Beyond self-eating: The control of nonautophagic functions and signaling pathways by autophagy-related proteins

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The identification of conserved autophagy-related proteins (ATGs) that mediate bulk degradation of cytosolic material laid the foundation for breakthroughs linking autophagy to a litany of physiological processes and disease conditions. Recent discoveries are revealing that these same ATGs orchestrate processes that are related to, and yet clearly distinct from, classic autophagy. Autophagy-related functions include secretion, trafficking of phagocytosed material, replication and egress of viral particles, and regulation of inflammatory and immune signaling cascades. Here, we define common processes dependent on ATGs, and discuss the challenges in mechanistically separating autophagy from these related pathways. Elucidating the molecular events that distinguish how individual ATGs function promises to improve our understanding of the origin of diseases ranging from autoimmunity to cancer.

Introduction

Twenty five years ago in the *Journal of Cell Biology*, Professor Yoshinori Ohsumi and colleagues published the first of several landmark papers demonstrating molecular control of macroautophagy in response to nutrient starvation in *Saccharomyces cerevisiae* (Takeshige et al., 1992). Thereafter, several groups identified autophagy-related proteins (ATGs), evolutionarily conserved molecules that control fundamental aspects of the macroautophagy pathway, including the formation of autophagosomes, double membrane vesicles that capture cellular cargo and subsequently deliver them to the lysosome for degradation (Tsukada and Ohsumi, 1993; Thumm et al., 1994; Harding et al., 1996). Since the discovery of ATGs, an explosion of research on autophagy has led to seminal advances in understanding the molecular regulation of the autophagy trafficking process, dissecting how autophagy controls cell survival and metabolic fitness in response to countless stressors, and illuminating the diverse functions of the autophagy pathway in both normal physiology and disease (Choi et al., 2013; Kaur and Debnath, 2015). At the same time, we have begun to appreciate that various ATGs and other autophagy regulators are deployed in assorted fundamental processes that are distinct and separable from their well-established roles in mediating autodigestion via the lysosome. This review highlights this exciting new facet of autophagy research and summarizes our current understanding of these autophagy-related functions and signaling pathways mediated by individual ATGs as well as entire cell biological subroutines using multiple components of the autophagy machinery.

Classic autophagy versus autophagy-related pathways

Autophagy consists of three cellular self-eating mechanisms that converge on the lysosome: microautophagy, chaperone-mediated autophagy, and macroautophagy. Among these, macroautophagy (hereafter called autophagy) is the most well studied and genetically controlled by ATGs. Classic autophagy proceeds through multiple "canonical" steps that include (1) initiation by an autophagy-inducing signal, (2) nucleation of an isolation membrane or phagophore assembly site, (3) elongation and sealing of this double membrane around the cargo to be sequestered to form an autophagosome, (4) docking and fusion of the autophagosome with the lysosome to form an autolysosome, and (5) degradation of the vesicle contents by lysosomal enzymes (Fig. 1 A). Initiation, nucleation, and elongation require the hierarchical recruitment and activity of ∼15 ATGs and other proteins to the phagophore assembly site to construct the autophagosome (Codogno et al., 2011; Mizushima et al., 2011). In this context, the term "noncanonical autophagy" refers to the formation of classic double membrane autophagosomes that does not require the activity of one or more key ATGs. Nonetheless, both canonical and noncanonical autophagy are fundamentally autodigestive pathways requiring autophagosome formation, followed by fusion with the lysosome (Codogno et al., 2011).

Adding to this complexity, ATG proteins, both individually and as part of larger networks, control pathways that either do not involve the formation of a classic autophagosome or do not terminate in lysosomal degradation and nutrient recycling (Subramani and Malhotra, 2013). Although not inclusive, the list of autophagy-related processes includes secretion and exocytosis, LC3-associated phagocytosis (LAP), viral replication

