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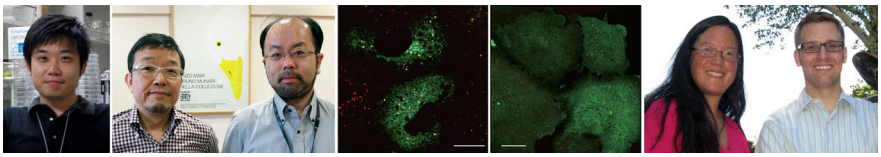
Casting the autophagy net

Two studies investigate how cells regulate the formation of autophagosomes.

Focal Point



Two groups examine how cells regulate formation of the autophagosome, the double-membraned vesicle that engulfs cytoplasmic components and targets them to the lysosome for degradation. (Left to right) Kohichi Matsunaga, Tamotsu Yoshimori, Takeshi Noda, and colleagues determine that Atg14L targets a PI3-kinase complex to the ER, where it generates the phosphoinositide PI3P to initiate autophagosome biogenesis. Overexpression of Atg14L (green) induces autophagosome (red) production (left), whereas an ER-localization mutant does not (right). Meanwhile, (right photo) Charleen Chu, Sam Cherra, and co-workers find that LC3—a protein required to expand and close autophagosomes after they're initiated—is kept in check by protein kinase A. LC3 is dephosphorylated upon autophagy induction, boosting its incorporation into autophagosome membranes.

Cells can degrade cytoplasmic components by capturing them inside a double-membraned vesicle called the autophagosome that then delivers them to lysosomes to be broken down and recycled. This process, known as autophagy, occurs at basal levels in all cells, and is upregulated in response to starvation or other stresses as a cell survival mechanism. But autophagy is a double-edged sword that can damage the cell if left unchecked, so the process must be carefully regulated. Two papers now address how cells control two key stages of autophagosome biogenesis (1, 2).

The first step in autophagy is the specification of the autophagosomal membrane. Although the site of this event is controversial, it may involve generating the phosphoinositide PI3P to form a subdomain of the ER called the omegasome that gives rise to the autophagosome (3). "Phosphoinositides often serve as landmarks within the cell," says Takeshi Noda from Osaka University in Japan. "Autophagy is a great example of this concept where PI3P triggers autophagosome formation."

Recently, Tamotsu Yoshimori's group, including Noda, were one of several labs to identify a mammalian PI3-kinase complex required for autophagy (4). To investigate whether this complex could generate PI3P at the ER, the researchers focused on Atg14L (1), the only subunit of the PI3-kinase exclusively involved in autophagy (the other subunits having other cellular functions as well).

> **"LC3 phosphorylation can modulate the pathway's response to injury."**

Knocking down Atg14L impaired omegasome formation in starved cells, whereas overexpressing the protein induced omegasomes and autophagosomes, even in nutrient-rich conditions. Atg14L localized to the ER and accumulated in omegasomes upon autophagy induction. Matsunaga et al. identified four N-terminal cysteines that localize Atg14L to the ER. When these residues were mutated, the protein could no longer induce autophagosomes nor restore autophagy to cells lacking wild-type Atg14L. "Then we added an exogenous ER-targeting motif to mutant Atg14L, and that recovered the protein's autophagic potency," Noda explains.

Atg14L thus targets the PI3-kinase complex to the ER, where it generates the PI3P required for omegasome and autophagosome formation. "Our paper strengthens the ER and omegasome model, though mitochondria have also been implicated as the site of autophagosome formation," Noda says. "But these two models might not be mutually exclusive."

Wherever autophagosome membranes initiate, they then expand to engulf cytoplasmic components. The protein LC3 is essential for this step and targets to autophagosome membranes by covalently linking to phospholipids (5).

"Almost everything that induces macroautophagy promotes LC3 lipidation," says Charleen T. Chu from the University of Pittsburgh. "It defines the pathway's activation." Little is known about how stress signals link directly to this critical step, but Chu and her team found that LC3 is phosphorylated by protein kinase A (PKA), and that this phosphorylation is reduced upon autophagy induction (2).

Phosphorylated LC3 was largely nonlipidated and not incorporated into autophagic membranes. However, a mutation in LC3 that blocked its phosphorylation by PKA caused an increase in the protein's lipidation and membrane association. LC3 phosphorylation might therefore act as a brake on autophagy to limit the pathway's degradative capacity. The authors propose that dephosphorylation mobilizes a reserve LC3 pool to meet increased autophagic needs, but certain stimuli can release this brake with devastating consequences. The neurotoxin MPP^+ and a mutant version of the kinase LRRK2 can both induce autophagy in neurons, causing neurite shortening that might contribute to Parkinson's disease. Cherra et al. found that an LC3 mutant mimicking PKA phosphorylation blocked neurite retraction induced by MPP^+ and LRRK2, as did activation or overexpression of PKA.

"LC3 phosphorylation doesn't block the essential housekeeping functions of basal autophagy," says Chu. "But it can modulate the pathway's response to injury, perhaps by affecting LC3's interaction with the lipidation machinery." This would alter LC3's ability to expand autophagosomes, though Chu wonders whether the protein also has additional functions in neurons, which express the protein at particularly high levels.

1. Matsunaga, K., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200911141.
2. Cherra, S. J., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201002108.
3. Axe, E.L., et al. 2008. *J. Cell Biol.* 182:685–701.
4. Matsunaga, K., et al. 2009. *Nat. Cell Biol.* 11:385–396.
5. He, C., and D.J. Klionsky. 2009. *Annu. Rev. Genet.* 43:67–93.