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Epr1, a UPR-upregulated soluble autophagy receptor for reticulophagy

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ABSTRACT

The endoplasmic reticulum (ER) is a major site of protein folding. Perturbations in the folding capacity of the ER result in ER stress. ER stress triggers autophagic degradation of the ER (reticulophagy). Molecular mechanisms underlying ER stress-induced reticulophagy remain largely unknown. Our recent study identified a soluble protein, Epr1, as an autophagy receptor for ER stress-induced reticulophagy in the fission yeast *Schizosaccharomyces pombe*. Epr1 can interact simultaneously with Atg8 and a VAP family integral ER membrane protein, and thereby act as a bridging molecule between them. VAP family proteins contribute to reticulophagy by not only connecting Atg8 to the ER membrane through Epr1, but also by supporting the ER-plasma membrane contact. The expression of Epr1 is upregulated during ER stress in a manner dependent on the unfolded protein response (UPR) regulator Ire1. Ire1 promotes reticulophagy by upregulating Epr1.

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The endoplasmic reticulum (ER) is an essential organelle that supports protein and lipid biosynthesis, maintains ion homeostasis, and forms contact sites with other organelles. To ensure proper ER functions, cells need to efficiently detect and combat ER stress. ER stress is sensed by the sensor proteins of the unfolded protein response (UPR) signaling pathways. The UPR sensors, including Ire1, initiate downstream events that boost the ER's folding capacity and reduce the protein folding load of the ER.

A common cellular response to ER stress is the induction of reticulophagy. In the budding yeast *Saccharomyces cerevisiae*, ER stress induces micro-reticulophagy that depends on the ESCRT machinery. In mammals, ER stress induces macro-reticulophagy that requires the conventional autophagy machinery. Molecular mechanisms and physiological importance of ER stress-induced reticulophagy to a large extent remain unclear.

We have been using the fission yeast *Schizosaccharomyces pombe* as a model to study autophagy. Inspired by the discovery of budding yeast reticulophagy receptors through identifying Atg8-binding proteins, we applied affinity purification coupled with mass spectrometry (AP-MS) analysis to fission yeast Atg8. Among the Atg8-binding proteins identified by this analysis, the only ER-localizing protein is a previously uncharacterized protein that we named Epr1 in our recently published study [1]. We found that Epr1 is required for autophagic sequestration of ER membranes into phagophores during ER stress. As with other typical selective autophagy receptors, Epr1 interacts with Atg8 through an Atg8-interacting motif (AIM) in a disordered region. The AIM of Epr1 is required for its reticulophagy function.

Interestingly, different from dedicated reticulophagy receptors in budding yeast and mammals, which are transmembrane proteins, Epr1 is a soluble protein. To understand how Epr1 localizes to the ER, we resorted to AP-MS analysis again, and uncovered interactions between Epr1 and the vesicle-associated membrane protein-associated protein (VAP) family proteins Scs2 and Scs22. VAPs interact with soluble cytosolic proteins containing the FFAT motif and tether them to the ER membrane. We found that Epr1 possesses an FFAT motif, and this motif is required for VAP binding, for the ER localization of Epr1, and for the reticulophagy function of Epr1. We further demonstrated that a 42-amino-acid disordered region of Epr1, which harbors the AIM and the FFAT motif, is necessary and sufficient for reticulophagy. This 42-amino-acid fragment of Epr1 can interact simultaneously with Atg8 and Scs2 in vitro. In vivo, Epr1 can be bypassed by an artificial tether that connects Atg8 and VAPs. These results demonstrate that the main role of Epr1 in reticulophagy is to bridge an Atg8-VAP connection.

In fission yeast, VAPs are required for maintaining the ER-plasma membrane (PM) contact. Interestingly, this role of VAPs is required for reticulophagy, as VAPs can only be bypassed by introducing both an artificial tether linking Atg8 to the ER membrane and an artificial ER-PM tether. In the future, it will be interesting to identify the exact cellular event that contributes to reticulophagy in a manner that requires the ER-PM contact but does not require VAPs per se.

Loss of either Epr1 or VAPs results in reduced cell survival against chronic ER stress, and artificial tethers bypassing their reticulophagy functions can fully restore the survival,

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demonstrating the physiological significance of Epr1- and VAP-mediated ER stress-induced reticulophagy.

During our study, we found that the protein level of Epr1 is upregulated by ER stress, and this upregulation requires Ire1. Loss of Ire1 results in a severe defect in ER stress-induced reticulophagy, and this defect can be rescued by artificially increasing the expression level of Epr1. Thus, the main role of Ire1 in ER stress-induced reticulophagy is to upregulate Epr1. Intriguingly, unlike previously known regulatory targets of Ire1 in fission yeast, the upregulation of Epr1 does not appear to occur through the alteration of its mRNA. Further research will be needed to uncover how Ire1 exerts an effect on Epr1. Regardless of the exact mechanism, our finding unveils a molecular link between UPR signaling and ER stress-induced reticulophagy.

Disclosure statement

The authors declare no conflict of interest.

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