Ubiquitin-independent degradation of nutrient transporter proteins by the ILF pathway

Tom Kazmirchuk, Erin. K. McNally, Ziheng Jiang, and Christopher. L. Brett

Lysosomes are tasked with recycling biomaterials in all eukaryotic cells. To perform this function, lysosomal nutrient transporter proteins mobilize lumenal products of catabolism for reuse by the cell. But we know very little about lysosomal transporter physiology. Using *S. 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: Polytopic proteins are sorted into a "boundary" area of membrane encircled by fusion machinery assembled between docked vacuoles. Upon organelle fusion, protein-laden boundary membranes are internalized as an intralumenal fragment and degraded by lumenal hydrolases. But how these proteins are labelled for selective degradation by the ILF pathway remains unknown. The objective of this study was to determine whether or not ubiquitylation machinery labels nutrient transporters for degradation by ILF pathway. We first screened yeast strains missing genes encoding E3-ubiquitin ligases (e.g. PIB1), adaptor proteins (e.g. SSH4) or chaperone proteins (e.g. YDJ1) to identify mutations that abolished sorting, internalization, and degradation of GFP-tagged ILF-client proteins Fth1 (an iron transporter) or Fet5 (an iron oxidase) after misfolding by heat shock (HS) or treatment with cycloheximide (CHX). Using a HILO microscopy and western blot analysis, we found that Fth1-GFP sorting and degradation triggered by HS requires YDJ1, whereas Fet5-GFP requires PIB1. In response to CHX, Fet5 sorting and degradation depends on SSH4. Next, we introduced a K603R mutation to prevent possible ubiquitylation of the only lysine present in the cytoplasmic C-terminal domain of Fet5. We found that K603 is dispensable for Fet5 degradation triggered by HS or CHX. Whereas deleting the entire cytoplasmic domain of Fet5 prevented its degradation by CHX, but not by HS. If not cleared, polytopic proteins misfolded by HS can aggregate promoting lysosome membrane permeability (LMP) and cell death. Consistent with other results, deleting YDJ1 or PIB1 promotes LMP and cell death after HS. All things considered, we conclude that ILF client protein labeling for degradation is stimulus-dependent: For HS, we speculate that degradation is mediated by chaperones, such as Ydj1. Whereas for CHX, adaptor proteins such as Ssh4 are required. However, neither seem to require ubiquitylation for nutrient transporter protein degradation by the ILF pathway.