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Cell Host & Microbe
Review

Self and Nonself: How Autophagy Targets Mitochondria and Bacteria

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http://dx.doi.org/10.1016/j.chom.2014.03.012

Autophagy is an evolutionarily conserved pathway that transports cytoplasmic components for degradation into lysosomes. Selective autophagy can capture physically large objects, including cell-invading pathogens and damaged or superfluous organelles. Selectivity is achieved by cargo receptors that detect substrate-associated "eat-me" signals. In this Review, we discuss basic principles of selective autophagy and compare the "eat-me" signals and cargo receptors that mediate autophagy of bacteria and bacteria-derived endosymbionts—i.e., mitochondria.

The maintenance of cellular homeostasis requires the controlled elimination of cellular components. Autophagy is of particular importance in this respect, since, in contrast to the proteasome and other cytosolic degradation machinery, autophagy can achieve the degradation of physically large and chemically diverse substrates including protein aggregates, cellular organelles, and even cytosol-invading pathogens (Deretic et al., 2013; Levine et al., 2011; Mizushima and Komatsu, 2011; Randow and Münz, 2012). Evolutionarily, autophagy is thought to have originated as a starvation-induced pathway that nonselectively degrades cytosolic compounds into building blocks and thereby provides energy and maintains essential anabolic processes even when external resources are limiting. How autophagy engulfs specific cargo is a particularly interesting problem for which much progress has been achieved recently. In this Review, we will discuss and compare how autophagy eliminates cytosol-invading bacteria and damaged or excess mitochondria, a conundrum conceptually related to the immune system's task of distinguishing self from non-self and further complicated by the evolutionary relatedness of mitochondria and bacteria. We therefore will focus on how "eat-me" signals and cargo receptors provide specificity for these cellular processes.

Overview of Autophagy

Macroautophagy (hereafter autophagy) is an evolutionarily conserved cellular activity that delivers cytosolic material into double-membrane vesicles, called autophagosomes, that eventually fuse with late endosomes or lysosomes (Mizushima and Komatsu, 2011). Autophagosome biogenesis proceeds along a stereotypical path (Weidberg et al., 2011). Initially a crescent-shaped double membrane forms, which is known as an isolation membrane or phagophore. The phagophore subsequently grows and sequesters cytosolic material, which, upon fusion of the phagophore edge, becomes fully enclosed inside the autophagosome. Autophagosomes finally mature into organelles competent to fuse with lysosomes, whereupon lysosomal enzymes degrade the autophagosome contents including the inner autophagosomal membrane.

Autophagosome biogenesis requires the coordinated action of about 15 "core" autophagy-related or ATG genes, several of which associate into protein complexes (Mizushima et al., 2011). ATG9, the only polytopic transmembrane protein essential for autophagy, and the ULK complex are independently recruited to nascent phagophores upon amino acid starvation. Then ULK kinase activity recruits the VPS34 lipid kinase complex that produces membrane patches rich in phosphatidylinositol 3-phosphate (PI(3)P) (Russell et al., 2013). Phagophores are generated de novo from these PI(3)P-enriched domains at ER-mitochondria contact sites under the control of PI(3)P-binding proteins such as WIPI1/2 (Hamasaki et al., 2013). Phagophore biogenesis requires extensive membrane remodeling, including the formation of ER-derived, PI(3)P-enriched omegasomes marked by DFCP1, another PI(3)P-binding protein (Axe et al., 2008).

The elongation and ultimate closure of phagophores relies on the conjugation of two ubiquitin-like proteins, ATG12 and ATG8, to ATG5 and the lipid phosphatidyl ethanolamine (PE), respectively (Mizushima et al., 2011). To catalyze the lipidation of ATG8 the ATG12~ATG5 conjugate associates with ATG16 into an E3-like enzyme complex, whose localization, together with more upstream components, specifies the site of autophagosome biogenesis. While yeasts encode only a single ATG8 gene, humans harbor six orthologs that cluster into the LC3 and GABARAP subfamilies (Weidberg et al., 2011). Membrane-associated LC3/GABARAP provide docking sites for receptors that deliver specific cargo to phagophores during selective autophagy (Boyle and Randow, 2013; Johansen and Lamark, 2011).

Selective Autophagy

Starvation-induced autophagy is a nonselective process that degrades randomly engulfed cytosolic components in order to fuel the cell in lean times and to provide building blocks for anabolic activities. In contrast, the task of selective autophagy is the elimination of specific cytosolic objects in the maintenance of cellular homeostasis, such as bacteria, damaged organelles, or protein aggregates (Weidberg et al., 2011). Selectivity is achieved by receptors that enforce physical proximity between cargo and

