.

Keywords: endoplasmic reticulum, autophagy, Arabidopsis, IRE1, ER stress

Abbreviations: BiP, binding protein; DIC, differential interference contrast; DTT, dithiothreitol; ER, endoplasmic reticulum; HAC1, histone acetyltransferase of the CBP1 family; JNK, c-Jun N-terminal kinase; NBR1, neighbor of BRCA1; TM, tunicamycin; TOR, target of rapamycin

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First, upon treatment of Arabidopsis seedlings with tunicamycin (TM) or dithiothreitol (DTT) to induce ER stress, autophagosomes accumulate in root cells, indicating that autophagy is activated by ER stress in plants. Second, confocal, differential interference contrast (DIC) and electron microscopy analyses indicated that portions of the ER are engulfed by autophagosomes and delivered to the vacuole for degradation. This evidence together suggests that plant autophagy is involved in ER degradation in response to ER stress. Third, to investigate the upstream signaling pathway that activates ER stress-induced autophagy, mutants lacking components of the known ER stress signaling pathways were tested for autophagy induction upon ER stress. Of the components tested, only IRE1b, one of the ER stress sensors, is required for activation of autophagy by TM or DTT. Autophagy is activated normally in an *ire1b* mutant in response to starvation, demonstrating that IRE1b regulates autophagy specifically in response to ER stress. IRE1b is a splicing factor that splices the mRNA encoding the bZIP transcription factor bZIP60. Spliced bZIP60 in turn activates expression of ER stress-response genes. To further characterize the IRE1b-dependent autophagy pathway, a mutant lacking the only known splicing target of IRE1b, bZIP60, was tested for autophagy induction. The *bzip60* mutant is capable of inducing autophagy in response to ER stress, suggesting that ER stress-induced autophagy does not rely on the splicing activity of IRE1b.

Although this evidence identified IRE1b as an upstream regulator of autophagy during ER stress in plants, the detailed regulatory pathway is still unclear. In yeast, ER stress-triggered autophagy is regulated by the endoribonuclease splicing activity of IRE1 toward mRNA encoding histone acetyltransferase of the CBP1 family (HAC1). In contrast, in animals ER stress-triggered autophagy is regulated by the kinase activity of IRE1 through the c-Jun N-terminal kinase (JNK) pathway, and not its splicing activity. Reminiscent of the situation in animals, ER stress-induced autophagy in Arabidopsis is also dependent on IRE1b function, but not its splicing activity toward bZIP60. However, the JNK pathway does not appear to exist in plants, which implies that either IRE1b has other splicing targets besides bZIP60, or IRE1b has unidentified functions in

addition to its splicing activity, possibly via an as yet unidentified kinase pathway. Future experiments are needed to address this issue.

In yeast, animals and plants, the target of rapamycin (TOR) kinase has been identified as an upstream negative regulator of autophagy. In animals, several studies showed that TOR may function to regulate ER stress responses; constitutive activation of TOR triggers ER stress, and ER stress induces autophagy through the inhibition of TOR activity. Studies in *Chlamydomonas reinhardtii* also suggested that the phosphorylation state of the binding protein (BiP) chaperone, a key component of the ER stress response, is regulated by TOR. Although these data implied an interaction between ER stress and TOR, how TOR senses ER stress and activates autophagy is still not clear. The potential interaction between TOR and IRE1b as upstream regulators of ER stress-induced autophagy should prove a fruitful area of future study.

Another intriguing question is how forming autophagosomes recognize the ER for degradation. Although autophagy has generally been considered to be nonselective, there is now considerable research showing that cargo can be selectively enclosed within autophagosomes. It is possible that during ER stress autophagosomes engulf fragmented ER membranes nonselectively. ER is not enclosed into autophagosomes during starvation-induced autophagy, and one possibility is that the fragmentation of ER during ER stress allows it to be incorporated into autophagosomes. It is also possible that ER fragments containing misfolded proteins are recognized by a selective autophagy cargo receptor. In both animals and plants, such cargo receptors have been identified, with the best studied being NBR1 (for neighbor of BRCA1 gene) and SQSTM1/p62 and their homologs. These receptors typically bind the autophagosomal membrane protein Atg8/LC3 via an Atg8-interacting motif in addition to interacting with the cargo, thus recruiting the cargo into autophagosomes, although the nature of the cargo is not yet known in plants.

In animals and yeast, several types of organelle-specific autophagy have been identified; for example, degradation of mitochondria and peroxisomes by selective autophagy-related mechanisms has been well studied. However, in plants, few examples of selective autophagy have been reported. Our study shows that the ER membranes can be degraded by autophagy, which is potentially another example of organelle-specific autophagy in plants, although the selectivity of the process has yet to be confirmed. We further identified IRE1b as an upstream regulator of ER stress-induced autophagy; the details of this regulatory pathway still need to be elucidated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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