Non-selective autophagy is like a storm to a cell. Organelles and cytosolic contents are swept up and packaged into double-membrane vesicles called autophagosomes, delivered to lysosomes/vacuoles, and degraded so component amino acids can be reclaimed for the synthesis of essential new proteins. Multiple nutritional stresses can trigger this storm, including depletion of amino acids, nitrogen, and glucose, but the extent to which other cellular metabolites serve as signals for induction of autophagy remains unclear.

Zinc is an important cellular nutrient that plays structural and functional roles in proteins in all organisms (1, 2). As a result, cells have mechanisms to maintain zinc homeostasis when available zinc supplies decrease; in particular, the transcription factor Zap1, the major zinc sensor, responds to limited zinc by increasing the expression of genes such as transporters that increase zinc influx. Autophagy has also been implicated in the cellular response to zinc starvation. In mammalian cells, zinc depletion, for example by zinc chelation, causes a significant suppression of autophagy whereas zinc addition stimulates autophagy in human hepatoma cells across several stimuli (3–7). Although the forecast for other species might be expected to follow this precedent, Kawamata et al. (8) now report the unexpected finding that zinc starvation triggers autophagy in yeast.

Kawamata et al. (8) demonstrate that autophagy is robustly activated upon zinc starvation using zinc-depleted media as shown by two autophagic markers, GFP-atg8 and API. Although the time course of activation is delayed compared with the prompt induction of autophagy upon nitrogen starvation, the magnitude of the two responses is comparable. The zinc chelator TPEN (N,N,N,N-tetrakis-[2-pyridyl-methyl]ethylenediamine) also induces autophagy, consistent with zinc starvation causing the observed phenotype. The authors conclude that zinc depletion-induced autophagy is an effort of yeast cells to rescue themselves from zinc starvation–induced growth retardation, as this growth retardation is much worse when autophagy is blocked.

The authors provide several additional lines of evidence to confirm that autophagy is caused by a shortage of zinc directly rather than via indirect effects on other nutrients. First, zinc starvation specifically decreases zinc concentrations without lowering the amounts of other divalent cations such as iron and copper. Second, the level of glucose was increased in the zinc-free medium. Third, complementation of glucose or other nutrients had no effect on zinc depletion–triggered autophagy. Finally, induction of autophagy by zinc starvation was reversed by adding zinc to the cellular medium. All these data indicate that zinc is a unique metal ion that can induce autophagy as profoundly as amino acids.

The authors next questioned whether autophagy selectively degraded zinc-binding proteins, which might resolve the zinc deficit most efficiently, or alternatively captured a general cross-section of cellular contents through a non-selective process. The answer was rather surprising: Kawamata et al. (8) tested a series of strains with proteins from autophagy pathways knocked out and found that zinc starvation–induced autophagy relies on the essential non-selective autophagy protein ATG2 and key autophagic degradation proteins Pep4/Prb1, but not the selective autophagy proteins ATG11/ATG19. They also observed the accumulation of ribosomes and mitochondria in autophagic vacuoles by electron microscopy. Moreover, TORC1, known to negatively regulate non-selective autophagy by phosphorylating Atg13, is inhibited upon zinc starvation-induced autophagy, whereas constitutively active TORC1 delayed autophagy. Based on these observations, the authors concluded that zinc depletion induces autophagy non-selectively. The authors also examined whether the canonical zinc sensor, Zap1, might be the link between zinc status and TORC1 function; surprisingly, Zap1 is not required for zinc starvation–induced autophagy. Thus, the zinc sensing mechanism that controls TORC1 remains to be determined.

Even though the overall process was determined to be non-selective, the authors wondered whether zinc-binding proteins might still be preferred targets for degradation. Indeed, the authors provided some evidence to support this notion. For example, electron microscopy data showed that large quantities of ribosomes, a rich zinc source since each complex binds at least eight zinc ions, were sequestered in autophagic bodies. A closer examination of GFP-tagged ribosomal proteins RPL37A and RPL37B confirmed they are degraded by autophagy under zinc starvation. However, the authors pointed out that...
autophagic degradation is not limited to zinc-binding proteins, as the glycolytic protein Pgk1, which does not bind zinc, also was degraded in these experiments. As the authors did not exhaustively report on the proteins targeted by zinc depletion-induced autophagic degradation, it remains unclear whether the recruitment of zinc-binding proteins or organelles to autophagosomes uses zinc-specific adaptors in a mechanism akin to selective autophagy. Substrate profiles might also vary based on the stimulus employed. It will be interesting to see what large-scale quantitative proteomics might reveal along these lines in future studies.

These combined data lead the authors to suggest a model for zinc starvation-induced autophagy, which proposes that the purpose of this process is to increase the level of free zinc in cells (Fig. 1). To provide initial support for this proposal, the authors used Pho8, a vacuolar enzyme whose activity and concentration are controlled by zinc status, as a reporter of zinc release. They discovered that Pho8 levels were lower in an autophagy-deficient mutant than wild-type cells, consistent with the notion that the intracellular zinc level is increased by autophagy. However, additional data are required to further support this conclusion.

This study provides a potential mechanism through which the storm of autophagy releases zinc from proteins, increasing the level of free ions for use in other crucial contexts. It is not entirely clear whether such a scenario also occurs in mammals. The potential role and biochemical mechanisms of autophagy in zinc homeostasis need to be further investigated in mammalian cells and yeast. Nevertheless, this study opens new avenues to study the larger climate connecting zinc and autophagy.

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