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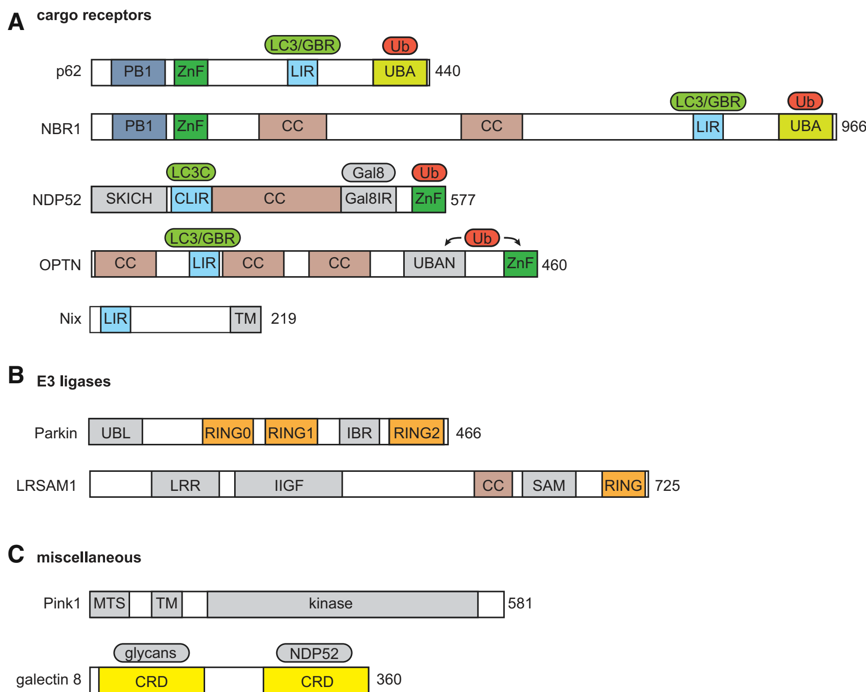

Figure 1. Domain Structure and Ligands of Cargo Receptors, E3 Ligases, and Miscellaneous Proteins Mediating Mitophagy and Antibacterial Autophagy

Shared domains and ligands are color coded; unique domains and ligands in gray. (A) is modified from Boyle and Randow (2013).

autophagy machinery due to simultaneous binding of ''eat-me'' signals on the prospective cargo and LC3/GABARAP on phagophores (Boyle and Randow, 2013; Johansen and Lamark, 2011). Cargo receptors have emerged by convergent evolution and subsequent gene duplication events; currently known are at least five members (p62 and its paralog NBR1, NDP52 and its paralog T6BP, and optineurin) (Figure 1). The interaction of cargo receptors with LC3/GABARAP relies on the formation of an intermolecular β sheet to which the cargo receptor contributes a single strand, the so-called LC3-interacting region (LIR). Negatively charged residues adjacent to the LIR motif contribute to the interaction, sometimes in a phosphorylation-dependent and therefore regulable manner (Wild et al., 2011). Cargo receptors displaying consensus variants of the LIR motif W/FxxI/L/V interact promiscuously with most if not all LC3/GABARAP family members. However, specificity for individual LC3/GABARAP proteins can be provided by more extreme variants of the LIR motif, such as the ILVV peptide occurring in NDP52, which binds selectively to LC3C (von Muhlinen et al., 2012). This selectivity of NDP52 for LC3C entrusts an essential role to LC3C in NDP52-dependent selective autophagy. Why NDP52 in contrast to other cargo receptors relies selectively on LC3C remains unknown but preferential binding could enable NDP52 to control a specific step of autophagosome biogenesis—a suggestion that supports the general concept of specific functions for the LC3 and GABARAP subfamilies in phagophore elongation and maturation, respectively, although species specific differences exist (Weidberg et al., 2010)

Mitophagy

Mitochondria are eliminated by autophagy when the demand for metabolic capacity declines, for example in yeast when they change from log-phase growth to the stationary phase (Abeliovich, 2011) and in cone visual cells during hibernation (Remé and Young, 1977). Mitochondria are completely cleared by autophagy during the differentiation of specialized tissues, such as eye lens fiber cells (Costello et al., 2013) and red blood cells (Heynen et al., 1985). Another mode of mitophagy occurs in many metazoan cell types to selectively cull damaged mitochondria from the intracellular pool, apparently to help maintain quality control (Youle and van der Bliek, 2012).

The molecular mechanisms of mitophagy during the clearance of mitochondria upon reticulocyte differentiation in mammalian cells are becoming understood. The mitochondrial outer-membrane protein, NIX/BNIP3L, was found to be dramatically upregulated during reticulocyte differentiation into mature red blood cells (Aerbajinai et al., 2003). Subsequent work revealed that circulating red blood cells in NIX knockout mice atypically retain mitochondria that are normally removed by mitophagy, establishing an important function in mitochondrial clearance for this mitochondrial membrane protein (Sandoval et al., 2008; Schweers et al., 2007). Although Nix was found to have a consensus LC3 interaction region (LIR) motif that binds to both LC3 and GABARAP (Novak et al., 2010), suggesting it functions to recruit mitochondria into isolation membranes/phagophores, in vivo experiments indicate additional unknown functions for Nix during mitophagy more important than LIR-mediated docking to LC3 (Zhang et al., 2012).

