

**E3-ubiquitin ligases and adaptor proteins mediate
lysosome/vacuole nutrient transporter protein degradation by the ILF pathway**

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Lysosomes are tasked with recycling biomaterials in all eukaryotic cells. To perform this function, they rely on nutrient transporter proteins to return products of catabolism to the cytoplasm for reuse by the cell. But we know very little about lysosomal transporter physiology, including their regulation and degradation. Using *S. cerevisiae* and its lysosomal vacuole as models, our group recently discovered a process called the IntraLumenal Fragment (ILF) pathway that selectively degrades lysosomal transport proteins in response to stimuli: These polytopic proteins are sorted into an area of membrane encircled by fusion machinery that forms the boundary between docked vacuoles. Upon membrane fusion, the protein-laden boundary membranes are internalized as an intraluminal fragment and degraded by hydrolases. But how these proteins are labelled for degradation by the ILF pathway remains unknown. The objective of this study was to determine if the protein ubiquitylation machinery labels proteins for degradation by ILF pathway. We first screened yeast strains missing genes encoding E3-ubiquitin ligases (e.g. PIB1) or adaptor proteins (e.g. SSH4, PIB1) to identify mutations that abolished sorting and degradation of GFP-tagged ILF client proteins Fth1 (an iron transporter) or Fet5 (a copper oxidase) after misfolding by heat stress. Using HILO microscopy, we found that Fth1-GFP sorting into boundary membranes after heat stress requires YDJ1 and PIB1, whereas Fet5-GFP sorting requires SSH4, and demonstrate that these proteins no longer decorate ILFs formed during vacuole fusion events in live mutant cells, blocking their degradation in vivo. Although needed for Fth1-GFP degradation after heat stress, YDJ1 was not required for degrading misfolded Fet5-GFP, and does not mediate Fth1-GFP degradation in response to other stimuli, suggesting that ubiquitylation by YDJ1 is protein- and stimulus- specific. If not cleared, misfolded polytopic proteins aggregate and promote lysosome membrane permeability (LMP) and cell death. Consistent with other results, we find that deleting YDJ1 or PIB1 promotes LMP and cell death after misfolding was stimulated by heat stress. All things considered, we conclude that ubiquitylation by the E3-ligase PIB1 and adapter proteins YDJ1 and SSH4 mediates lysosomal nutrient transporter protein labeling required for degradation by the ILF pathway.