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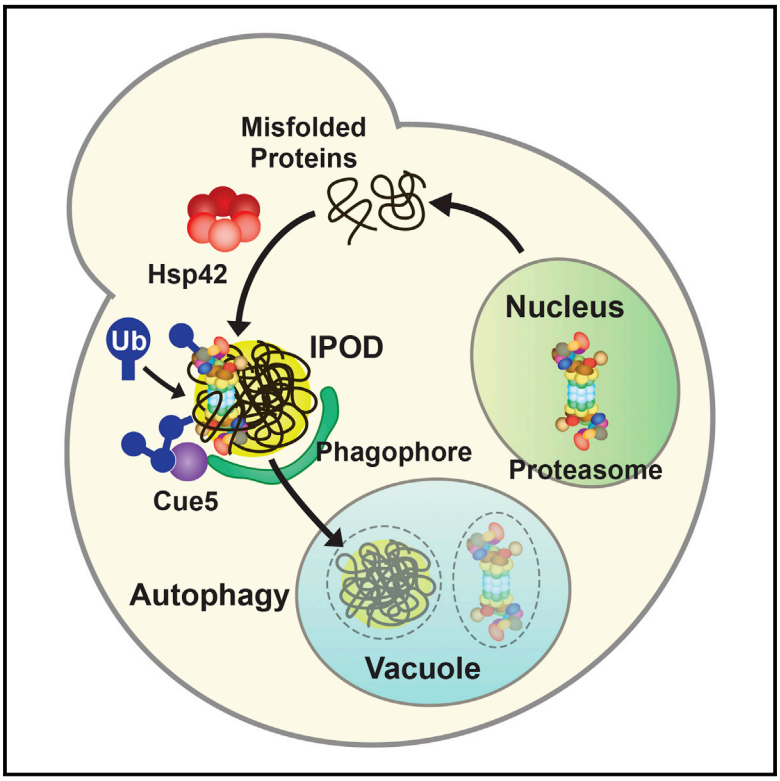
# Autophagic Turnover of Inactive 26S Proteasomes in Yeast Is Directed by the Ubiquitin Receptor Cue5 and the Hsp42 Chaperone

## Graphical Abstract

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## In Brief

Marshall et al. find that 26S proteasomes are degraded by autophagy in yeast, a process stimulated by inactivation or nitrogen starvation. Proteasome inhibition is accompanied by both Hsp42-mediated aggregation and ubiquitylation of the complex, which is then targeted to autophagic membranes by the ubiquitin binding autophagy receptor Cue5.

## Highlights

- The yeast 26S proteasome is degraded by Atg8-mediated autophagy
- Nitrogen starvation and inactivation stimulate proteaphagy via distinct pathways
- Proteasome inhibition is accompanied by extensive ubiquitylation of the complex
- Proteaphagy engages the Cue5 autophagy receptor and the Hsp42 chaperone

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# Autophagic Turnover of Inactive 26S Proteasomes in Yeast Is Directed by the Ubiquitin Receptor Cue5 and the Hsp42 Chaperone

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## SUMMARY

**The autophagic clearance of 26S proteasomes (proteaphagy) is an important homeostatic mechanism within the ubiquitin system that modulates proteolytic capacity and eliminates damaged particles. Here, we define two proteaphagy routes in yeast that respond to either nitrogen starvation or particle inactivation. Whereas the core autophagic machineries required for Atg8 lipidation and vesiculation are essential for both routes, the upstream Atg1 kinase participates only in starvation-induced proteaphagy. Following inactivation, 26S proteasomes become extensively modified with ubiquitin. Although prior studies with *Arabidopsis* implicated RPN10 in tethering ubiquitylated proteasomes to ATG8 lining the autophagic membranes, yeast proteaphagy employs the evolutionarily distinct receptor Cue5, which simultaneously binds ubiquitin and Atg8. Proteaphagy of inactivated proteasomes also requires the oligomeric Hsp42 chaperone, suggesting that ubiquitylated proteasomes are directed by Hsp42 to insoluble protein deposit (IPOD)-type structures before encapsulation. Together, Cue5 and Hsp42 provide a quality control checkpoint in yeast directed at recycling dysfunctional 26S proteasomes.**

## INTRODUCTION

Constant re-modeling of proteomes is critical for developmental transitions, maintenance of cellular homeostasis in response to environmental challenges, and robust nutrient recycling. In eukaryotes, these adjustments are mainly performed by two proteolytic routes, the ubiquitin-26S proteasome system (UPS) and autophagy. Together, they direct the turnover of a wide array of targets, ranging from single proteins whose control is necessary for proper growth and development, to whole organelles when they become defective or unnecessary. The UPS consists of a highly polymorphic enzymatic cascade that attaches multiple ubiquitins to selected target proteins, which enables their recognition and subsequent degradation by the 26S proteasome (Bhattacharyya et al., 2014; Finley et al., 2016; Lu et al., 2015). In contrast, autophagy is uniquely designed to eliminate larger structures, which are encapsulated and delivered in bulk from the cytoplasm to either vacuoles (plants and fungi) or lysosomes (mammals) for breakdown (Klionsky and Schulman, 2014; Reggiori and Klionsky, 2013).

Autophagy occurs continuously at a basal level, but is upregulated when extensive recycling is required, such as during nutrient starvation or programmed cell death. It is initiated at the phagophore assembly site (PAS), where a collection of factors builds the engulfing phagophore membrane, which then seals to trap cargo within a double membrane-bound autophagosome (Lamb et al., 2013; Reggiori and Klionsky, 2013). Autophagosomes fuse with the limiting membrane of the vacuole to release the internal vesicle as an autophagic body, which is then eliminated by resident hydrolases. Central to this process is the ubiquitin-fold protein Atg8 (or LC3), which becomes modified at its C terminus with phosphatidylethanolamine (PE) via a conjugation cascade analogous to ubiquitylation. The Atg8-PE adduct decorates the expanding phagophore, thus providing docking sites for proteins that promote vesicle closure and for receptors that recruit specific cargo. By virtue of an expansive list of such receptors, autophagy can selectively remove large protein complexes, insoluble aggregates, whole organelles, and even invading intracellular pathogens (Khaminets et al., 2016; Lu et al., 2014; Mochida et al., 2015; Rogov et al., 2014; Sica et al., 2015).

Although the UPS and autophagy were initially thought to operate independently, recent work has revealed considerable overlap between the two systems. In particular, many targets of selective autophagy first require ubiquitylation, which allows their recognition by receptors that simultaneously bind both Atg8 and ubiquitin through an Atg8-interacting motif (AIM) and various ubiquitin-binding domains, respectively. Examples include optineurin and NDP52, which are recruited to both ubiquitylated pathogens and mitochondria (Lazarou et al., 2015; Wild et al., 2011), p62/SQSTM1 and NBR1, which bind to various ubiquitylated cargo including protein aggregates, pathogens, and peroxisomes (Khaminets et al., 2016; Rogov et al., 2014), and Cue5 from the yeast *Saccharomyces cerevisiae* and its mammalian counterpart Tollip, which bind ubiquitylated protein