The molecular mechanisms mediating quality-control mitophagy in mammalian cells have become understood in recent years (Twig and Shirihai, 2011; Youle and van der Bliek, 2012). The mitochondrial kinase, PINK1, detects damaged mitochondria and subsequently recruits and activates the RBR E3 ubiquitin ligase, Parkin (Matsuda et al., 2010; Narendra et al., 2010). Parkin, in turn, ubiquitinates proteins on the outer mitochondrial membrane surface that likely initiate autophagosome isolation membrane encapsulation of the damaged mitochondria (Figure 2). This selective autophagy of damaged mitochondria is thought to mediate quality control (Narendra et al., 2008). Interestingly, autosomal recessive mutations in either PINK1 or Parkin cause early onset Parkinson's disease, suggesting that insufficient mitochondrial quality control may be to blame. PINK1 is able to ''sense'' mitochondrial ''quality'' based on its turnover mechanism; PINK1 undergoes rapid and constitutive degradation in healthy mitochondria by the inner mitochondrial membrane protease PARL following import through the TOM and TIM membrane translocation complexes. When the

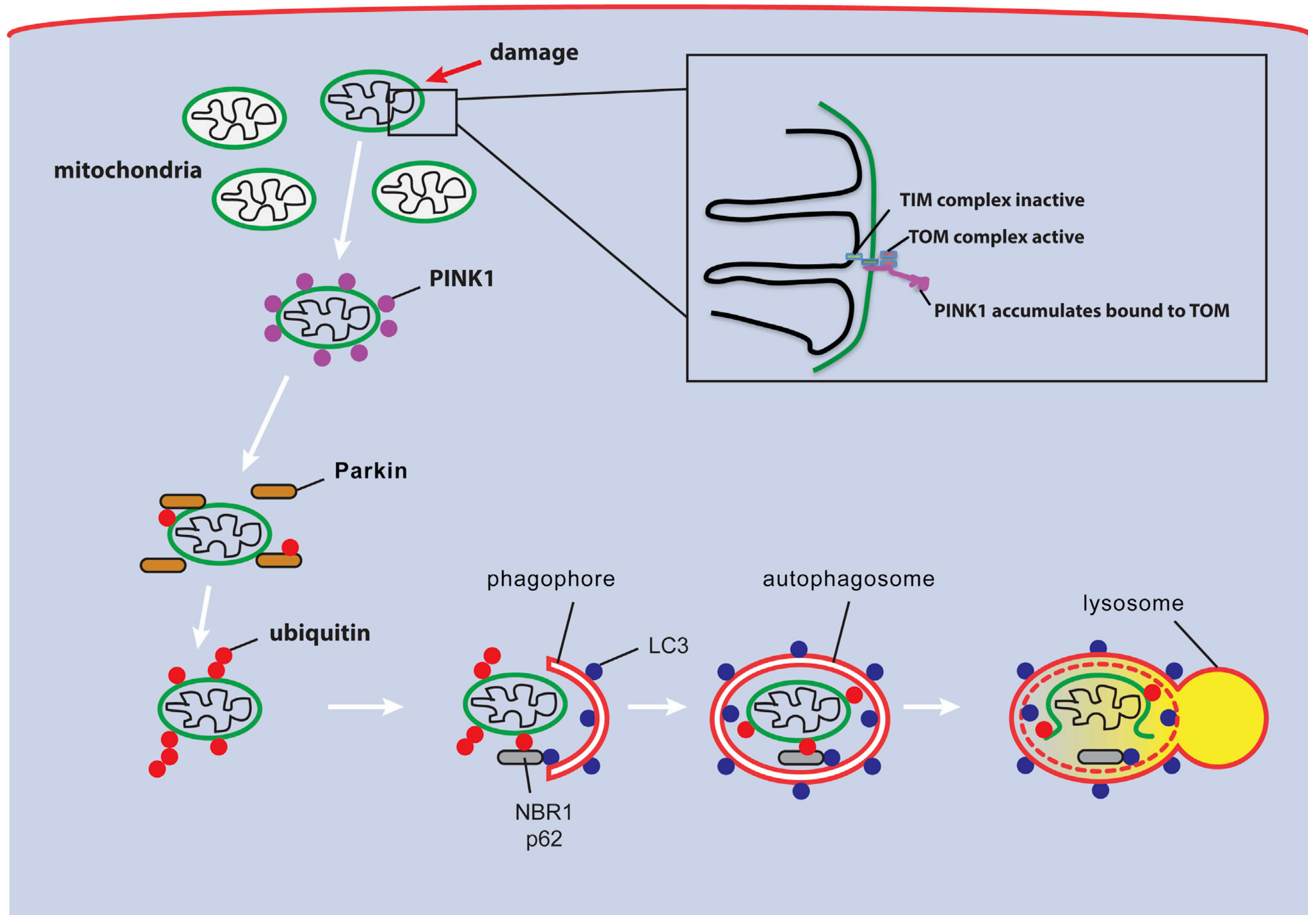

Figure 2. PINK1 and Parkin Mediate Mitochondrial Quality Control by Inducing Mitophagy
(Upper-right inset) PINK1 is constitutively degraded in healthy mitochondria through import via TIM and TOM translocation complexes and cleavage by PARL in the inner membrane followed by proteosomal degradation. Mitochondrial damage prevents PINK1 import and cleavage, allowing the kinase to accumulate on the outer membrane. (Top left to bottom right) When a mitochondrion loses membrane potential or accumulates misfolded proteins, PINK1 accumulates on the outer mitochondrial membrane. The PINK1 kinase recruits Parkin to mitochondria from the cytosol and activates the E3 ligase to ubiquitinate outer mitochondrial membranes. These ubiquitinated proteins act as "eat-me" signals for cargo adaptors that signal autophagosome engulfment of the mitochondrion.

