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Receptors make the pathway choice for protein degradation

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ABSTRACT

Damaged or aggregated proteins and organelles accumulate with age and contribute to various age-related pathologies including Alzheimer, Parkinson or Huntington diseases. In eukaryotic cells, there are 2 major pathways for degradation of the cytoplasm: The ubiquitin–proteasome system (UPS) and macroautophagy/autophagy. Both pathways can share the characteristic of initiating the process by ubiquitination of the substrate, but they utilize different ubiquitin receptors. In a paper described in a punctum in this issue, Lu et al. used the yeast *Saccharomyces cerevisiae* to demonstrate that the decision to use a particular pathway is made through a mechanism that depends on the receptors rather than the specific type of substrate ubiquitination.

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It has been proposed in previous studies that the choice of which degradation pathway to use is made based on different ubiquitin chain linkages.[1] For example, Lys63-linked ubiquitin chains typically promote autophagic degradation, whereas the Lys48 linkage is the signal for targeting to the UPS.[2,3] However, Lu et al. proposed that this pathway choice is made after substrate ubiquitination.[4] To understand the determinant, they first tested the substrate specificity of the ubiquitin receptors Dsk2 and Cue5, which function in the UPS and autophagy, respectively, by looking at the ubiquitination of heat-induced abnormal proteins and the short-lived UPS model substrate ubiquitin (Ub)-gal. They found that ubiquitinated soluble proteins are mainly recognized by Dsk2 and targeted to the proteasome. In contrast, the insoluble protein aggregates are recognized by Cue5 and targeted for autophagic degradation. Similarly, the aggregation-prone protein TARDBP/TDP-43 that is implicated in amyotrophic lateral sclerosis colocalizes predominantly with Cue5 but not Dsk2.

Moreover, both soluble and insoluble abnormal proteins are stable in cells lacking Ubc4 (one of the main E2 ubiquitin-conjugating enzymes) or Rsp5 (an E3 ubiquitin ligase), components that are traditionally associated with the UPS. Lu et al. also showed that Lys63-linked ubiquitin chains are not the distinguishing mark for autophagy because the ubiquitination and degradation pattern of abnormal proteins, including TARDBP and the human HTT (huntingtin)-related construct HTT103Q, are the same in wild-type cells as those expressing Ub^{K63R}. These findings indicate that both receptors, Dsk2 and Cue5, can recognize misfolded proteins, but depending on which receptor binds, the substrates are targeted to different pathways. Accordingly, the authors hypothesized that both autophagy and the UPS share the same ubiquitination machinery for modifying their target substrates. They confirmed this hypothesis by co-immunoprecipitation of substrates with Dsk2 or Cue5 and also found that these 2 receptors compete for substrate. Isothermal titration calorimetry was next used to analyze ubiquitin-binding affinities; Dsk2 displays a significantly higher affinity for Ub compared with Cue5 for various types of ubiquitin chains, but neither exhibits a specific preference for polyUb chains composed of Lys48 or Lys63 in vitro.

In addition to this difference in ubiquitin-binding affinities, the authors discovered that Cue5 displays a strong capacity for self-interaction, mediated by its CUE domain similar to other CUE-domain proteins, which is critical for ubiquitin binding.[5] This interaction allows Cue5 to assemble into higher-order oligomers, a feature that is seen with other autophagic receptors that bind Ub, including SQSTM1/ p62 and NBR1; this oligomerization is important for promoting stable binding of the receptor to Ub and LC3/Atg8.[6]

Finally, the authors used a synthetic biology approach to determine the minimal requirements of each receptor; this method allowed them to overcome possible in vivo differences between targeting to the UPS or autophagy. Three sets of synthetic receptors were engineered with the first set containing a Ub-like domain that is used for proteasome targeting, the second containing an Atg8-interacting motif for autophagy, and the third harboring both motifs and thus having the potential to enter either pathway. Additionally, a segment of FKBP (FK506 binding protein), was included in all 3 sets of receptors; the wild-type FKBP domain is monomeric, but a mutant version is dimeric. The authors expressed these synthetic receptors in yeast cells lacking Dsk2 and Cue5 and examined the degradation of the UPS substrate Ub-gal. They found that oligomeric receptors that are targeted into the UPS pathway are deficient in soluble protein degradation; however, oligomeric receptors targeted for autophagy support the degradation of insoluble proteins. Furthermore, only the oligomeric receptor is able to clear heat-induced aggregated proteins by autophagy to the

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level seen with an endogenous receptor. In addition, cells expressing the oligomeric synthetic receptor exhibit resistance to TARDBP-induced toxicity, similar to the result seen with Cue5, and this receptor colocalizes to the protein aggregates that contain TARDBP. Based on these data, the authors conclude that receptor oligomerization instead of substrate modification is necessary for autophagic targeting. Thus, with regard to protein quality control, ubiquitination is a general signal that leads to degradation via the UPS or autophagy. This mechanism allows increased flexibility with regard to the different types of stress a cell might encounter, and also provides a greater option for crosstalk between these 2 degradative pathways.

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