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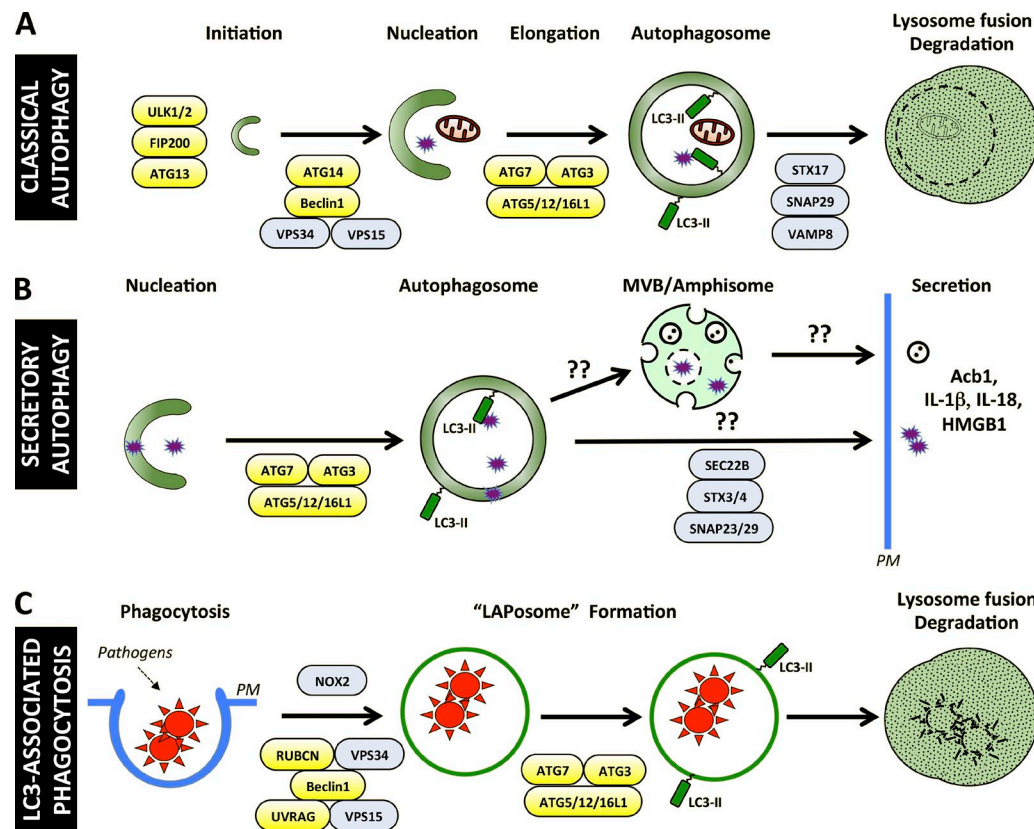
Figure 1. **Classic autophagy compared with related trafficking pathways. (A)** Classic autophagy: Diverse stimuli elicit the hierarchical recruitment and activity of multiple ATGs (yellow) and other regulatory proteins (blue) to construct the double membrane autophagosome. The lipidation of LC3 (LC3-II) is crucial for the capture of autophagic cargo and to stabilize of the inner autophagosomal membrane. The autophagosome subsequently fuses with the lysosome in a STX17-dependent manner, resulting in degradation of the vesicle contents by lysosomal enzymes. **(B)** Secretory autophagy: ATGs mediate the unconventional secretion of multiple proteins (e.g., Acb1 in yeast, and IL-1β, IL-18, and HMGB1 in mammalian cells) that lack an N-terminal signal sequence. These targets are postulated to be released via several putative mechanisms. First, the ATG conjugation machinery promotes the formation of an LC3+ autophagosome-like intermediate, and the contents enwrapped within the inner membrane of autophagosome are released extracellularly instead of degraded in lysosomes. Second, targets of secretory autophagy, such as IL-1β, are translocated into the intramembrane space of an LC3+ double membrane vesicular intermediate that fuses directly with the plasma membrane or fuses with a MVB intermediate that is secreted. Last, although formal experimental evidence is lacking, secretory autophagy may involve an MVB/amphisome intermediate and the exocytic release of small extracellular microvesicles. Regardless of the exact pathway, recent work indicates that secretory autophagy proceeds through a dedicated SNARE machinery, which diverts secreted targets away from the lysosome and toward the plasma membrane (PM). **(C)** LAP: the phagocytosis of pathogens and other prey in certain cell types (e.g., macrophages and dendritic cells) recruits UVRAG and Rubicon (RUBCN), thereby activating the Beclin-1–VPS34 complex to generate phosphatidylinositol 3-phosphate and NOX2, an NADPH oxidase that generates ROS inside the phagosome. This subsequently triggers the recruitment and activation of the ATG conjugation machinery, which mediates LC3-II at the single membrane phagosome. LC3-II expedites fusion to lysosomes and degradation of the offending pathogen.

and exit, antigen presentation, and ATG-mediated regulation of inflammatory and immune signaling. Importantly, these processes are fundamentally distinct from classic autophagy, and certainly, many can be construed as "noncanonical" functions for the individual ATG proteins that are involved. However, they are not by definition "noncanonical autophagy" in its strictest sense. Hence, to avoid confusion, the term "noncanonical" should be avoided when referring to these autophagy-related processes. In the following sections, we overview our current understanding of this diverse collection of autophagy-related processes that are distinct from classic autophagy.

Secretory autophagy
In addition to its established role in lysosomal degradation, the autophagy machinery controls extracellular secretion. Evidence to date most notably implicates ATGs in unconventional secretion of proteins lacking an N-terminal signal sequence (Dupont et al., 2011; Deretic et al., 2012; Malhotra, 2013; Subramani and Malhotra, 2013). Whereas the majority

of eukaryotic secretory proteins classically transit to the surface via the ER and Golgi apparatus, a growing list of proteins traffic through unconventional mechanisms that do not require insertion into the ER and/or bypass the Golgi (Rabouille et al., 2012; Malhotra, 2013). In addition, some classically secreted proteins appear to be preferentially rerouted through unconventional pathways to facilitate trafficking during stress (Gee et al., 2011). Studies to date have uncovered clear genetic requirements for two proteins originally implicated in the stacking of the Golgi apparatus, GRASP55 and GRASP65, as well as ATGs in mediating these alternative secretory pathways (Rabouille et al., 2012; Malhotra, 2013; Zhang and Schekman, 2013). ATGs have been genetically linked to the unconventional secretion of acyl-CoA-binding protein Acb1 in yeast (AcbA in *Dictyostelium discoideum*; Duran et al., 2010; Manjithaya et al., 2010), several inflammatory mediators in mammalian cells, including IL-1β and IL-18; the high mobility group protein B1 (HMGB1); and finally, the plasma membrane trafficking of the integral membrane protein ΔF508 CFTR (Dupont et al., 2011;

