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A ravenous defense: canonical and non-canonical autophagy in immunity

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Abstract

While classically considered a survival mechanism employed during nutrient scarcity, the autophagy pathway operates in multiple scenarios wherein a return to homeostasis or degradative removal of an invader is required. Now recognized as a pathway with vast immunoregulatory power, autophagy can no longer serve as a "one size fits all" term, as its machinery can be recruited to different pathogens, at different times, with different outcomes. Both canonical autophagy and the molecularly- related, yet divergent pathways non-canonical autophagy are key players in proper host defense and allow us an opportunity to tailor infectious disease intervention and treatment to its specific pathway.

Introduction

In 2016, Nobel Assembly at Karolinska Institutet awarded Yoshinori Ohsumi with the Nobel Prize in Medicine and Physiology for his groundbreaking work unraveling the molecular mechanisms that underlie the tightly regulated catabolic process of macroautophagy (herein referred to as autophagy). We now recognize that the reach of autophagy extends far beyond nutrient deprivation, into cellular quality control and host defense against internalized pathogens. While canonical autophagy likely evolved as a homeostatic response to cellular stress and/or nutrient deprivation, non-canonical autophagic functions are unified in the ancient theme of containment and suppression of inflammation. Similarly, efferocytosis, the immunotolerant clearance of dying host cells by tissue phagocytes, has recently been shown to rely upon recruitment of autophagy effectors to the phagosome through a non-canonical autophagic pathway called LC3-associated phagocytosis (LAP). Taken together, emerging evidence indicates that autophagy, through both canonical and non-canonical pathways, has diversified into a host defense mechanism, capable of confronting immunological and pathogenic stress and mediating immunological self-tolerance to both intracellular and extracellular threats.

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Canonical Autophagy

Autophagy is the highly conserved process by which eukaryotic cells scavenge their own cytoplasmic contents through sequestration into a phagophore and subsequent fusion with a lysosome for degradation. This process of “self-eating” is classically thought of as non-selective in response to nutrient deprivation and is largely orchestrated by the ATG family of proteins [1]. Upon starvation, autophagy progresses in 6 stages: inactivation of mTOR and pre-initiation complex formation, vesicle/phagophore nucleation, vesicle elongation, autophagosome formation, lysosome fusion, and component degradation [2] (Figure 1).

Extensive research has shown AMP-activated kinase (AMPK) to be the main energy sensing rheostat regulating the cell’s response to ATP/AMP imbalance [3]. When ATP levels decrease and AMP levels rise, AMPK becomes activated and inhibits mTOR complex 1(mTORC1) activity [4], leading to nuclear localization of TFEB and Gln3, two autophagy-related transcription factors [5, 6]. AMPK directly controls autophagy factors ULK1 (ATG1) and ATG13 through phosphorylation and sequestration [4]. Once free and active, ULK1 forms the autophagy pre-initiation complex with ATG13, FIP200, and ULK2 and phosphorylates ATG9 within nearby phospholipid membranes [7].

The Beclin-1-binding partner, Ambra1, directly connects the activity of this preinitiation complex, considered the most upstream regulator of the autophagic process, to the Class III PI3K complex. Ambra1 binds the core components of the Class III PI3K complex, Beclin 1 and VPS34, at the cytoskeleton through an interaction with the dynein motor complex. Upon autophagy induction, ULK1 phosphorylates Ambra1, allowing it and its bound partners to re-localize to the ER and initiate vesicle nucleation. The activity and localization of the Ambra1 complex further supports the role of the ER in autophagosome formation [8, 9]. Interestingly, Ambra1 can act in an mTORC1-sensitive positive-feedback loop to promote K63-linked ubiquitination of ULK1 through recruitment of the E3-ubiquitin ligase TRAF6 [10].

In addition to Beclin 1 and VPS34, the Class III PI3K complex consists of ATG14 or UVRAG in a mutually exclusive manner [11]. VPS34, the class III PI3 kinase in the complex, generates PI3P (phosphatidylinositol 3-phosphate), which serves as a critical recruitment signal for the two downstream ubiquitin-like conjugation systems. These two systems, the ATG5-12 system and the LC3-PE system, are required for vesicle nucleation, elongation, and curvature of the forming autophagosomes [2]. E3-ligase complex ATG7 and ATG10 mediates the conjugation of ATG5 to ATG12 in association with ATG16L1 to form a multimeric complex. Subsequently, this ATG5/12/16L1 complex is critical for the generation of LC3-PE (or LC3-II), the lipidated form of LC3 (or LC3-I). Cytosolic LC3-I is cleaved by ATG4, and conjugated to phosphatidylethanolamine (PE) via the activity of ATG7 and ATG3 [12]. This lipidated LC3-PE is bound to the autophagosomal membrane and is require for subsequent fusion to lysosomes, wherein the autophagosomal contents are degraded and recycled [13, 14].

