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Autophagy and innate immunity ally against bacterial invasion

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The precise mechanisms by which autophagy participates in the control of intracellular infection have only lately begun to emerge. In a recent issue of *Science*, Wild *et al* demonstrate how innate immunity and autophagy cooperate in the clearance of cytosolic *Salmonella*, thereby shedding new light on the molecular regulation of xenophagy.

Macroautophagy (hereby referred to as autophagy) is a finely tuned, evolutionary conserved pathway whereby intracellular components are sequestered within double-membraned organelles (autophagosomes) and delivered to lysosomes for bulk degradation. Although autophagy may occasionally facilitate cell death (Kroemer *et al*, 2007), most often autophagy functions as a stress-inducible cytoprotective mechanism (Kroemer *et al*, 2010). Moreover, baseline levels of autophagy contribute to cellular homeostasis (thus exerting anti-ageing and oncosuppressive functions) by limiting the accumulation of aggregate-prone proteins or damaged organelles (Green *et al*, 2011). Of note, whereas starvation-induced autophagy occurs in a rather unselective manner, cells can respond to specific types of stress by activating highly selective autophagic pathways including mitophagy and xenophagy, which target mitochondria and invading pathogens, respectively (Kraft *et al*, 2010). Although the

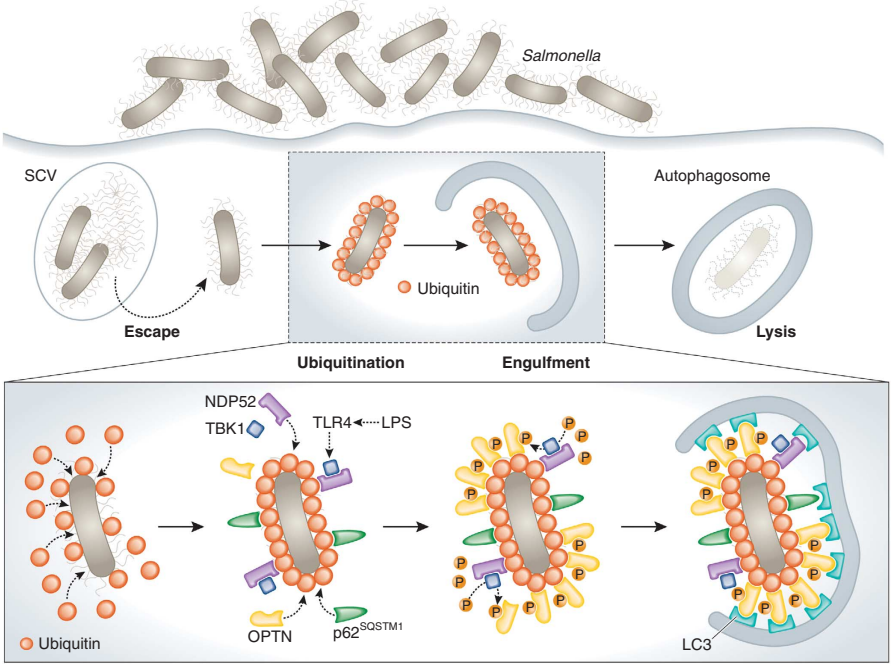

Figure 1 TBK1-phosphorylated optineurin targets intracellular bacteria to xenophagic degradation. Upon infection, most *Salmonella* are sequestered into *Salmonella*-containing vacuoles (SCVs), but some can escape from SCVs and proliferate in the cytosol. To prevent this, cytosolic bacteria are rapidly ubiquitinated, leading to the recruitment of several autophagic receptors including NDP52 and $p62^{SQSTM1}$ as well as that of the TANK-binding kinase 1 (TBK1). Until recently, the mechanism by which TBK1 would limit bacterial proliferation was not fully understood. Now, it has been discovered that, in response to bacterial products such as lipopolysaccharide (LPS), the pattern recognition receptor Toll-like receptor 4 (TLR4) activates TBK1 leading to the phosphorylation of another autophagic receptor, optineurin (OPTN), on Ser177. Phosphorylated OPTN has a high affinity for the autophagic protein LC3, thereby guiding (together with NDP52 and $p62^{SQSTM1}$) ubiquitinated bacteria to the autophagic machinery and allowing for their elimination by xenophagy.

underlying molecular mechanisms have only recently begun to emerge, selective autophagy appears to rely on a set of cytoplasmic receptors that link specific cargoes to autophagosomes. In a recent issue of *Science*, Wild *et al* (2011) have shed new light on this issue by describing how optineurin (OPTN), an ubiquitin-binding protein implicated in the pathogenesis of glaucoma, can function as an autophagic receptor at the crossroad between innate immunity and xenophagy.