Gee et al., 2011). To date, apart from these limited targets, the broader autophagy-dependent secretome remains uncharacterized. Accordingly, recent quantitative proteomic analysis of the secretome from ATG5-deficient macrophages has uncovered new leaderless proteins that may be secreted in an autophagy-dependent manner (Kimura et al., 2017). However, it remains unclear from these genetic loss-of-function studies whether the observed secretory defects represent a direct versus indirect consequence of impaired autophagy.

The mechanistic underpinnings of secretory autophagy are only beginning to emerge, and numerous questions remain unaddressed. First, despite the genetic interconnections between ATGs and Grh1, the yeast GRASP orthologue, in *S. cerevisiae*, recent work questions whether autophagy and Grh1 truly converge on a common secretory pathway (Cruz-Garcia et al., 2014). Second, although ATGs that promote early autophagosome formation are genetically required for unconventional secretion, it is unclear whether secretory autophagy targets are actually captured into the autophagosomal lumen (Fig. 1 B). In fact, recent work demonstrates that IL-1β secretion requires ATGs but proceeds via translocation into the intermembrane space of the autophagosome (Zhang et al., 2015; Fig. 1 B). Third, it remains unclear how secreted targets are transported to the cell surface. Because evidence supports that contents of multivesicular bodies (MVBs) can be directly exported to the cell surface, most notably the release of small extracellular microvesicles (exosomes), a role for the late endocytic pathway seems attractive (Colombo et al., 2014; Fig. 1 B). At the same time, recent evidence demonstrates interconnections between autophagy and the retromer complex, a protein assembly implicated in plasma membrane exocytosis of diverse molecules from early endosomes (Steinberg et al., 2013). During metabolic stress, the induction of autophagy elicits LC3+ autophagic compartments that bind and sequester a key inhibitor of retromer complex, the RabGAP protein TBC1D5; as a result, autophagy activates the retromer-driven translocation of proteins to the plasma membrane surface, most notably the glucose transporter GLUT1/SLC2A1 (Roy et al., 2017). Last, defining the mechanisms by which targets of secretory autophagy are diverted away from lysosomal degradation remains an important question for further study. Indeed, recent work indicates that secretory autophagy involves autophagosome-like vesicles that bypass STX17-dependent fusion with lysosomes; rather, they use the SNARE protein SEC22B in combination with plasma membrane syntaxins to complete cargo secretion (Kimura et al., 2017; Fig. 1).

In addition to unconventional secretion, ATGs promotes the efficient egress of secretory lysosomes in osteoclasts (De-Selm et al., 2011) and the conventional secretion of cytokines during oncogene-driven cancer cell invasion and senescence (Narita et al., 2011; Lock et al., 2014). Further dissecting the cellular mechanisms through which autophagy mediators facilitate these diverse secretory processes remains an important topic for future study.

LAP

Studies of LAP poignantly illustrate how key elements of the core autophagy machinery can be redirected toward lysosomal pathways that are distinct from the canonical autophagosome-to-lysosome cascade. LAP represents a process akin to macroautophagy in which phagosomes engulf extracellular contents, such as microorganisms or dying cells, which are subsequently trafficked to the lysosome (Sanjuan et al., 2007).

During LAP, elements of the autophagy machinery are recruited to phagosomes, upon which they facilitate maturation and the digestion of phagosomal contents (Fig. 1 C). Originally discovered during phagocytosis of particles containing Toll-like receptor (TLR) ligands, LAP involves the recruitment of the Beclin-1 (BECN1)–VPS34 complex to the phagosome, resulting in production of phosphatidylinositol 3-phosphate and the subsequent formation of phosphatidylethanolamine lipidated, membrane-bound LC3 (LC3-II; (Sanjuan et al., 2007). LC3-II facilitates phagosome maturation in LAP, probably by recruiting molecules that enhances fusion to the endolysosomal compartment, such as the homotypic fusion and protein sorting complex involved in Rab7-mediated lysosomal fusion (McEwan et al., 2015).