membrane potential across the inner mitochondrial membrane that is normally generated by oxidative phosphorylation deteriorates, PINK1 import into the inner mitochondrial membrane and cleavage by PARL are blocked. PINK1 instead starts to accumulate on the outer mitochondrial membrane with its kinase domain facing the cytosol where Parkin resides (Figure 2). On the outer mitochondrial membrane PINK1 associates in a 2:1 molecular complex with the TOM import machinery (Lazarou et al., 2012; Okatsu et al., 2013). PINK1 also accumulates on the outer mitochondrial membrane when misfolded proteins aggregate in the matrix compartment (Jin and Youle, 2013), suggesting that mitochondrial import or PINK1 proteolysis are shut down in response to mitochondrial stress. PINK1 therefore selectively accumulates only on those mitochondria within a cell population that are dysfunctional and thus flags them for elimination (Narendra et al., 2010).

The accumulation of active PINK1 on mitochondria recruits Parkin and activates its latent HECT/RING hybrid mechanism of ubiquitin transfer. The crystal structure of Parkin shows how the enzyme is held in the cytosol in an autoinhibited form (Riley et al., 2013; Trempe et al., 2013; Wauer and Komander, 2013). Although the structure of active Parkin remains unknown, it appears to form a dimer or multimer upon activation. PINK1 kinase activity is required for Parkin activation, but it is not clear what the essential PINK1 substrate is. PINK1 ectopically placed on peroxisomes recruits Parkin to peroxisomes ruling out mitochondria-specific PINK1 substrates as essential intermediates of Parkin activation (Lazarou et al., 2012). Other models indicate that PINK1 autophosphorylation (Okatsu et al., 2012) or Parkin phosphorylation (Kondapalli et al., 2012) are involved or that an unknown cytosolic protein is the essential PINK1 substrate mediating Parkin translocation.

Once activated, Parkin ubiquitinates scores of substrates on the mitochondria and in the cytosol (Sarraf et al., 2013). Which, if any, of these individual substrates is essential for autophagy remains unknown. Ubiquitin chain linkage or ubiquitin chain density above a certain threshold may be as or more important than the identity of the ubiquitinated substrate—as discussed below in relation to the role of ubiquitin in xenophagy. Parkin appears to attach several ubiquitin chain linkages types, including K48-,

K63-, and K27-linked chains, to proteins located on the outer mitochondrial surface (Chan et al., 2011; Geisler et al., 2010; van Wijk et al., 2012). The K63-linked ubiquitin chains are likely to be important for recruitment of the cargo receptor p62 (Geisler et al., 2010) and other adaptor proteins that can engage phagophore-bound LC3 and GABARAP via LIR motifs. The K48-linked ubiquitin chains are likely involved in the recruitment of the AAA+ ATPase p97 (Tanaka et al., 2010) and the proteasome (Chan et al., 2011) to mitochondria, which respectively mediate the extraction and proteosomal degradation of ubiquitinated outer mitochondrial membrane proteins. The robust proteosomal elimination of outer mitochondrial membrane proteins appears capable of rupturing the outer membrane and may yield a membrane damage signal that triggers mitophagy and recruitment of autophagosome machinery downstream of Parkin (Yoshii et al., 2011).

Parkin-mediated mitophagy also involves noncanonical adaptor proteins that guide autophagic targeting of mitochondria. Notably, two RabGAPs, TBC1D15 and TBC1D17, which are bound to the outer mitochondrial membrane protein, Fis1, interact with LC3/GABARAP and participate in isolation membrane formation during Parkin-mediated mitophagy (Yamano et al., 2014). Despite identical core LIR motifs, TBC1D15 and TBC1D17 bind differentially to LC3 and GABARAP members of the ATG8 family. Interestingly, both require their RabGAP activity in the conserved TBC domain to restrict excessive LC3 protein accumulation during mitophagy. This stems from excessive Rab7 activity in the absence of RabGAP activity that appears normally to be involved in LC3 membrane recruitment and trafficking to mitochondria during mitophagy but not during starvation-induced autophagy.

