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Autophagy As A Stress Response And Quality Control Mechanism—Implications for Cell Injury and Human Disease

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Abstract

Autophagy, a vital catabolic process that degrades cytoplasmic components within the lysosome, serves as an essential cytoprotective response to pathologic stresses that occur during diseases such as cancer, ischemia, and infection. In addition to its role as a stress response pathway, autophagy plays an essential quality control function in the cell by promoting basal turnover of long-lived proteins and organelles as well as selectively degrading damaged cellular components. This homeostatic function protects against a wide variety of diseases including neurodegeneration, myopathy, liver disease, and diabetes. This review discusses our current understanding of these two principal functions for autophagy as a physiologic stress response and quality control mechanism within mammalian cells and details how alterations in autophagy promote human disease.

Keywords

Macroautophagy; lysosome; mitophagy; inflammation; neurodegeneration; myopathy

INTRODUCTION

Autophagy (literally "self-eating") is an essential catabolic pathway that degrades cellular components within the lysosome. There are three principal routes of autophagic degradation, mainly differing in the means of cargo delivery to the lysosome: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (1). During macroautophagy, bulk cytoplasmic components are sequestered in a double membrane structure called the autophagosome (AP); the AP is subsequently trafficked to the lysosome where its outer membrane fuses to the lysosome, leading to degradation of its contents. In microautophagy and CMA, cargo is directly taken up by the lysosome, either through invagination of the lysosomal membrane (in microautophagy) or by unfolding and translocation of proteins with a specific signal sequence that is recognized by the LAMP2A receptor on the lysosome (in CMA) (1). This review focuses on macroautophagy, hereafter called "autophagy".

Autophagy was first identified in mammalian cells by electron microscopy studies in the 1960s, but an understanding of the molecular pathways involved was not achieved until almost thirty years later, following the discovery of the first autophagy genes (ATGs) through landmark genetic screens in yeast. These initial experiments identified a set of highly conserved ATGs that control autophagy (2), many of which have mammalian orthologues. Further studies have elucidated the molecular pathways that control autophagy

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in mammalian systems (Figure 1) and have revealed the importance of autophagy in both normal physiology and disease (1). The identification of yeast ATGs and their mammalian homologues has also enabled experimental manipulation of the autophagy pathway and the development of assays for monitoring autophagic activity and flux (Figure 2) (3).

Autophagy machinery components are mutated in a variety of human diseases, highlighting the importance of autophagy in human health (Table 1). Tissue-specific deletion of ATGs and autophagy-related genes in mice has also provided further insight into the role of autophagy in maintaining normal tissue homeostasis and in disease pathogenesis (Table 2). In this review, we will discuss the role of autophagy in the cellular stress response and in cellular homeostasis, and how alterations in autophagy promote disease pathogenesis.

AUTOPHAGY IN STRESS RESPONSE

Autophagy is an essential pro-survival pathway induced by a wide variety of stresses including nutrient deprivation, growth factor withdrawal, oxidative stress, infection, and hypoxia (4). During periods of stress, autophagy maintains cellular biosynthetic capacity and ATP levels by supplying amino acids for de novo protein synthesis and providing substrates for the tricarboxylic acid (TCA) cycle, such as amino acids and free fatty acids (5). In the mouse, autophagy is upregulated in almost all tissues except the brain following starvation (6). Mice lacking the essential autophagy genes *Atg5* or *Atg7* (Figure 1B) have decreased plasma and tissue amino acid concentrations and die within one day after birth, likely due to nutrient depletion during the neonatal starvation period (7, 8). Figure 1 details the molecular functions of ATGs discussed throughout this review. The importance of autophagy as a pro-survival catabolic pathway has also been demonstrated in cell culture systems. In Bax/Bak double knockout cells, which are unable to undergo apoptosis, intact autophagy protects cells from death following prolonged growth factor withdrawal. In growth factor deprived cells, RNAi-mediated Atg7 depletion or treatment with the autophagy inhibitors 3-methyladenine (3-MA) or chloroquine (CQ) (Figure 1) leads to cell death. Supplying autophagy-deficient cells with the TCA cycle substrate methylpyruvate rescues ATP production and cell viability (9), demonstrating that the catabolic function of autophagy likely mediates cytoprotection. Importantly, self-eating does not protect a cell indefinitely; rather, autophagy is proposed to function as a "battery" that buys cells time, allowing them to survive if the stress is resolved in a timely manner. In this context, autophagy is viewed as a salvage mechanism that provides basic components to sustain core metabolic functions during starvation or stress (10).