In addition to Beclin-1, multiple ATGs are required for LAP, including all key elements of the LC3 conjugation machinery (ATG3, ATG5, ATG7, ATG12, and ATG16L; Martinez et al., 2011). Despite this common utilization of multiple ATGs, LAP is a mechanistically distinct process. During classic autophagy, LC3 lipidation occurs on early double membrane structures, called phagophores, and functions in the capture of autophagic cargo and to enhance the stability of the inner autophagosomal membrane (Stolz et al., 2014; Tsuboyama et al., 2016). In contrast, during LAP, components of the autophagy conjugation machinery recruit LC3-II directly onto phagosomes, which are single membrane organelles. As a result, LAP does not use the ULK (unc-51–like autophagy activating kinase) complex that initiates canonical autophagy (Martinez et al., 2011). Notably, similar pathways direct LC3 conjugation onto single-membrane organelles during entosis and macropinocytosis, two processes that also converge on the lysosome (Florey et al., 2011). Furthermore, LAP uses a unique VPS34 complex, composed of Beclin-1, UVRAG, and Rubicon (RUBCN), a protein that is inhibitory to classic autophagy (Martinez et al., 2015). Last, generation of reactive oxygen species (ROS) by NADPH oxidase–2 (NOX2) has also been critically implicated in the control of LAP (Martinez et al., 2015).

LAP serves as a host defense system targeting several pathogens, including *Aspergillus fumigatus* and *Salmonella typhimurium* (Huang et al., 2009; Martinez et al., 2015) and has emerged as a key regulator of inflammation and immunity. Previous studies have implicated autophagy in antigen presentation to T cells by major histocompatibility complex (MHC) molecules (Dengjel et al., 2005; Schmid et al., 2007). However, optimal MHC-II presentation of peptides from phagocytosed pathogens requires LC3 targeting to phagosomes by ATGs and NOX2, suggesting that LAP mediates presentation of extracellularly derived antigens (Romao et al., 2013). Importantly, LAP directs the production of type I IFN (IFN-I), namely IFN-α, in response to host-DNA containing immune complexes by plasmacytoid dendritic cells (Henault et al., 2012). In addition to controlling the immune response, LAP is required for the phagocytosis and degradation of photoreceptor outer segments by retinal pigment epithelial cells in mice, which is essential for proper vision (Kim et al., 2013). Overall, these studies reinforce the physiological importance of LAP as an autophagy-related pathway.

ATGs in host–pathogen interactions

In addition to LAP, studies have uncovered a growing list of nonautophagic functions mediated by ATGs that modulate the infection of host cells and tissues. In this section, we focus
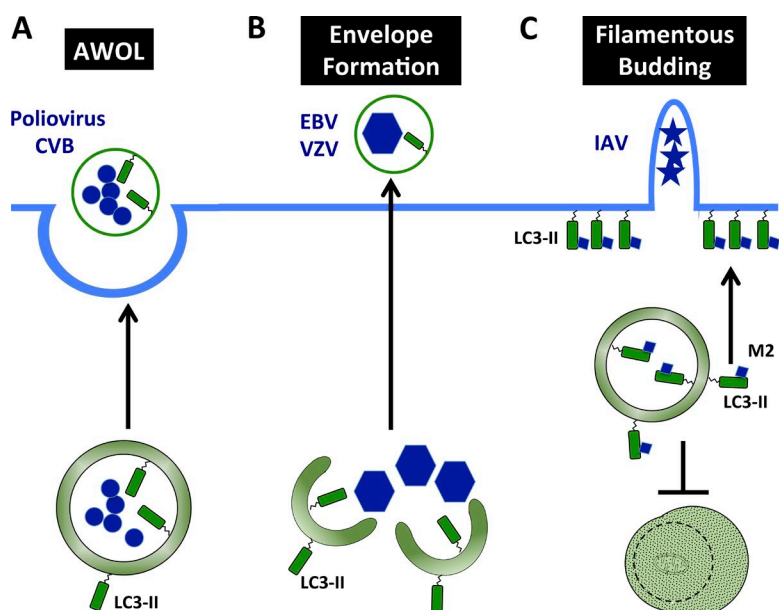
Figure 2. **LC3-conjugated membranes support viral exit. (A)** Picornavirus (circles), such as poliovirus and CVB, promote the formation of LC3-II⁺ double membrane vesicles. LC3-conjugated membranes support viral replication as well as autophagosome-mediated exit without lysiss (AWOL), the exocytic release of multiple virions within a LC3⁺ membrane-bound coat. **(B)** For certain enveloped viruses (hexagons), such as the herpesviruses Epstein-Barr virus (EBV) and varicella-zoster virus (VZV), LC3-coupled membranes are incorporated into the viral envelope and promote viral release during lytic infection. **(C)** During IAV (stars) infection, the viral product matrix protein 2 (M2, diamonds) interacts with lipidated LC3 (LC3-II) to block autophagosome-to-autolysosome maturation in the host cell as well as redirect LC3-II to the cell surface. The translocation of LC3-conjugated membranes to the plasma membrane is important for the filamentous budding of IAV and the stability of virions in the extracellular milieu.

on how individual ATGs can either augment or diminish the propagation of assorted viruses as well as how ATGs restrict intracellular pathogens via pathways that are distinct from both classic autophagy and LAP.