Traditionally, autophagy is considered a cell survival process, however it is important to note that the autophagy machinery can serve as a switch from survival to death. Beclin 1 can bind

pro-survival members of the BCL2 family, specifically BCL2, thus preventing its inhibition of BAX and allowing apoptosis to proceed. Importantly, BCL2-bound Beclin 1 cannot participate in autophagy [15]. Similarly, ATG proteins (ATG5, ATG3, ATG4D), cleaved by calpain or caspases have been shown to be pro-apoptotic, and mutation of the cleavage sites in these same proteins prevents the pro-apoptotic effect [16].

While the ability to self-eat evolved as a cellular response to metabolic stress and a need to return to intracellular homeostasis, autophagy has diverged in to combat infection and is a pivotal regulator of the inflammatory response. In both unicellular and multicellular organisms, autophagy can regulate different steps of the immune response, with immune signaling pathways eliciting an autophagic response to aid in defense [17–20]. Autophagy functions not only as a response to cellular stress, but is also important for pathogen recognition, pathogen degradation, antigen presentation, and regulation of pathways for cytokine production. Unsurprisingly, defects in the autophagic pathway have been strongly associated with inflammatory and autoimmune disorders, as well as infectious susceptibility [21]. However, we now recognize that the autophagic machinery serves many non-canonical functions that are critical for host defense.

Non-canonical Autophagy

While canonical autophagy is considered a non-specific process that sequesters and degrades cytoplasmic contents in bulk, the autophagy machinery can also be selectively targeted to internal cellular substrates. Selective autophagy can be triggered for a variety of stimuli, such as damaged organelles (mitophagy for mitochondria) [22], macromolecules (lipophagy for lipids) [23], aggregated proteins (aggrephagy) [24], intracytoplasmic microbes (xenophagy), or phagocytosed particles such as dying cells or extracellular pathogens (LC3-associated phagocytosis or LAP) (Figure 2) [25–27].

Xenophagy

Hosts have evolved to utilize the autophagy machinery to detect and eliminate intracellular pathogens, such as viruses, bacteria and protozoa [17, 28]. Xenophagy (from the Greek for "strange" and "eating") is a selective form of non-canonical autophagy wherein pathogens are targeted and directed to the autophagosome for subsequent degradation via the autophagolysomal pathway [18, 19]. In addition to cytosolic detection by autophagic elements, some pathogens, such as *Mycobacterium tuberculosis* (*Mtb*) and *Salmonella enterica serovar* Typhimurium, can be eliminated by the fusion of the pathogen-containing vesicles to the autophagolysosome [17, 19, 20, 29].

Xenophagy is initiated by the ubiquitination of either the pathogen substrate, thus sealing its fate. Either the pathogen itself or the ruptured pathogen-containing vacuole can be ubiquitinated, as occurs during *Salmonella ser*. Typhimurium infection [30–33]. The process is mediated by a family of ubiquitinating enzymes comprised of the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). Leucine Rich Repeat and Sterile Alpha Motif Containing 1 2 3 5 RING-Type E3 Ubiquitin Transferase (LRSAM1) and Parkin are two E3 ubiquitin ligases involved in xenophagy, suggesting that

the host autophagy machinery has devised a mechanism against the invading bacteria without a prompt from the pathogen [20, 34, 35].

Once ubiquitinated, the pathogen substrate is now capable to connecting to LC3-containing autophagosomal membranes via recruitment by autophagy receptors, namely SQSTM1 (p62), NDP52, NBR1, and optineurin (OPTN), which contain both a ubiquitin-binding domain and LC3-interacting region (LIR), thus bridging the ubiquitinated substrate to the autophagy machinery [19, 20]. The receptors p62, NBR1, and OPTN bind to LC3 isoforms through their LIR [36–38]. Some LC3 isoform specificity exists, as p62 selectively binds to LC3B and NDP52 binds to LC3C [36, 38, 39]. LC3 itself is a ubiquitin-like protein that conjugates with PE on the autophagosomal membrane [38, 39]. NDP52 is critical to binding ruptured endosomal membranes, as it binds to galectin-8, a β-galactose binding lectin that translocates to the endosomal membrane after its rupture [20]. The mechanisms that govern their function in xenophagy, however, are still under investigation.

Ubiquitinated pathogen substrates also recruit autophagic proteins like ULK1, ATG9L1, ATG16L1, ATG14L, and however, the mechanisms involved are currently not well understood [40]. This recruitment can trigger the formation of an autophagosomal membrane around pathogens or pathogen-containing vacuoles, even in the absence of LC3-bound membranes, indicating that xenophagy can occur independently of the autophagy receptors [40, 41]. Collectively, the goal of xenophagy is targeting of pathogens with ubiquitin, the assembly of the autophagy machinery at the autophagosomes, and degradation of the cargo [20].