Additionally, recent evidence suggests that autophagic machinery can be recruited to targeted mitochondria independent of LC3. Ulk1, Atg14, DFCP1, WIPI-1, and Atg16L1 (Itakura et al., 2012) are recruited to autophagosomes associated with Parkin-bound and ubiquitin-labeled mitochondria even in the absence of membrane bound LC3. Ulk1 and Atg9A recruitment to damaged mitochondria are downstream of Parkin activity but independent of one another. What signals the independent recruitment of autophagy machinery proteins to mitochondria-associated isolation membranes is unknown but may stem from different linkage types of ubiquitin chains.

Mitochondrial fission is associated with mitophagy either to reduce the size of elongated mitochondria to facilitate engulfment by autophagosomes or to prevent damaged mitochondria from fusing with healthy mitochondria and impairing them by the exchange of damaged proteins and lipids (Twig et al., 2008). Interestingly, Parkin ubiquitinates the mitochondrial fission proteins Mfn1 and Mfn2 possibly to actively prevent mitochondrial refusion in both *Drosophila* and mammalian cells (Gegg et al., 2010; Poole et al., 2010; Tanaka et al., 2010; Ziviani et al., 2010). This conclusion is corroborated by genetic studies in *Drosophila* where promotion of mitochondrial fission compensates for loss of PINK1 and Parkin and inhibition of fission exacerbates the phenotype of PINK1 and Parkin loss (Deng et al., 2008; Park et al., 2009; Poole et al., 2008; Yu et al., 2011). Mitochondrial trafficking is inhibited by Parkin-mediated ubiquitination and proteosomal degradation of the adaptor protein Miro that links mitochondria to kinesin motors, which may facilitate autophagic engulfment by stalling organelle mobility (Wang et al., 2011; Weihofen et al., 2009). Although recent results in *Drosophila* support the model that PINK1 and Parkin mediate mitophagy in vivo (Burman et al., 2012; Pimenta de Castro et al., 2012; Vincow et al., 2013), whether defects in mitophagy cause Parkinson's disease remains unclear.

Antibacterial Autophagy (Xenophagy)

Autophagy and autophagy genes have been implicated through unbiased genome-wide association studies in antibacterial defense and in inflammatory conditions such as Crohn's disease (Deretic et al., 2013). Susceptibility genes for Crohn's disease include NOD2, which mediates the cytosolic response to peptidoglycan fragments, IRGM, the sole human member of a large family of antimicrobial GTPases, and ATG16L1, a core autophagy gene. Exon sequencing of patients with Crohn's disease recently identified a missense mutation in the cargo receptor NDP52, present at low frequency in the general population, as a potential risk factor (Ellinghaus et al., 2013). Functional activities associated with these risk factors, for example the NOD2-mediated recruitment of ATG16L1 to the site of bacterial entry, suggest that autophagy may provide functionally important defense against cell-invading bacteria in Crohn's disease (Travassos et al., 2010). However, the role of ATG16 in antibacterial defense might be more complex as the disease-associated allele is of high prevalence and a hypomorphic ATG16 allele enhances resistance of mice to *Citrobacter rodentium*, an intestinal pathogen, and to uropathogenic *Escherichia coli* (Marchiando et al., 2013; Wang et al., 2012). Exposure to multiple pathogens and/or nonautophagy related functions of ATG16L1, for example the secretion of antimicrobial peptides from Paneth cells, may be additional confounding factors (Cadwell et al., 2008). Despite this apparent complexity, deletion of ATG5 from intestinal epithelial cells has recently provided direct experimental evidence for a protective in vivo role of autophagy against invasion of the intestinal epithelium by both opportunistic invasive commensals (*Enterococcus faecalis*) and intestinal pathogens (*Salmonella enterica* serovar Typhimurium [*S.* Typhimurium]) (Benjamin et al., 2013).

These and other intracellular bacteria and parasites inhabit specific compartments. Most dwell inside vacuoles, which they manipulate and in many cases prevent from fusing with lysosomes, the cell's major degradative organelle and a source of potent antimicrobial effectors. In contrast, the cytosol with its vast abundance of nutrients is inhabited by a comparably small number of bacterial species. This apparent paradox is largely caused by cell-autonomous antibacterial effector mechanisms, in particular by autophagy (Randow et al., 2013), although it should be noted that compartmentalization is not absolute and that temporary breaches of phagosomal membranes may be part of the life cycle of several bacterial pathogens (Huang and Brumell, 2014). Autophagy represents a fundamental host cell response to invasion by a variety of bacteria including *Shigella flexneri* (Ogawa et al., 2005), *Listeria monocytogenes* (Py et al., 2007), *S.* Typhimurium (Birmingham et al., 2006), and *Mycobacterium tuberculosis* (Gutierrez et al., 2004). The impressive degree to which autophagy antagonizes bacterial invasion of the cytosol is, at least in part, due to the existence of multiple