Subversion during viral replication and transmission. Growing evidence supports that viruses use LC3⁺ membranes to exit host cells via exocytic pathways analogous to secretory autophagy (Fig. 2). Infections by poliovirus and coxsackievirus B (CVB), two nonenveloped RNA viruses, result in the formation of LC3⁺ double membrane vesicles in an ATG-dependent manner, which serve as scaffolds for viral replication complexes (Jackson et al., 2005; Wong et al., 2008). Instead of degradation in the lysosome, sequestered virions are released from cells within a membrane coat through a process termed autophagosome-mediated exit without lysis (Fig. 2 A; Taylor et al., 2009). The "envelope" acquired during egress shields virion clusters from immune recognition and aids entry into neighboring cells (Bird et al., 2014; Chen et al., 2015b). Consistent with these studies that an autophagy-like process is required for viral replication and spread, ATG5 deletion in pancreatic acinar cells of mice leads to a 2,000-fold reduction in CVB replication and protection from pancreatitis (Alirezaei et al., 2012). In addition to these picornaviruses, lipidated LC3 also contributes to the envelopment and exocytosis of certain herpesviruses during lytic infection. Epstein-Barr virus and varicella-zoster virus acquire LC3-conjugated membranes during envelope acquisition in the cytosol, which can be detected in purified virions (Fig. 2 B; Nowag et al., 2014; Buckingham et al., 2016). Accordingly, inhibiting LC3 lipidation via *ATG12* or *ATG16L1* knockdown impairs viral exit and results in the accumulation of viral DNA in the cytosol (Nowag et al., 2014). Finally, influenza A virus (IAV) encodes an ion channel protein, matrix protein 2, that blocks lysosomal degradation of the virion and facilitates viral egress through binding and redirecting LC3-II to the plasma membrane. Notably, although the improved filamentous budding of IAV is dependent on ATG conjugation pathways that lipidate LC3, the overall infectious virus production remains intact (Fig. 2 C; Gannagé et al., 2009; Beale et al., 2014).

In addition to these effects on viral exocytosis, interactions with LC3 are also required for the processing and inclusion of HIV Gag into the virion, whereas HIV Nef inhibits Beclin-1 to prevent virion degradation through autophagy (Kyei et al., 2009). In contrast, the multilamellar membranes that harbor coronavirus replication complexes are decorated with the non-lipidated form of LC3; down-regulation of LC3 impairs viral replication, but ATG7 deletion has no effect (Reggiori et al., 2010). Thus, viruses subvert LC3-mediated membrane trafficking events through different ways.

Although we focus on strategies by which viruses coopt ATGs for their own benefit, some viruses are restricted by autophagy. For example, autophagic degradation of ER, reticulophagy, restricts dengue and Zika virus replication on ER-derived membranes. The NS3 protease encoded by these and other flaviviruses counteract this inhibition by cleaving the reticulophagy receptor FAM134B (Lennemann and Coyne, 2017). Also, multiple herpesviruses encode proteins that bind and inhibit Beclin-1 to avoid autophagy-mediated targeting to the lysosome and antigen presentation to T cells (Deretic and Levine, 2009). In other situations, it may be nonautophagic functions of ATGs that determine virulence, as suggested by an siRNA screen comparing the requirement of diverse ATGs during infection by six viruses (Mauthe et al., 2016). This screen revealed that many of these proteins augment or diminish the replication of individual viruses independent of other ATGs, consistent with the conclusion that nonautophagic functions of ATGs are pervasive during viral infection.

When antimicrobial immunity is not xenophagy or LAP. Xenophagy, in which an internalized microbe is sequestered in an autophagosome and targeted to the lysosome, is an established form of cell-autonomous defense (Cadwell, 2016). However, mechanisms by which ATGs restrict intracellular pathogens are sometimes radically different from classic autophagy or LAP. The cytokine IFN-γ inhibits *Toxoplasma gondii* replication in macrophages by triggering a process referred to as Targeting by AutophaGy proteins (TAG), which requires ATGs involved in LC3 conjugation, but not the lysosome. Instead of promoting acidification, the ATG8 orthologue GABARAPL2 (GATE-16) recruits IFN-inducible GTPases to the

parasitophorous vacuole where they disrupt the membrane and destroy the replicative niche of *T. gondii* (Choi et al., 2014; Park et al., 2016b; Sasai et al., 2017). A similar mechanism disrupts the membranous structures on which noroviruses replicate (Biering et al., 2017). Additionally, though xenophagy inhibits *Mycobacterium tuberculosis* replication in cultured macrophages, experiments using cell type-specific knockout mice revealed a surprising autophagy-independent function of ATG5 in neutrophils. Deletion of ATG5, but not the other ATGs involved in LC3 conjugation, increases the amount of neutrophils that infiltrate and damage the lung during *Mycobacterium tuberculosis* infection (Kimmey et al., 2015). As with viruses, bacteria can benefit from selective components of the autophagy machinery. The multimembrane vacuoles that support *Brucella abortis* replication are generated by ATGs that control PI3 kinase activity (ULK1, Beclin-1, and ATG14), but not ATGs required for LC3 conjugation (ATG7, ATG16L1, ATG5, LC3B, and ATG4B) (Starr et al., 2012). In addition to these cell-autonomous functions of ATGs during infection, nonautophagic ATG functions are important for immune signaling pathways that contribute to multi-cellular immunity, which is discussed in the following section.

