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UVRAG: orchestrating the initiation of reticulophagy

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ABSTRACT

Reticulophagy is a selective autophagy of the endoplasmic reticulum (ER) mediated by cargo receptors. It plays a crucial role in ER quality control, yet the mechanisms that initiate reticulophagy remain poorly understood. Our study identified the multifunctional protein UVRAG (UV radiation resistance associated gene) as a novel regulator of reticulophagy. UVRAG interacts with sheet and tubular reticulophagy receptors, regulates the oligomerization of receptors and facilitates their interaction with LC3/GABARAP, critical for ER fragmentation and autophagosome targeting. Remarkably, we found that UVRAG's function in reticulophagy initiation is independent of its traditional role in macroautophagy. Furthermore, UVRAG enhances the degradation of ER-associated mutant proteins linked to diseases like diabetes. Our findings offer insights into the mechanisms of reticulophagy initiation and highlight UVRAG's therapeutic potential in ER-related diseases.

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Autophagy is a cellular 'self-clearance' process that degrades superfluous or damaged organelles and proteins to recycle macromolecules, thus maintaining cellular homeostasis. Dysfunction in the endoplasmic reticulum (ER), a critical organelle, is associated with a range of diseases, including tumors, metabolic disorders, and neurodegenerative diseases. Reticulophagy is a selective process that degrades ER membrane and lumenal proteins via autophagy, playing a key role in ER quality control. However, the molecular mechanisms that initiate reticulophagy remain unclear.

To identify new genes with reticulophagy functions, we performed a genetic screen looking for genes whose overexpression can specifically promote reticulophagy. We observed that high expression of the UVRAG specifically enhances reticulophagy without affecting non-selective macroautophagy or other types of selective autophagy, such as mitophagy or aggrephagy. Immunoprecipitation mass spectrometry results indicated that UVRAG interacts with multiple reticulophagy receptors, which concentrate at ER subdomains to initiate reticulophagy. These results suggest that UVRAG likely regulates the initiation of reticulophagy through interactions with receptors.

Separate co-immunoprecipitation experiments revealed that UVRAG can interact with sheet reticulophagy receptors RETREG2/FAM134A, RETREG1/FAM134B, RETREG3/FAM134C, and tubular reticulophagy receptors ATL3 and RTN3L, but not TEX264, CCPG1, SEC62, or CALCOCO1. We then focused our research on the widely studied receptors RETREG1, ATL3, and RTN3L. Analysis through domain dissection showed that UVRAG binds to the cytoplasmic domains of RETREG1, ATL3, and RTN3L through its N-terminal proline-rich (PR) domain. This interaction does not depend on the receptor's LC3-interacting region/GABARAP-interacting motif (LIR/GIM), the essential domain necessary for receptor binding to LC3/GABARAP.

Early in cellular starvation, UVRAG is upregulated at both transcriptional and protein expression levels, enhancing its interaction with receptors. UVRAG colocalizes with WIPI2, an early autophagosome marker, and reticulophagy receptors in the ER. UVRAG knockout significantly reduces the colocalization of WIPI2 and receptors, suggesting that UVRAG is indispensable for the initiation of reticulophagy. Further mechanistic studies revealed that the absence of UVRAG impairs the oligomerization of these receptors, a process known to facilitate ER fragmentation by increasing membrane curvature. Conversely, elevated UVRAG levels enhance receptor oligomerization. Although UVRAG can bind to LIR/GIM mutated receptors, it does not promote oligomerization of these mutants, indicating that LC3/GABARAP's binding to receptors enhances their oligomerization. Furthermore, LC3/GABARAP's binding to receptors acts as a bridge connecting the ER to phagophores, initiating their formation at specific ER subdomains and providing an external force to promote ER fragmentation. Overexpressing UVRAG enhances the receptor's binding to LC3/GABARAP, whereas knocking out *UVRAG* hinders the starvation-induced increase in this binding. In summary, UVRAG promotes both receptor oligomerization and binding with LC3/GABARAP, leveraging this dual action to initiate reticulophagy.

UVRAG is often considered a subunit of the class III phosphatidylinositol 3-kinase complex II (PtdIns3KC3-II) in the macroautophagy pathway. To determine if UVRAG's role in initiating reticulophagy depends on its classical function, we created a UVRAG-6E mutant (L233E L240E L247E L251E L265E L272E), featuring mutations in the key amino acids of the coiled-coil (CC) domain that are essential for BECN1 interaction and PtdIns3KC3-II formation. While UVRAG-6E loses its ability to interact with BECN1, it retains the capacity to bind to reticulophagy receptors, effectively promoting their association with LC3/GABARAP and facilitating reticulophagy. Additionally, we