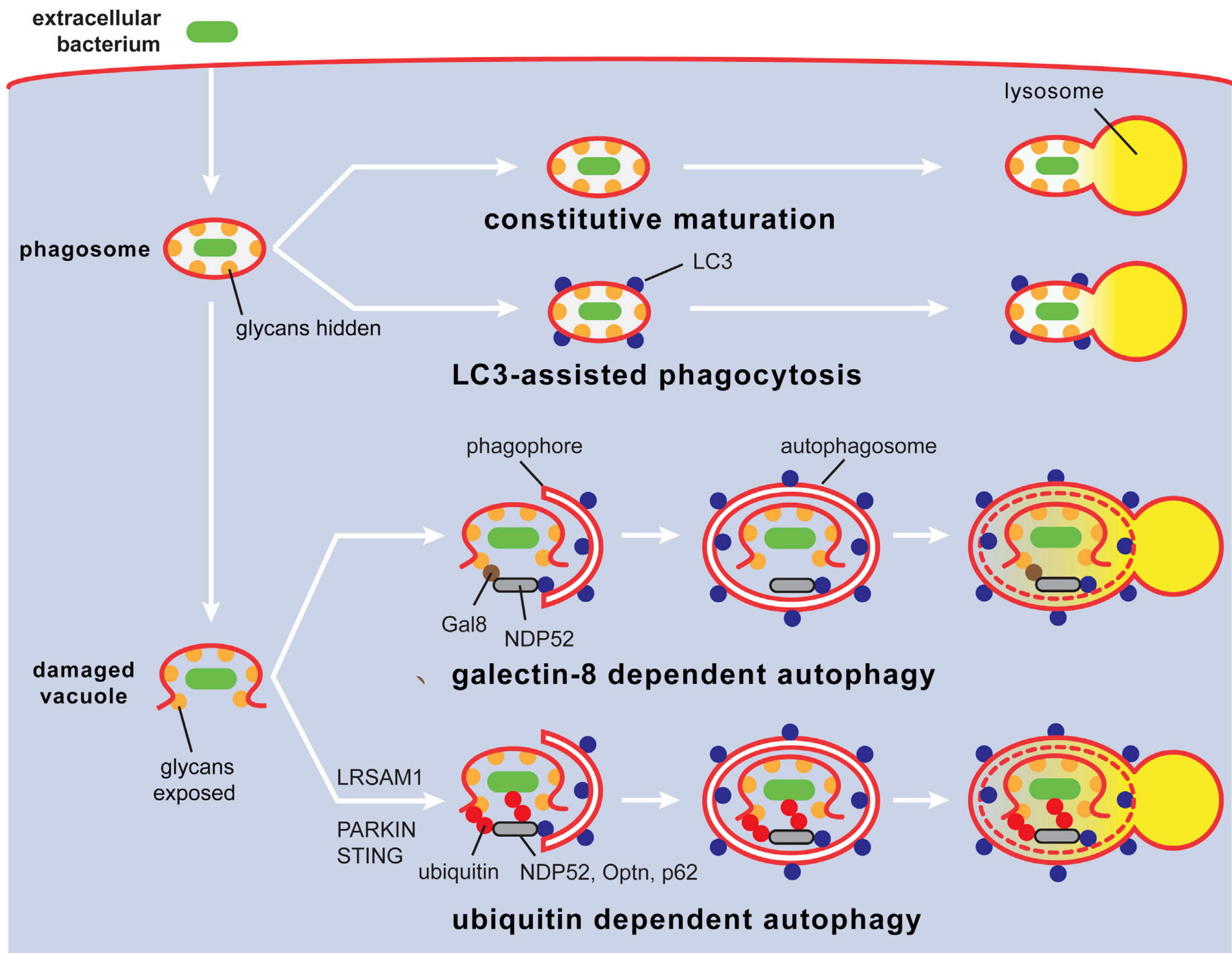

Figure 3. Targeting of Intracellular Bacteria for Lysosomal Destruction by Xenophagy
(Top) Phagosomes mature constitutively and ultimately deliver their bacterial cargo to lysosomes. During LC3-assisted phagocytosis (LAP), conjugation of LC3/GABARAP to the limiting phagosomal membrane promotes phagosome maturation. (Bottom) On damaged vacuoles, exposure of otherwise hidden glycans recruits the danger receptor galectin-8, whose accumulation provides an "eat-me" signal for the cargo receptor NDP52, thereby inducing autophagy. The ubiquitin coat deposited by LRSAM1 and Parkin around cytosol-exposed bacteria (which may still be in association with vacuolar membrane remnants) serves as an alternative "eat-me" signal for multiple cargo receptors (NDP52, Optn, p62), thereby inducing autophagy.

autophagy-related pathways that together establish a multilayered and synergistic defense network (Figure 3) (Boyle and Randow, 2013; Deretic et al., 2013; Levine et al., 2011). Evolutionary evidence for the importance of autophagy is provided by the variety of bacterial adaptations that inhibit or even usurp autophagy (Huang and Brumell, 2014).