ATG-mediated inflammatory and immune signaling

ATGs have a fundamental role in suppressing immune signaling (Cadwell, 2016). Among the first indications that autophagy exacts inhibitory functions came from population genetic studies implicating a coding variant of *ATG16L1* in Crohn's disease, a type of inflammatory bowel disease (IBD) associated with an abnormal immune response to the gut microbiota. Consistent with this genetic association, ATG16L1 deletion causes macrophages to overproduce the cytokine IL-1β, which mediates intestinal inflammation (Saitoh et al., 2008). Although increased production of immune effectors is pathological in this setting, *Atg16l1* mutation unexpectedly enhances innate immune resistance to oral infection by the Gram-negative bacterium *Citrobacter rodentium* (Marchiando et al., 2013). Additionally, deletion of other ATGs throughout the autophagy pathway leads to a general increase in cytokine levels that promote resistance to influenza virus infection (Lu et al., 2016) and inhibition of herpesvirus reactivation from latency (Park et al., 2016a). In this section, we provide examples highlighting different mechanisms by which ATGs reduce signaling downstream of cytosolic pathogen sensors.

ATGs restrict retinoic acid–inducible gene I (RIG-I) antiviral signaling. The presence of nucleic acid in the cytoplasm derived from intracellular pathogens induces transcription of IFN-I. The magnitude of this response is regulated by ATGs through both autophagy-dependent and -independent mechanisms. RIG-I binds short double-stranded RNA with a 5′ppp moiety, which exposes the caspase activation and recruitment domain (CARD) to allow interaction with the CARD of mitochondrial antiviral signaling protein (MAVS; Yoneyama et al., 2004; Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005). After this CARD–CARD interaction, MAVS oligomerizes on the mitochondrial outer surface to activate TBK-1, which in turn leads to the phosphorylation and nuclear translocation of the transcription factors IFN regulatory factor 3 (IRF3), IRF7, and NFκb (Honda et al., 2005; Seth et al., 2005). The ATG5–ATG12 conjugate inhibits this RNA recognition pathway by binding the CARDs of RIG-I and MAVS (Fig. 3; Jounai et al.,
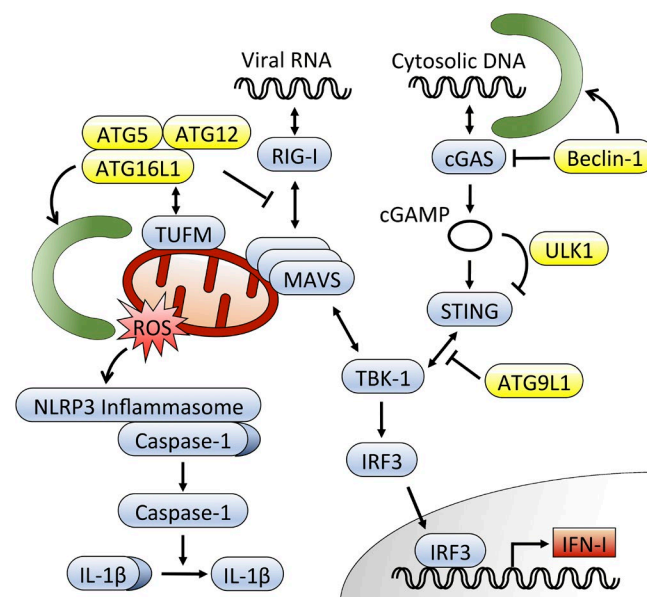

Figure 3. **ATGs in inflammatory and immune signaling.** ATGs regulate immune signaling cascades through autophagy-dependent and -independent mechanisms. The mitochondrial protein TUFM recruits the ATG16L1–ATG5–ATG12 complex to mediate the autophagic removal of mitochondria that produce ROS, an activator of RIG-I signaling and the NLRP3 inflammasome. By targeting mitochondria, autophagy further inhibits IFN-I production by removing the signaling intermediate MAVS, which aggregates on mitochondrial surfaces downstream of viral RNA recognition by RIG-I. Also, the ATG5–ATG12 complex inhibits RIG-I and MAVS through an inhibitory binding event. Beclin-1 prevents sustained signaling by inducing the autophagic removal of cytosolic DNA and inhibiting cGAS through direct binding. cGAMP generated by cGAS activates ULK1 to inhibit STING in a negative feedback loop. ATG9L1 interferes with the trafficking of STING to prevent continuous TBK-1 activation.