The autophagy machinery can also orchestrate the capture of viral components for the removal of the both RNA and DNA viruses [42, 43], a process termed virophagy. Viral receptors such as endosomal toll-like receptors (TLR3, 7–9) or cytosolic nucleic acid sensors (RIG-I, STING, DAI, etc.) are essential for detection of a variety of viral pathogens like measles virus, human herpesvirus 6, adenovirus, and bovine viral diarrhea virus (BVDV) and triggering autophagy in antigen presenting cells [43]. In addition, there exists crosstalk between viral recognition and the autophagy machinery. For example, the lentivirus-encoded protein Nef binds Beclin 1 and induces autophagy [20, 44, 45].

There exist autophagy-independent roles for ATG proteins during host defense. Pathogens such as *Listeria monocytogenes*, *Shigella flexneri*, *Mycobacterium marinum*, HIV, herpesviruses, and Influenza A virus have developed strategies to circumvent detection by the host autophagy apparatus and remain hidden in the cytosol or in the vacuole [17, 18, 20, 46]. In the case of *S. flexneri*, the endosomal membrane is ubiquitinated after the bacteria has escaped the endosome, resulting in delivery of an empty vacuole to the autophagic machinery [30–32]. Other bacteria like *Listeria monocytogenes*, *Mycobacterium marinum*, and *Francisella tularensis* disrupt the formation of initial phagosome and enter the cytosol without host detection [18, 20, 32]. Strikingly, *Mycobacterium tuberculosis* (*Mtb*) colocalizes with multiple autophagy proteins, like ATG5, ATG12, ATG16L1, p62, NDP52, Beclin 1, and LC3, yet only ATG5 in polymorphic mononuclear cells (neutrophils) is required for resistance to *Mtb in vivo*, suggesting a novel autophagy-independent role for ATG5 in tuberculosis pathology and *Mtb* replication [47].

Moreover, bacteria have evolved to advantageously utilize the autophagy machinery for survival [18]. *Brucella abortus* recruits ULK1, Beclin 1, and ATG14L to form LC3-negative Brucella-containing vacuoles [48, 49]. Like bacteria, viruses (such as herpesviruses, HIV, and influenza A virus) have also adapted to opportunistically commandeer the autophagy machinery for their own purposes [42, 43, 50–52]. For example, Sindbis virus can degrade autophagy sensors/proteins like STQSM1 and ATG5, thereby evading detection [44]. As the autophagosome may or may not be decorated by LC3, careful consideration of the processes at play during host defense should be considered before characterizing the outcome as autophagy [20].

LC3-associated Phagocytosis

Whereas xenophagic processes are initiated once the pathogen is within the cell, the autophagic machinery can be actively recruited upon phagocytosis of a pathogen via signaling and sensing by an extracellular receptor. LC3-associated phagocytosis (or LAP) is a form of non-canonical autophagy that is initiated by the engagement of an extracellular receptor, such as Toll-like receptors (TLR), by a pathogen during phagocytosis. LAP can also be triggered by the uptake of dying cells (via phosphatidylserine receptors [PtdSer-R]) or immune complexes (via FcR), therefore LAP can be viewed as a conserved mechanism for mediating control and tolerance over exogenous threats. Receptor signaling results in the recruitment of some, but not all, of the autophagy machinery to the cargo-containing, single-membraned vesicle, which facilitates its decoration with lipidated LC3-PE [14, 25, 27]. The LC3-decorated, cargo-containing structure, or LAPosome, then fuses to lysosomes to mediate the rapid destruction of the cargo and modulation of the pursuant immune response [14].

While LAP and other autophagic immune responses share much of the same machinery, LAP is a process molecularly and functionally distinct from both canonical autophagy and xenophagy. Firstly, LAP results in a single-membraned LAPosome, whereas canonical autophagy and xenophagy create a double-membraned autophagosomes. Furthermore, the pre-initiation complex, described above, Ambra1, and WIPI2 are critical mediators of autophagy and xenophagy, yet completely dispensable for LAP [14, 25, 53]. The most upstream autophagic players required for successful execution of LAP are the components of the Class III PI3K complex (Beclin1, VPS34, and VPS15) [27, 54]. Whereas the Class III PI3K complex can contain with ATG14 or UVRAG during autophagy, LAP exclusively utilizes the UVRAG-containing Class III PI3K complex [14]. Similar to canonical autophagy, Class III PI3K complex-mediated PI(3)P on the LAPosome facilitates downstream recruitment of the ubiquitin-like conjugation systems, the ATG5-12 and LC3-PE conjugation systems required for successful LAP. LC3 bound to the LAPosome is required for subsequent fusion to the lysosome and degradation of the engulfed cargo [14].