Xenophagy participates in the first-line defence against viral, bacterial and parasitic infections, and defects in autophagy/xenophagy reportedly result in increased susceptibility to infectious diseases. In line with this notion, invading pathogens have evolved multiple mechanisms for avoiding xenophagic elimination including autophagy-inhibitory proteins and strategies for escaping autophagosomes and lysosomes (Deretic, 2011). However, how invading pathogens would be selectively recognized and targeted to degradation by the xenophagic machinery has remained largely obscure until recently, when it was discovered that components of the innate immune system physically interact with proteins from the autophagic machinery, thus directing the formation of autophagosomes to bacterial entry sites or targeting ubiquitinated bacteria to autophagic degradation (Thurston *et al*, 2009; Galluzzi *et al*, 2010; Travassos *et al*, 2010).

Now, Ivan Dikic's group has added one important piece to this puzzle by outlining how Toll-like receptor 4 (TLR4), a pattern recognition receptor involved in innate immunity, can elicit an OPTN-dependent signalling cascade for the elimination of *Salmonella* that escape intracellular vacuoles (Wild *et al*, 2011). Wild *et al* discovered that OPTN can bind the essential autophagic protein LC3 via an N-terminal LC3-interacting region, thus physically bridging ubiquitin-coated cytosolic *Salmonella* to nascent autophagosomes and favouring its xenophagic clearance. Thus, OPTN acts as a novel autophagic receptor. Subsequent *in silico* studies led Wild *et al* (2011) to hypothesize that OPTN would be subjected to phosphorylation-dependent regulation by an NF-κB-activating kinase, namely TANK-binding kinase 1 (TBK1).

TBK1 was previously reported to respond to bacterial products such as lipopolysaccharide (LPS) by limiting the replication of cytosolic *Salmonella* (Radtke *et al*, 2007). The underlying molecular mechanisms were largely elusive, yet some data pointed the involvement of another autophagic receptor, NDP52 (Thurston *et al*, 2009). *In vitro* kinase assays and *in cellula* SILAC-based mass spectrometry demonstrated that, in response to LPS, TLR4-activated TBK1 phosphorylates OPTN at Ser177, thereby increasing its affinity for LC3. In line with these observations, a phospho-mimicking version of OPTN (in which 5 Ser residues were mutated to Asp) bound to LC3 with a higher affinity than its wild-type counterpart, while a non-phosphorylatable version of the protein (in which 5 Ser were substituted by Ala) was strongly impaired in its LC3-binding ability. Of note, OPTN co-localized with TBK1 and NDP52 (but not with yet another autophagic receptor, $p62^{SQSTM1}$) on the surface of cytosolic *Salmonella*, suggesting that there are multiple partially overlapping mechanisms by which ubiquitinated bacteria are targeted to xenophagic degradation. The knockdown of OPTN in HeLa cervical cancer cells resulted in a striking increase in cytosolic *Salmonella* proliferation, further underscoring the functional relevance of OPTN-mediated xenophagy (Wild *et al*, 2011).

In conclusion, Wild *et al* have provided novel insights into the crosstalk between components of the innate immune system such as TLR4 and the molecular machinery for xenophagy (Figure 1), as they identified a novel autophagic cargo receptor, OPTN. Moreover, they elucidated one potential mechanism by which autophagic receptors can be regulated during cargo-specific autophagy. Future studies will have to elucidate whether and how such autophagic receptors can be pharmacologically manipulated for the treatment of infectious diseases or other autophagy-related pathologies.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Deretic V (2011) Autophagy in immunity and cell-autonomous defense against intracellular microbes. *Immunol Rev* **240:** 92–104

Galluzzi L, Kepp O, Zitvogel L, Kroemer G (2010) Bacterial invasion: linking autophagy and innate immunity. *Curr Biol* **20:** R106–R108

Green D, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* (in press)

Kraft C, Peter M, Hofmann K (2010) Selective autophagy: ubiquitin-mediated recognition and beyond. *Nat Cell Biol* **12:** 836–841

Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. *Physiol Rev* **87:** 99–163

Kroemer G, Marino G, Levine B (2010) Autophagy and the integrated stress response. *Mol Cell* **40:** 280–293

Radtke AL, Delbridge LM, Balachandran S, Barber GN, O'Riordan MX (2007) TBK1 protects vacuolar integrity during intracellular bacterial infection. *PLoS Pathog* **3:** e29

Thurston TL, Ryzhakov G, Bloor S, von Muhlinen N, Randow F (2009) The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. *Nat Immunol* **10:** 1215–1221

Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG, Yuan L, Soares F, Chea E, Le Bourhis L, Boneca IG, Allaoui A, Jones NL, Nunez G, Girardin SE, Philpott DJ (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* **11:** 55–62

Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B, Korac J, Waidmann O, Choudhary C, Dotsch V, Bumann D, Dikic I (2011) Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. *Science* **333:** 228–233