2007). Although it is unclear how these interactions prevent MAVS function, the direct binding of ATG5–ATG12 to CARDs suggests that the mechanism is autophagy-independent. An alternative mechanism involves increased IFN-I production upon disruption of mitophagy. ROS from damaged mitochondria accumulate upon ATG5 deletion, which enhances RIG-I signaling (Fig. 3; Tal et al., 2009). Mitophagy also mediates the concurrent degradation of MAVS upon its translocation to mitochondria. The recruitment of the ATG16L1–ATG5–ATG12 complex to mitochondria by NLRX1 and elongation factor Tu (TUFM) decreases MAVS activity (Fig. 3; Lei et al., 2012). The importance of this pathway is highlighted by the observation that the human parainfluenza virus type 3 matrix protein induces mitophagy through interaction with TUFM, leading to decreased immune signaling and increased viral replication (Ding et al., 2017). Also, a single base substitution that confers the ability of the viral polymerase basic protein 2 to bind TUFM and induce autophagy allows avian influenza virus to infect human cells (Kuo et al., 2017). Most likely through a similar process, the mitochondrial protein COX5B binds ATG5 and MAVS, and reduces ROS and IFN-I production during viral infection (Zhao et al., 2012). The commonality in these studies is that inhibiting the ATG5–ATG12 conjugate increases IFN-I and decreases replication of RNA viruses.

ATG control of innate immune DNA-sensing pathways. Cyclic GMP-AMP synthase (cGAS) generates the dinucleotide cyclic GMP-AMP (cGAMP) upon binding double-stranded DNA in the cytosol (Sun et al., 2013; Wu et al.,

2013). Similar to RNA sensing by RIG-I, STING (Stimulator of interferon genes) mediates a TBK-1 dependent signaling cascade in the presence of cGAMP (Chen et al., 2016). Deletion of ATG9L1, but not other ATGs, increases the colocalization between STING and TBK-1 to enhance IFN-I production in response to cytosolic DNA (Fig. 3; Saitoh et al., 2009). Also, generation of cGAMP by cGAS activates ULK1, which phosphorylates and inhibits STING while also inducing autophagy (Fig. 3; Konno et al., 2013). Similarly, Beclin-1 can bind and inhibit cGAS and simultaneously mediate the degradation of cytoplasmic DNA through autophagy (Fig. 3; Liang et al., 2014). Thus, Beclin-1 prevents sustained cGAS activation through both direct inhibition and depriving the enzyme of its substrate. In these examples, conserved autophagy proteins (ATG9L1, ULK1, and Beclin-1) display functions independent of autophagy-related processes, although these functions do not necessarily preclude their role in classic autophagy.

ATGs and inflammasome activation. Inflammasomes are multiprotein complexes that induce the cleavage of pro–IL-1β and pro–IL-18 by caspase-1 to generate the activate forms of these cytokines (Sharma and Kanneganti, 2016). The NLRP3 inflammasome responds to a variety of stimuli that directly or indirectly cause the release of ROS and DNA from leaky mitochondria (e.g., bacterial toxins and microcrystalline substances). Inhibiting mitophagy, therefore, leads to accumulation of damaged mitochondria that induce pathological cytokine production downstream of NLRP3 inflammasome activation (Fig. 3; Saitoh et al., 2008; Zhong et al., 2016). ATGs also inhibit the caspase-11 inflammasome, which is activated by the bacterial cell wall component lipopolysaccharide in the cytosol. When IFN-inducible GTPases attack the *Salmonella*-containing vacuole as part of the host defense mechanism, LC3 is recruited to the damaged membrane by the linker protein NDP52 to mediate the autophagic sequestration of bacteria and lipopolysaccharide, which inhibits inflammasome activation (Meunier et al., 2014). Hence, it is likely that removal of damaged vesicles and organelles (or their contents) through classic autophagy restrains inflammasome activity, thereby explaining why inhibiting autophagy proteins such as ATG16L1 enhance IL-1β and IL-18 production.

ATGs in inflammatory diseases

Here, we use IBD, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) as examples of how ATGs contribute to complex inflammatory diseases. IBD includes Crohn's disease and ulcerative colitis, and is frequently a debilitating condition that involves chronic inflammation in the small intestine or colon, although any part of the gastrointestinal tract can be affected. Many genetic variants that increase risk of IBD, including several known to affect autophagy, are also found in individuals without disease (Lassen and Xavier, 2017). A particularly common polymorphism in *ATG16L1* (up to 50% heterozygosity in certain populations) linked to Crohn's disease introduces a caspase-3 cleavage site that destabilizes the protein product (ATG16L1^{T300A}), thereby causing a reduction in autophagy (Lassen et al., 2014; Murthy et al., 2014). ATG16L1^{T300A} is associated with structural defects in Paneth cells, intestinal epithelial cells that produce antimicrobial granules (Cadwell et al., 2008). Additional inflammatory pathologies in the intestinal epithelium of *Atg16L1* mutant mice is triggered by a commensal enteric virus (Cadwell et al., 2010; Kernbauer et al., 2014), or by dual deletion of ATG16L1 and the ER stress transcription factor XBP-1 in Paneth cells (Adolph et al., 2013). These findings are consistent with epidemiological observations suggesting that IBD is caused by the confluence of multiple genetic and environmental susceptibility factors.