Rubicon (RUN domain protein as Beclin-1 interacting and cysteine-rich containing) was recently identified as a protein required for LAP, yet not required for canonical autophagy. Rubicon acts as an inhibitor of autophagy, via its negative regulation of VPS34 [55, 56] or GTPase Rab7 activation [57]. Rubicon associates constitutively with the UVRAG-containing Class III PI3K complex and seems to serve two critical roles during LAP both – promoting

the generation and localization of PI(3)P by the Class III PI3K complex and the production of ROS via the NOX2 complex, the major NADPH oxidase in phagocytes which is also required for LAP [14, 58, 59]. Rubicon stabilizes NOX2 by interacting with the $p22^{phox}$ subunit, and PI(3)P generated via Rubicon's activity on VPS34 binds the $p40^{phox}$ subunit of NOX2 for optimal ROS production [60]. Further cementing the role for NOX2 in LAP are its interactions with Beclin1 via its CCD domain [58] and VPS34 via its RUN domain [61]. Collectively, Rubicon promotes the localization and activity of the Class III PI3K complex with the LAPosome and stabilizes the active NOX2 complex to promote optimal ROS production, which is also required for successful LAP [14]. As LAP is a recently described phenomenon, work uncovering its role in host defense is ongoing. Clearance of *Saccharomyces cerevisiae* [27], *Listeria monocytogenes* [58], and *Aspergillus fumigatus* is severely compromised in LAP-deficient macrophages and animals [14]. In response to fungal β-glucans and engagement of Dectin-1, Rubicon associates with CARD9 to displace it from the NF-κB complex needed for antifungal immunity. Similar scenarios exist during viral infection, wherein RNA viruses, such as VSV, Sendai virus, and influenza A (IAV), engage RIG-I and result in Rubicon disrupting the CARD9- NF-κB complex [62]. During HBV, IAV, and VSV infection, Rubicon can negatively regulate type I interferon signaling by interacting with IRF3 and IRF 7 [63, 64]. Furthermore, Rubicon binds to NEMO and suppresses ubiquitination, thus enhancing viral replication [64]. Hence, in these pathogenic scenarios, Rubicon-deficiency affords a survival advantage.

While LAP certainly plays a critical role in the degradation of engulfed pathogens, LAP is also an important mediator of the immunotolerant response. Cells and animal models with Rubicon deficiency produce significantly increased levels of pro-inflammatory cytokines, such as IL-6, IL-1β, and IL-12, in response to a variety of pathogens [14, 58, 62]. Strikingly, this increase in inflammation is observed in response to pathogens that require LAP for clearance (such as *Aspergillus fumigatus* [14], *Listeria monocytogenes* [58]) and pathogens that do not (fungal β-glucans, Sendai virus, HBV, IAV, and VSV [62–64]. The role of LAP in maintaining the immunotolerant state is exemplified during the clearance of dying cells, a process termed efferocytosis. Animals with LAP deficiency (i.e. Rubicon deficiency), but not canonical autophagy only-deficiency (i.e. ULK1 deficiency), develop lupus-like pathology with age, with increased serum levels of pro-inflammatory cytokines, autoantibodies, and kidney dysfunction [26]. Therefore, LAP represents a conserved cellular rheostat for shaping the appropriate immune response to engulfed pathogens and dying cells.

Mitophagy

The autophagic machinery can also target damaged organelles, such as mitochondria, for degradation, highlighting the quality control function that autophagy plays. In this sense, the degradative clearance of endosymbiont mitochondria (mitophagy) mirrors the clearance of intracellular pathogens (xenophagy) in that mitochondrial components, such as mtDNA, can mimic bacterial molecules and elicit autoinflammatory activation of cells. Both involve the ubiquitination of autophagy substrates resulting in the recruitment of autophagy machinery. Many of the adaptors utilized by xenophagy are also used in Parkin-dependent mitophagy [22].

Several mechanisms for mitophagy have been describe in the literature, all of which involve recognition of mitochondrial distress signals (depolarization, exposed cardiolipin, ubiquitination), and targeting of distressed mitochondria to LC3-containing phagophores via autophagy receptors, such as p62, OPTN, and NDP52 [65]. The most well characterized mitophagy pathway utilizes the kinase PINK1 and the E3 ubiquitin ligase Parkin. After depolarization of the mitochondrial membrane, PINK1 translocates and is stabilized on the outer mitochondrial membrane, wherein it phosphorylates ubiquitin chains that have tagged the damaged mitochondria and elicits the recruitment of Parkin from the cytosol. Here, Parkin is transformed into an active phospho-Ub-dependent E3 ligase that ubiquitinates itself as well as many different mitochondrial substrates. These ubiquitinated substrates are subsequently phosphorylated by PINK1, which fueling a feed forward amplification cycle of further PARKIN recruitment and activation [66]. These ubiquitinated, damaged mitochondria are then delivered to the $LC3^+$ autophagosomes via autophagy receptors [22, 66].