Signaling pathways that control autophagy

Stress-induced autophagy is primarily controlled at two critical nodes: mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK). TOR was initially identified as a negative regulator of autophagy in yeast and has been corroborated as a major regulator of mammalian autophagy (4). In mammalian cells, under normal nutrient conditions active mTOR phosphorylates Ulk1 (the mammalian Atg1 orthologue) and sequesters it in a complex with Atg13 and FIP200, inhibiting autophagy (11–13). Starvation, amino acid deprivation, or growth factor withdrawal inhibit mTOR activity, leading to autophagy induction (Figure 3A) (14). AMPK is a major positive regulator of autophagy that is activated by a high ratio of AMP to ATP (4). Under conditions of low intracellular energy, activated AMPK induces autophagy both by phosphorylating Ulk1, activating it, and by inhibiting mTORC1 via phosphorylation of Raptor (Figure 3A) (11, 15). Both AMPK and mTOR also control cell growth and metabolism, coupling autophagy to these processes.

Other important stress response pathways that induce autophagy include HIF-1 signaling in response to hypoxia, p53 signaling downstream of DNA damage, and pattern recognition

receptor (PRR) signaling following infection (4). The transcription factor HIF-1 is stabilized under hypoxic conditions, leading to induction of a hypoxia-associated gene expression pattern. The BH3-only protein BNIP3 is one major HIF-1 target, and it is essential for autophagy induction following hypoxia. BNIP3 induces autophagy by binding to Bcl-2 and consequently disrupting the inhibitory interaction between Bcl-2 and Beclin 1 (the mammalian orthologue of yeast Atg6) (Figure 3B) (16). The tumor suppressor p53 is induced by a wide variety of cellular stresses including DNA damage and plays dual roles in autophagy induction. Multiple transcriptional targets of p53 activate autophagy, including BAX and PUMA. On the other hand, cytosolic p53 also has transcription-independent functions, and cytosolic p53 inhibits autophagy (4). The consequences of this balance between the pro- and anti-autophagic roles of p53 are not well understood. Finally, PRRs recognize molecular patterns associated with different pathogens and induce autophagy following infection (Figure 3C) (17). The signaling pathways leading from PRR activation to autophagy induction remain unclear, but there is evidence for AMPK and Beclin 1 as downstream effectors in some contexts (18, 19). The induction of autophagy via PRRs is discussed in greater detail below.

AUTOPHAGY ACTIVATION IN RESPONSE TO DISEASE-RELATED CELL STRESS

Given the central role that autophagy plays in responding to cellular stresses, it is not surprising that autophagy is an essential response to disease-induced stress. Autophagy generally plays a pro-survival role during disease, although there are also contexts in which either overactive autophagy or autophagy induction coupled to lysosomal or maturation defects may be cytotoxic, as discussed below. Stresses such as starvation, hypoxia, and oxidative or genotoxic stress contribute to the etiology of multiple diseases including cancer, stroke, heart disease, and infection. Notably, growing evidence supports key roles for autophagy during cancer progression and metastasis. As a result, there is immense interest in discerning how to most effectively modulate autophagy to treat cancer. Accordingly, the multifaceted roles of autophagy in cancer progression and response to therapy have been recently covered in multiple excellent reviews (10, 20). Below, we focus on three specific examples that poignantly illustrate the diverse roles for autophagy as a response to disease-induced cell stress: 1) cardiac ischemia-reperfusion injury, 2) infection, and 3) chronic inflammation during Crohn's disease.

Role of autophagy during cardiac ischemia and reperfusion injury

Cardiac ischemia-reperfusion (I/R) injury results from a blockage in blood supply to the heart. It is characterized by two phases: ischemia, during which impaired coronary circulation leads to a state of nutrient and oxygen deprivation, and reperfusion, during which circulation is restored. The primary tissue damage occurs during the reperfusion phase (21).

Autophagy induction during ischemia is likely a response to nutrient withdrawal and subsequent energy depletion caused by impaired blood supply. Since starvation is a classical inducer of autophagy, it is not surprising that autophagy plays an important role in responding to the starved state during I/R. Moreover, HIF-1 signaling and BNIP3 upregulation following hypoxia also may also induce autophagy following ischemia (Figure 3B), although the relative role of this pathway in cardiac ischemia is unknown (16). Autophagy during the ischemic phase is thought to be cytoprotective, because it is able to supply amino acids for use in the TCA cycle to maintain cellular ATP levels. Autophagy may also protect against oxidative damage during ischemia by supplying amino acids for glutathione synthesis (21). Using glucose deprivation in primary cultured myocytes as an in vitro model of ischemia, Matsui et al. demonstrated that autophagy induction following

ischemia is AMPK-dependent (Figure 3A). Inhibiting AMPK or inhibiting autophagosome formation with 3-MA during glucose deprivation severely depletes cellular ATP and reduces cell viability (22). Further confirming this result in vivo, transgenic mice with cardiac-specific expression of a dominant-negative AMPK have reduced autophagy induction during ischemia and increased infarct size (23). Although these data suggest that autophagy is upregulated following ischemia and plays a cytoprotective role, there may be certain pathological contexts in which an ischemic cell is unable to induce autophagy. In the cardiomyocyte tumor line HL-1, although autophagy is induced by glucose deprivation alone, autophagosome formation is severely impaired upon combining glucose deprivation with hypoxia (24). Autophagy induction may depend on the degree of energy depletion following prolonged or severe ischemia, since autophagy is an ATP-dependent process (21).