During host cell invasion, bacteria are initially taken up into a membrane-surrounded compartment. Upon detection of microorganisms inside the (undamaged) vacuole by Toll-like receptors (TLRs), LC3/GABARAP can become directly conjugated to the limiting membrane of the bacterium-containing vacuole in a process termed LC3-assisted phagocytosis (LAP). LAP requires only a subset of ATGs, for example the ATG5/12/16 complex, but not the most upstream ATGs, such as FIP200 in the ULK complex, since conjugation of LC3 to the vacuolar membrane does not involve de novo phagophore formation (Martinez et al., 2011). Conjugation of LC3/GABARAP to pathogen-containing vacuoles promotes content killing by enhancing lysosomal fusion.

Transition of bacteria from their vacuole into the cytosol, which is an essential step in the life cycle of all cytosol-dwelling bacteria, causes massive damage to the limiting vacuolar membrane and exposes glycans and other molecules normally hidden inside the vacuole to the cytosol. Cells detect breaches to the integrity of the endolysosomal compartment with the help of galectins, a family of cytosolic lectins specific for β(1-4)-linked galactosides that are present abundantly in post-golgi compartments but are lacking in the cytosol (Dupont et al., 2009; Thurston et al., 2012). Cytosolic detection of host-derived glycans via galectins is a remarkably versatile principle of pathogen detection as it enables cells to sense the entry of evolutionarily distant pathogens including Gram-negative and Gram-positive bacteria as well as nonenveloped viruses (Denard et al., 2012; Dupont et al., 2009; Thurston et al., 2012). Although mammals

encode about a dozen galectins, so far only galectins 1, 3, 8, and 9 have been found to sense damaged bacteria-containing vacuoles (Thurston et al., 2012). Accumulation of galectin-8 on damaged vesicles provides an ''eat-me'' signal for the cargo receptor NDP52, thereby triggering autophagy and restricting the ability of *S.* Typhimurium to enter the host cytosol (Li et al., 2013; Thurston et al., 2012).

A second ''eat-me'' signal is produced when cells coat bacteria with polyubiquitin (Perrin et al., 2004). The substrates of antibacterial ubiquitylation have not been identified so far. However, it seems likely that bacteria are directly ubiquitylated and that host proteins associated with bacteria, including proteins of the vacuolar remnants, are also substrate for ubiquitylation (Fujita et al., 2013). Whether ubiquitylation of any particular substrate is essential for antibacterial autophagy is unknown, although it seems likely that a larger number of bacteria-associated proteins become ubiquitylated and that therefore the ubiquitin coat per se is of greater importance than the identity of the ubiquitylated substrate. It is clear, however, that ubiquitin chains of different linkage types, including K48-, K63-, and M1-linked chains, constitute the bacterial ubiquitin coat (Collins et al., 2009; Manzanillo et al., 2013; van Wijk et al., 2012). The linkage type analysis is still preliminary as it relied on the availability of either linkage-specific antibodies or ubiquitin-binding proteins of appropriate specificity. Further chain types may therefore contribute to the bacterial coat and be detected in the future either by mass spectrometry or once additional probes are utilized. The bacterial ubiquitin coat is sensed by at least four cargo receptors, namely NDP52, p62, NBR1, and optineurin, of which all except NBR1 are essential to restrict bacterial proliferation and therefore execute unique functions (Mostowy et al., 2011; Thurston et al., 2009; Wild et al., 2011; Zheng et al., 2009). While the essential contribution of NDP52 might be explained by its unique abilities to sense the galectin-8 eat-me signal on damaged vacuoles (Thurston et al., 2012) and to selectively bind LC3C (von Muhlinen et al., 2012), the nonredundant roles of p62 and optineurin in antibacterial autophagy indicate that their function is also not limited to binding LC3/GABARAP and the ubiquitin ''eat-me'' signal (Wild et al., 2011; Zheng et al., 2009). The possibility that p62 and optineurin are essential solely because they sense different ubiquitin-linkage types appears unlikely since the cargo receptor NBR1, although accumulating on ubiquitin-coated bacteria, is not essential to restrict bacterial proliferation (Mostowy et al., 2011; Zheng et al., 2009).