In the absence of Parkin, several mitophagy receptors have been shown to facilitate the clearance of mitochondria during hypoxic stress in an LIR-dependent manner. LIR containing proteins NIX1 (also involved in Parkin-dependent mitophagy), BNP31, and FKBP8 have been shown to recruit lipidated LC3A to damaged mitochondria [67, 68]. FUNDC1 ubiquitination by MARCH5, a E3 ubiquitin ligase, on the outer membranes of mitochondria also targets mitochondria for mitophagy [69].

While clearance of defective mitochondria is a tool to limit unwanted inflammation and maintain homeostasis, mitochondria themselves play a crucial role in host defense by producing ROS, generating the necessary energy for an immune response, and providing a platform for host defense [70]. Studies involving RIPK2 have shown that mitophagy prevents hyperactivation of the NLRP3 inflammasome during infection [71]. In the absence of mitophagy, apoptotic mitochondria release mtDNA which can also trigger NLRP3 inflammasome activation [72] and promotes genomic instability and tumorigenesis [73]. The mitochondrial antiviral signaling (MAVS) protein localizes on the outer mitochondrial membrane where it interacts with RIG-I and MDA5 to activate downstream NF-κB and IRF signaling pathways for pro-inflammatory cytokine and type I IFN production. This mitochondrial localization makes MAVS a target for regulation via mitophagy. Ubiquitin ligases associated with mitophagy, like Smurf1 and Gp78, have been shown to also negatively modulate MAVS activity via both ubiquitin-dependent and -independent mechanisms, and in the absence of mitophagy, infection with VSV, a MAVS agonist, results in hyperstimulation [70]. Interestingly, other studies [74] have demonstrated that that healthy mitochondria are required to promote MAVS activity.

Conclusion

It is well established that defects in the autophagic machinery have been associated with aberrant host defense, inflammatory disease, and age-related disorders [21]. While initial interpretation implicates canonical autophagy in these pathologies, it is possible that the defect lies with non-canonical autophagic processes, such as LAP, rather than traditional autophagy. This is an emerging field in host defense and immunity, and our ability to

discriminate between these related, yet distinct processes will have far-reaching applications in our approach to tumorigenesis, autoimmunity, and infectious disease. As broad inhibition of autophagic processes could be more harmful than beneficial, selective manipulation of specific canonical or non-canonical autophagy pathways could prove to be an invaluable tool for immunomodulation. Therefore, it is imperative that the molecular mechanisms that distinguish these processes are differentiated, as well as the physiological scenarios in which each is required, thus allowing for the design of anti-inflammatory therapeutics that specifically target the appropriate pathway, while maintaining the quality control mechanisms of canonical autophagy.

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Highlights

- Whereas the stress induced during infection can induce canonical autophagy, invading organisms and viruses can be specifically targeted for degradation by the autophagic machinery in a process broadly termed selective autophagy.
- Xenophagy and LC3-associated phagocytosis (LAP) represent two forms of non-canonical selective autophagy. During xenophagy, intracellular pathogens are targeted for removal via ubiquitination and delivered to the LC3+ autophagosomes via autophagy receptors. During LAP, engagement of extracellular pathogen recognition receptors (PRR) trigger the recruitment of autophagy machinery and Rubicon to the pathogen-containing LAPosome for degradation.
- Autophagic processes function not only in the physical removal of pathogens, but also in the modulation of the subsequent immune response.

