Ubiquitin-Independent Degradation Of Lysosomal Transporter Proteins By The Intralumenal Fragment (ILF) Pathway

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Lysosomes are tasked with recycling biomaterials in all eukaryotic cells. Essential for this activity are transporter proteins embedded in lysosome membranes that export lumenal catabolites to the cytoplasm for reuse by the cell. By mobilizing an important nutrient store, they are critical mediators of metabolism and proteostasis. However, we know relatively little about lysosome transporter physiology. Using Saccharomyces cerevisiae and its lysosomal vacuole as models, our group recently discovered a new process for selective transporter protein degradation called the intralumenal fragment (ILF) pathway: In response to stimuli (e.g. TOR kinase activation, unfolding), some proteins are recognized and sorted into membrane microdomains that are internalized into the lumen and degraded upon vacuole fusion. Although independent of ESCRTs and the autophagy machinery, how proteins are labelled or selected for degradation by the ILF pathway remains unknown. The objective of this study was to uncover the mechanisms responsible by first determining if protein ubiquitylation machinery is involved. We studied the membrane-bound vacuolar iron-oxidase Fet5, which is degraded in response to TOR kinase activation by cycloheximide (CHX). Based on western blot analysis, GFP-tagged Fet5 did not show a migration pattern indicative of ubiquitylation prior to degradation triggered by CHX. We next performed a coimmunoprecipitation of Fet5-GFP and HA-tagged ubiquitin, and results suggested that Fet5 is not directly ubiquitylated. In support, introducing a K603R mutation to prevent ubiquitylation of the only lysine present in the cytoplasmic domain of Fet5 had no effect on its degradation in response to CHX. However, Fet5 degradation was blocked when we deleted the entire cytoplasmic domain suggesting a cytoplasmic mechanism is involved. Cytoplasmic adapter proteins, such as Ssh4, are implicated in binding transporter proteins to mediate their ubiquitylation by E3 ligases for degradation. But some reports suggest adapter proteins can drive cargo protein degradation without their ubiquitylation. Thus, we studied a small set of yeast strains missing genes encoding adaptor proteins and found that Fet5-GFP degradation by the ILF pathway in response to CHX requires the adapter protein SSH4. Moreover, this requirement is specific to both stimulus and cargo protein, as deleting SSH4 had no effect on Fet5-GFP degradation after heat stress (to trigger unfolding), nor did it affect degradation of GFP-tagged Fth1, a vacuole iron transporter, after CHX treatment. Based on these results, we conclude that some lysosome transporter proteins are recognized by adaptor proteins but are not directly ubiquitylated prior to being selectively degraded by the ILF pathway.