Several findings support the idea that the classic autophagy function of ATG16L1 is critical for protecting the epithelial barrier and preventing a sustained immune reaction. ATG-deficient Paneth cells display unresolved ER stress and mitochondrial damage that contribute to necroptosis, a type of programmed necrotic cell death (Diamanti et al., 2017; Matsuzawa-Ishimoto et al., 2017; Tschurtschenthaler et al., 2017). These observations suggest that the organelle homeostasis function of autophagy is important to counteract the secretory burden of this highly differentiated cell type and prevent inflammatory sequelae. Also, ATGs are generally required to protect the epithelial barrier, potentially through xenophagy or mediating mucus production by goblet cells (Benjamin et al., 2013; Conway et al., 2013b; Patel et al., 2013). An important role for autophagy in immune cells should also be considered. Increased inflammasome activity in macrophages and decreased differentiation of antiinflammatory T cells are both consequences of ATG16L1 inhibition, and can cause intestinal inflammation (Saitoh et al., 2008; Chu et al., 2016; Kabat et al., 2016). However, many of these studies rely on animal models in which classic autophagy and related processes are difficult to distinguish. ATG16L1^{T300A} disrupts secretory autophagy, leading to impaired exocytosis of lysozyme from Paneth cells during *Salmonella* infection (Bel et al., 2017), and the effect of ATG16L1^{T300A} on the necroptosis signaling complex likely involves disruption in ATG-mediating immune signaling (Matsuzawa-Ishimoto et al., 2017). In addition to being an unstable protein, ATG16L1^{T300A} displays impaired binding with TMEM59, a transmembrane protein that mediates the trafficking of LC3$^+$ vesicles through a process distinct from classic autophagy (Boada-Romero et al., 2016). Also, with the exception of graft-versus-host disease (Hubbard-Lucey et al., 2014), the IBD variant of *ATG16L1* is not linked to other inflammatory disorders associated with autophagy dysfunction, such as Vici syndrome (Lu et al., 2016). Thus, investigating the potential nonautophagic functions of ATG16L1 in maintaining the intestinal barrier remains a critically important future direction.

RA is an autoimmune disease that primarily affects the joints and is associated with the presence of autoantibodies that are reactive to citrullinated peptides that are presented by MHC-II molecules. In contrast to IBD, ATG function may promote RA. ATG5 is required for generation of citrullinated antigens by peptidylarginine deiminases, potentially by mediating the trafficking of these enzymes to MHC-II antigen loading compartments (Ireland and Unanue, 2011). CTLA4 on anti-inflammatory T cells binds B7 molecules on dendritic cells to inhibit LC3 expression and autophagy, which dampens the capacity to present antigens (Alissafi et al., 2017). The CTLA4-Ig fusion molecule abatacept, which is used to treat RA, also inhibits ATG-mediated antigen presentation by dendritic cells, suggesting that dampening autophagy is part of the mechanism of action of this drug (Alissafi et al., 2017). Because it is currently unclear when autophagy or an autophagy-related process such as LAP mediates antigen presentation, an important future direction will be to determine which ATGs are necessary for autoantigen presentation, and whether this differs among cell types.

Polymorphisms in a noncoding region of *ATG5* is associated with SLE, a multiorgan autoimmune disease characterized by antinuclear antibodies, indicative of improper immune

activation toward cellular contents (Harley et al., 2008). ATG5 in B cells is required for autoantibody generation in the *Lpr* and *Tlr7* transgenic mouse models of SLE (Weindel et al., 2015; Arnold et al., 2016), consistent with the role of autophagy in supporting the secretory burden and organelle homeostasis in differentiated B cells (Conway et al., 2013a; Pengo et al., 2013; Chen et al., 2014, 2015a). In contrast to this proposed role of autophagy in B cells that facilitates SLE pathogenesis, the genetic ablation of LAP-specific ATGs in phagocytic cells results in inefficient clearance of dead cells and their immunogenic contents, leading to a SLE-like disease in mice (Martinez et al., 2016). Thus, it is possible that inhibiting autophagy may ameliorate, whereas inhibiting LAP may exacerbate disease. Developing drugs that can exclusively target classic autophagy without effecting autophagy-related processes, or vice versa, may be necessary to treat certain disorders.

Concluding remarks

Overall, these studies illustrate the wide array of nonautophagic functions mediated by the ATG machinery. These diverse functions deepen our understanding of ATGs in enacting cell-autonomous and non–cell-autonomous biological functions beyond self-eating in both normal and disease states. They also broach the importance of revisiting phenotypes and functions, both in vitro and in vivo, that to date have been attributed to classic autophagy based on the genetic analysis of a single individual ATG. Going forward, it will be critical for future researchers to keep in mind alternative pathways, not just autophagy, upon discovering new ATG-dependent phenotypes. Importantly, in future studies to delineate how autophagy influences both normal physiology and disease, it will be incumbent on researchers to interrogate multiple ATGs controlling distinct elements of the autophagy trafficking pathway to definitively attribute a role for classic autophagy on cell fate and function. In a similar vein, as we develop more precise methods to therapeutically target specific autophagy machinery components, the role of alternative autophagy-related processes and signaling pathways merit thoughtful and rigorous consideration.

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