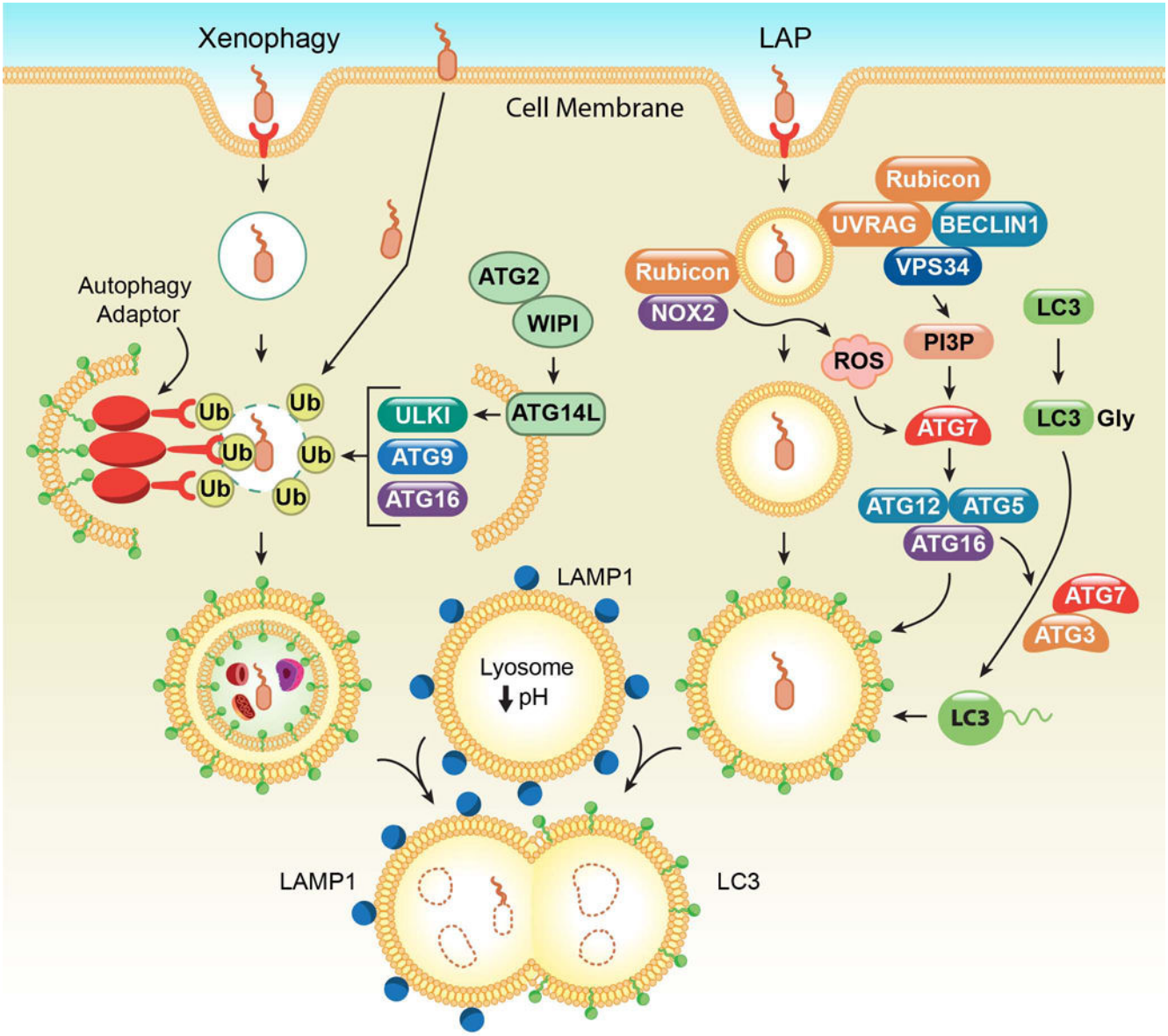

Figure 1. The molecular mechanisms of canonical autophagy

Normally held in check by mTOR, autophagy-inducing signals (such as nutrient deprivation) triggers the activation of AMPK, whose kinase activity simultaneously inhibits mTOR and activates the pre-initiation complex (ULK1/2, ATG13, FIP200). This complex then activates the Class III PI3K complex, composed of VPS34 and Beclin 1, along with either ATG14 or UVRAG. The Class III PI3K complex produces phosphatidylinositol 3-phosphate (PI3P), which acts as recruitment signal for the downstream ubiquitin-like conjugation systems, the ATG12-5 system and the LC3-PE system. The activity and coordination of these two systems facilitates the curvature and sealing of the autophagosome, as well as the lipidation and embedding of LC3-PE into the autophagosomal membrane.