Insights into the identity of the antibacterial E3 ubiquitin ligases and the nature of the ubiquitylation process have been obtained recently. LRSAM1, a RING-domain E3 ligase, has been found to generate the ubiquitin ''eat-me'' signal around *S.* Typhimurium in a manner dependent on its leucine-rich repeat (LRR) domain, a fold that mediates pathogen recognition in TLRs and other pattern-recognition receptors (PRR). If LRSAM1 is indeed a PRR, its ligand must be widely distributed as LRSAM1 colocalizes with both Gram-negative and Gram-positive bacteria, although its activity does not extend to *Mycobacterium tuberculosis (M. tuberculosis)* (Huett et al., 2012; Manzanillo et al., 2013). For the latter species, the RBR E3 ligase Parkin (PARK2), discussed above for its role in mitophagy, is required to generate the ubiquitin ''eat-me'' signal (Manzanillo et al., 2013). Parkin alleles that predispose to the development of Parkinson's disease (PARK2 T240R, P437L) are impaired in coating *M. tuberculosis* with ubiquitin, while polymorphisms in noncoding regions of PARK2 are associated with increased susceptibility to *Mycobacterium leprae* and *S.* Typhi (Ali et al., 2006; Manzanillo et al., 2013; Mira et al., 2004). In contrast to LRSAM1, Parkin acts on bacteria still contained in vacuoles as revealed by their inaccessibility to antibody staining (Huett et al., 2012; Manzanillo et al., 2013), although limited permeabilization of the vacuolar membrane seems likely to occur given the dependence of Parkin recruitment and ubiquitin deposition on ESX-1, the bacterial type VII secretion system. Ubiquitin coating of *M. tuberculosis* also requires STING (Watson et al., 2012), a receptor for cytosolic cyclic dinucleotides, i.e., cyclic di-GMP, cyclic di-AMP and cyclic GAMP (Danilchanka and Mekalanos, 2013). All three cyclic dinucleotides are bacterial second messengers, while cyclic GAMP is also generated by host-encoded cGAS, the recently identified cytosolic DNA receptor (Danilchanka and Mekalanos, 2013). Although the epistatic relationship of STING and Parkin has not been experimentally addressed yet, the requirement of either gene for the development of the ubiquitin coat around *M. tuberculosis* (Manzanillo et al., 2013; Watson et al., 2012) suggests that they act in the same pathway. Considering the established functions of the two proteins, Parkin acts most likely downstream of STING and is, based on the available literature (Manzanillo et al., 2013; Watson et al., 2012), predicted to be activated by *M. tuberculosis* DNA. However, how such DNA gains access to the host cytosol remains to be clarified.

Outlook

It is interesting to compare the mechanisms involved in the autophagy of endosymbiont mitochondria with those of pathogenic bacteria: self versus nonself. Mitophagy relies on dedicated sentinels such as Nix and PINK1 in mammals and ATG32 in yeast (Kanki et al., 2009; Okamoto et al., 2009), which are all membrane spanning proteins located on the outer mitochondrial membrane. In contrast, bacteria are recognized by cytosolic sensors such as STING and galectin-8 (Thurston et al., 2012; Watson et al., 2012), components of innate immunity pathways, that are recruited to the pathogens or their surrounding phagosome. Following these distinct initiation steps, mitophagy and xenophagy share some common themes. Notably, ubiquitination of mitochondria and bacteria (or their surrounding phagosome membranes) is a shared means to recruit autophagosomal machinery. The E3 ligase Parkin ubiquitinates mitochondria subsequent to its recruitment and activation by the mitochondrial kinase PINK1. Interestingly, Parkin also ubiquitin coats *M. tuberculosis* (or the surrounding phagosome) (Manzanillo et al., 2013). What recruits the normally cytosolic Parkin to the *Mycobacterium*-containing vacuole and derepresses the autoinhibited E3 ligase activity of Parkin during xenophagy remains to be identified. A kinase localized to the pathogen that functions as PINK1 does in mitophagy is a likely candidate. However, another E3 ubiquitin ligase, LRSAM1, is involved in ubiquitin coating of *S.* Typhimurium and there are likely more E3 ligases to be identified in xenophagy (Huett et al., 2012). Adaptor proteins such as p62 and NBR1 are recruited to mitochondria during Parkin-mediated mitophagy and to bacteria during xenophagy that in turn bind to LC3 on preautophagosomal membranes. While

p62, NBR1 and likely NDP52 recognize K63 linked ubiquitin chains on mitochondria, NDP52 also recognizes galectin-8 bound to bacterial permeabilized phagosomes independent of ubiquitin binding.

Likewise, p62 is recruited independently of ubiquitin binding to nascent phagophores (Itakura and Mizushima, 2011) during starvation-induced autophagy, further suggesting we have much more to learn about adaptor function in selective autophagy.

As outlined in this review, much progress has been made recently on the identity and generation of cargo-associated "eat-me" signals and on the cargo receptors that mediate selective autophagy. From these studies a simple model has emerged, in which selectivity is achieved by receptors that bridge cargo and phagophores. However, this model implies that cargo and pre-existing phagophores occur in each other's proximity. Such condition may occur frequently enough for selective autophagy of certain cargos, for example protein aggregates, or for cargos that could be safely transported to a location where phagophores are generated. However, in order to preempt the threat of bacterial proliferation, anti-bacterial autophagy needs to be initiated with high efficiency in exactly the location where the bacterium has been detected. Since even the most upstream autophagy components, for example ATG9 and the ULK kinase complex, colocalize transiently with invading *S.* Typhimurium and also depolarized mitochondria, it is tempting to speculate that the prospective cargo is indeed able to instruct the autophagy machinery to generate phagophores in its proximity (Itakura et al., 2012; Kageyama et al., 2011). While the membrane source for phagophore formation has been an important problem for the autophagy field in general, in selective autophagy the question becomes how phagophores are generated in situ.

ACKNOWLEDGMENTS

We apologize to all colleagues whose work could not be cited due to space limitations. Work in the authors' laboratories was supported by the MRC (U105170648, FR) and the National Institute of Neurological Disorders and Stroke intramural program (to R.Y.). We thank members of our laboratories for invaluable discussions.

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