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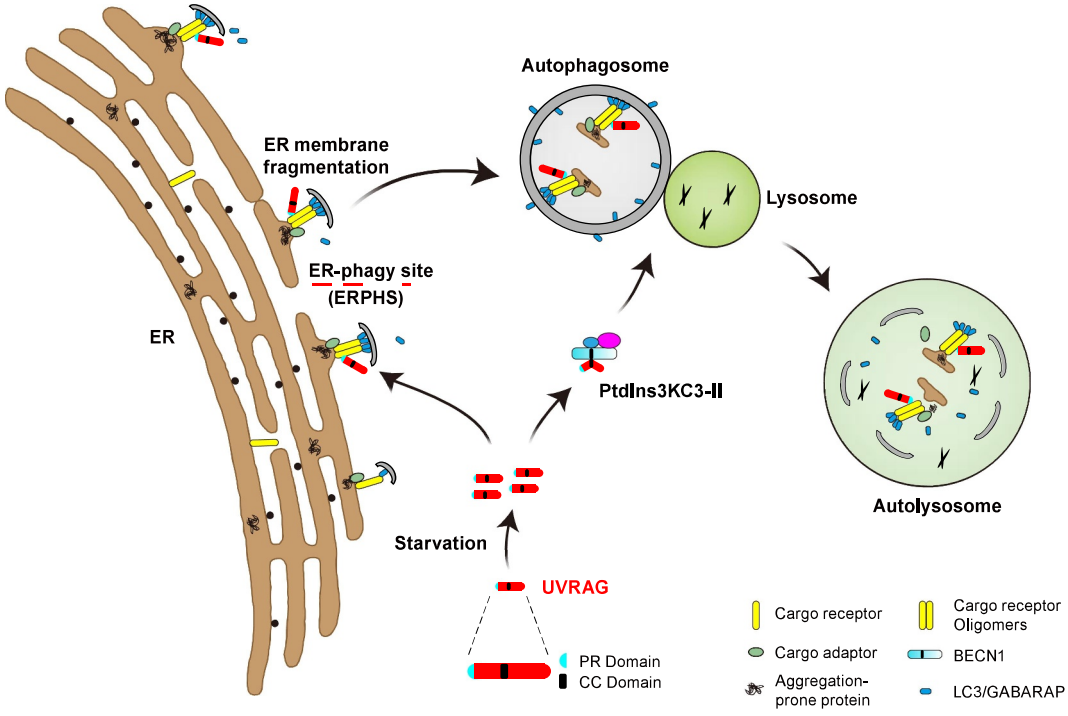

Figure 1. Schematic model describing dual functions of UVRAG in reticulophagy. To drive ER turnover through reticulophagy, UVRAG localizes to specific reticulophagy/ER-phagy sites (ERPHS), interacts with cargo receptors through its PR domain, enhances the recruitment of LC3/GABARAP to these receptors, and promotes the oligomerization of cargo receptors. This facilitates the scission of ER membranes and promotes the engulfment of ER fragments by phagophores. In the later stages, UVRAG binds with BECN1 via its CC domain to form PtdIns3KC3-II, promoting the maturation of ER-containing autophagosomes and their fusion with lysosomes.

discovered that the prolines within the PR domain of UVRAG are crucial for its interaction with reticulophagy receptors. Although UVRAG-PR mutants cannot restore reticulophagy in *UVRAG* knockout cells, they are capable of rescuing macroautophagy. Therefore, our study provides evidence that UVRAG's regulation of reticulophagy initiation via the PR domain is distinct from its classical role in macroautophagy.

Based on these findings, we propose that UVRAG has two distinct functions in reticulophagy (Figure 1) [1]. On the one hand, it orchestrates a series of crucial events during the assembly of the reticulophagy/ER-phagy sites (ERPHS): UVRAG binds to reticulophagy receptors, promotes receptor oligomerization, and enhances LC3/GABARAP recruitment, leading to ER fragmentation and its subsequent targeting into phagophores for degradation. This function in reticulophagy initiation is distinct from UVRAG's role as a PtdIns3KC3-II subunit. On the other hand, UVRAG, through its CC domain, interacts with BECN1 to form PtdIns3KC3-II, which promotes the fusion of ER-containing autophagosomes with lysosomes.

In summary, our study identifies UVRAG as a versatile ER quality controller, which interacts with both sheet and tubular reticulophagy receptors, thus regulating the autophagic degradation of various types of ER, maintaining its homeostasis. Second, UVRAG is a multifunctional protein regulating many important cellular processes. Our study characterizes a novel function of UVRAG in reticulophagy initiation, one that is surprisingly independent of its canonical role as a subunit of PtdIns3KC3-II. Third, our further study on the pathological significance of the UVRAG-mediated reticulophagy process found that it aids in clearing aberrantly accumulated *Akita* mutant precursor of insulin within the ER, which has been closely linked to early-onset diabetes.

Future work will be needed to uncover: 1) the specific signals that increase UVRAG transcription; 2) how UVRAG is directed to reticulophagy sites; 3) the basis of UVRAG's preference for different cargo receptors; 4) the process by which UVRAG promotes the oligomerization of its interacting receptors. Ultimately, finding ways to harness UVRAG-mediated reticulophagy to treat diabetes and related protein aggregation diseases is a key future step.

Disclosure statement

The authors have no conflicts of interest to disclose.

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