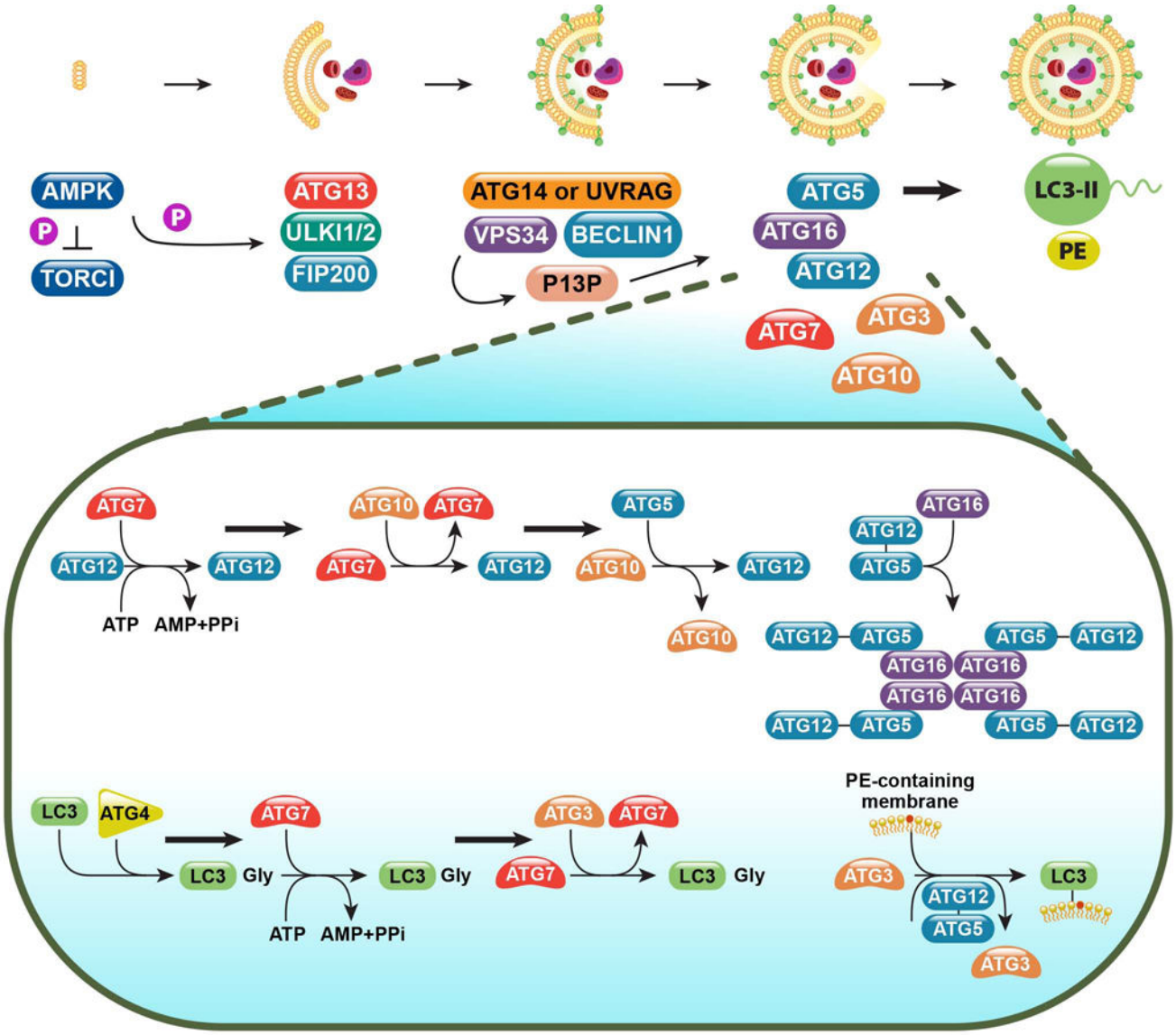

Figure 2. Xenophagy versus LC3-associated phagocytosis

(Left) During xenophagy, rupture of the pathogen-containing vesicle triggers the recruitment of ubiquitin to endosomal proteins or the pathogen itself. subsequently, autophagy adaptors, like p62, OPTN, and NDP52, are recruited and link these ubiquitinated pathogen substrates to the LC3-containing autophagosome. In addition, ATG proteins and other autophagy components are recruited via ubiquitin to mediate autophagosome formation. (Right) During LC3-associated phagocytosis (LAP), engagement of the PRRs during uptake of a pathogen triggers the recruitment of the Class III PI3K complex, comprised of VSP34, Beclin 1, UVRAG, and Rubicon, to the single membraned LAPosome. This complex is required for sustained and localized production of PI3P, which is needed for the recruitment of the downstream LAP machinery (like ATG5, ATG12, ATG16L, and ATG7) and stabilization of the NOX2 complex for ROS production. Both ROS and PI3P are required for successful

LC3-PE decoration of the LAPosome. In both scenarios, LC3-PE is required for fusion to the lysosome and subsequent degradation of its contents.

Table 1

Pathogens associated with the autophagic machinery

Bacteria	Autophagic machinery	Description	References
Mycobacterium Tuberculosis (Mtb)	ATG5, miRNA125a	Induction of autophagy promotes clearance; unique role for ATG5, but not other autophagy proteins, in PMN during infection *in vivo*	[20, 21, 47, 75]
		MtB infection elevates expression of miRNA-125a-3p (miR-125a) and targets UVRAG to inhibit autophagy and phagosomal maturation	
Mycobacterium bovis	ATG5	Induction of autophagy promotes clearance	[20, 75]
Burkholderia pseudomallei	LC3	LAP required for clearance	[76, 77]
		Bacterial Bsa T3SS effector proteins, bopA and bipD, increases bacterial survival by decreasing LC3 accumulation	
Group A Streptococcus (GAS)	ATG5, NDP52, p62, NDR1	LC3 decorated autophagosome containing SpeB cysteine protease degrades the ubiquitin-LC3 adaptor proteins NDP52, p62, and NDR1	[17, 20, 77, 78]
Listeria monocytogenes	p62, ATG5, Rubicon	Macroautophagy and LAP induced; mediates inflammatory responses to pathogen	[58, 79, 80]
		LLO blocks maturation of autophagosome and evades into cytosol by releasing ActA	
Bacteroides fragilis	ATG16L1, Rubicon	Outer membrane vesicles (OMVs) activate LAP for protection from colitis	[52]
Salmonella enterica serovar Typhimurium	NDP52, TBK1, OPTN, ATG9, ATG16L1	Delivery of ubiquitinated bacteria or bacterial substrates for degradation	[28, 37]
Shigella flexneri	ATG5, NBR1, NDP52	Secretes VirG which binds to ATG5 and activates autophagy; delivery of ubiquitinated bacteria or bacterial substrates for degradation	[19, 77, 80]
		IcsB secreted by Shigella competes with ATG5	
Legionella pneumophila	Beclin 1, LC3	Inhibition of Beclin 1 restricts autophagosome initiation and elongation.	[17, 81, 82]
		RavZ and LegA9 secreted from T4SS uncouples LC3 from autophagosome membrane and inhibits autophagosome elongation and maturation.	
		L. pneumophila secretes effector protein, SGPL1 targets host sphingolipid metabolism, inhibit autophagosome formation and causes starvation-induced autophagy for intracellular survival	
Adherent & invasive Escherichia coli (AIEC)	ULK1, LC3	HIF1α-mediated retention in LC3-II$^+$ vesicles and induces phosphorylation of ULK1	[83]
Uropathogenic E. coli	ATG16L1	Required for clearance	[84]
Yersinia Pseudotubercul osis	ATG5	Defect in acidification of the LC3$^+$ autophagosome-like vacuoles containing pathogen.	[17, 20]
Yersinia pestis	LC3	Resides in LC3$^+$ vesicles, yet prevents vacuole acidification	[17, 85]
Citrobacter rodentium	ATG16L1	Required for clearance	[86]
Pseudomonas aeruginosa	ATG7, Beclin 1	Autophagy mediated clearance	[87]
Klebsiella pneumoniae	ATG7	Autophagy mediated clearance	[88]
Franscisella tularensis	NOX2, Beclin 1, ATG5, ATG12, ATG16L, ATG7, ATG4	Disruption of NOX2-mediated ROS; downregulation of autophagy genes	[89]

Bacteria	Autophagic machinery	Description	References
Coxiella burnetii	Varies	Recruits autophagosomes to acquire nutrients or other factors that may trigger differentiation, and delays fusion with lysosomes for viral replication	[90, 91]
Brucella abortus	ULK1, Beclin 1, ATG14, VPS34	*S*electively recruits autophagy proteins to subvert clearance	[48, 49]
Viruses	**Autophagic machinery**	**Description**	**References**
HBV	Rubicon, ATG5	Rubicon reduces IFN production and binds to NEMO to suppress ubiquitination, delays autophagosome maturation and allows viral replication; autophagy inhibits viral clearance	[64]
VSV	Rubicon, ATG5, ATG12	Rubicon reduces IFN production and binds to NEMO to suppress ubiquitination and allows viral replication; autophagy inhibits antiviral response	[63, 64, 92]
IAV	Beclin 1, Rubicon	Influenza virus matrix protein 2 causes inhibition of beclin1 restricts autophagosome initiation and elongation.	[50, 63, 64]
		Rubicon reduces IFN production and bind to NEMO to suppress ubiquitination and allows viral replication	
HSV-1	Beclin 1	HSV inhibition of Beclin 1 to restrict autophagy	[50, 93]
Kaposi's sarcoma herpes virus	Beclin 1	BCL2-like proteins cause inhibition of Beclin 1 and restricts autophagy	[50, 94]
HIV	Beclin 1	Accessory protein Nef binds/inhibits Beclin1 restricts autophagosome initiation and elongation	[50, 95]
Zika Virus	mTOR	NS4A and NS4B destabilize mTOR signaling	[96]
Sindbis Virus	ATG5, Beclin 1	Defects in ATG5 impairs CNS clearance of Sindbis virus capsid	[50, 97]
		Ectopic Beclin 1 expression in Sindbis virus-infected neurons suppresses viral replication in the brain and reduces mouse mortality.	
Fungi	**Autophagic machinery**	**Description**	**References**
Aspergillus fumigatus	Rubicon, NOX2, LAP machinery	LAP-mediated degradation of and immune response to *A. fumigatus*	[14, 98]
Candida albicans	ATG5, Rubicon, NOX2	Rubicon binds to CARD9 and NEMO to suppress ubiquitination and allows for increased fungal burden	[62, 99]
Saccharomyces cerevisiae	ATG7	LAP required for fungal clearance	[27]
Cryptococcus neoformans	ATG5	ATG5 aids in delivering *C. neoformans* in $LC3^{+}$ autolysosome	[99]
Parasites	**Autophagic machinery**	**Description**	**References**
Toxoplasma gondii	ATG14, ATG9, ATG5, ATG7, ATG12, ATG16L1	Autophagy is required for targeting and degradation of *T. gondii*	[28, 77]
Plasmodium vivax	Beclin 1, VPS34, ATG5	LAP required for parasite control	[100]

Pathogens (Bacteria, viruses, fungi, and parasites) with known links to components of the canonical and non-canonical autophagic machinery. Descriptions highlighted in green represent scenarios where pathogen clearance requires components of the autophagic machinery. Descriptions highlighted in red represent scenarios where autophagic components impede pathogen clearance.