Autophagosome accumulation is further increased during the reperfusion phase (22). However, this accumulation is likely due to decreased AP turnover rather than increased induction, since I/R leads to a profound impairment in autophagic flux as measured by buildup of GFP-LC3 puncta following treatment with lysosomal inhibitors (Figure 2D) (24). The precise role of autophagy during reperfusion remains somewhat controversial. Beclin 1 is upregulated during reperfusion, and overexpression of Beclin 1 enhances autophagic flux in an in vitro model of I/R, which is cytoprotective (22, 24). However, inhibiting autophagy by treatment with 3-MA or Beclin 1 knockdown also increases cell viability in an in vitro model of I/R (22, 25), and *Beclin1* (Beclin 1) heterozygous mice have decreased infarct size following I/R (Table 2) (22). These conflicting data may be attributed to the dual roles of Beclin 1 in regulating both autophagosome formation and autophagic flux. Beclin 1 is an essential component of the phosphatidylinositol 3-kinase (PI3K) complex that mediates autophagosome initiation (Figure 1A); at the same time, Beclin 1 also promotes autophagic flux via interactions with Rubicon, although the precise mechanism for this process is unclear (Figure 1C) (26, 27). This suggests a model in which autophagy is cytoprotective during I/R, but the severe block in autophagic flux following reperfusion is cytotoxic; this block in autophagosome maturation leads to accumulation of APs, expansion of the lysosomal compartment, and eventual leakage of lysosomal proteases and cell death (21). Inhibiting autophagy at an early step by 3-MA treatment or RNAi-mediated depletion of Beclin 1 decreases autophagosome accumulation, increasing cell viability. Similarly, enhancing AP turnover via overexpression of Beclin 1 reduces AP accumulation and is also cytoprotective. Additional studies that more carefully monitor changes in autophagic flux and dissect the effects of inhibiting the early versus late steps in autophagy during I/R remain to be done.

Further evidence for the cytoprotective role of autophagy during I/R comes from studies of cardiac preconditioning. Cardiac preconditioning consists of repeated brief periods of ischemia induced before I/R and is one of the most reproducible methods of preventing tissue damage following I/R (21). Autophagy is upregulated during preconditioning, and perfusion of the dominant negative TAT (HIV-1 transactivator of transcription) fusion protein Atg5-K130R inhibits autophagy and blocks the protective effect of preconditioning (28). In vitro, the cardioprotective drugs uridine-5′-triphosphate, diazoxide, and ranolazine also induce autophagy, and Atg5-K130R expression similarly blocks their cytoprotective effects (28).

Overall, the current data suggest that autophagy plays a cytoprotective role during cardiac I/R, but that induction of autophagy coupled to a block in flux during reperfusion leads to a cytotoxic buildup of the lysosomal compartment. Because of this, therapies that induce AP formation without increasing autophagic flux may cause more harm than good in I/R; in such contexts, inhibitors that block early steps of autophagy may be cytoprotective, at least

during reperfusion. Further research is necessary to determine whether autophagy modulation is a viable therapy in cardiac I/R.

Autophagy and the host response to infection

Autophagy represents one of the most ancient innate immune responses and serves as the first line of defense against bacterial, protozoan, and viral pathogens (17). Xenophagy, or degradation of foreign pathogens by autophagy, is the most direct innate immune response in the cell, and multiple studies have confirmed this important role for autophagy following infection. For example, a subset of *Salmonella typhimurium* are targeted to APs following infection, and autophagy-deficient *Atg5* –/– cells are more permissive for cytosolic growth (29). Similarly, APs are targeted to Sindbis virus in infected neurons, and Atg5 knockdown leads to delayed viral clearance and increased cell death (30).

Intriguingly, numerous pathogens have evolved mechanisms to evade, suppress, or subvert the host autophagic machinery. Cytosolic *Listeria monocytogenes* evades autophagic recognition by recruitment of host cytoskeletal proteins; the bacterial surface protein ActA interacts with the Arp2/3 complex and actin, masking the bacteria behind host proteins. Unlike wild type *L. monocytogenes*, ActA mutant bacteria are efficiently targeted to APs (31). Likewise, *Shigella flexineri* evade autophagy by secreting IcsB, which competitively binds the bacterial surface protein VirG and prevents its interaction with the autophagy machinery component Atg5 (32). Alternatively, *Mycobacterium tuberculosis* blocks autophagosome maturation, suppressing autophagic flux altogether. Rapamycin treatment or starvation overcomes this block and leads to decreased intracellular survival of bacteria (33). Other pathogens such as *Coxiella burnetii* subvert the autophagic machinery and use autophagic vacuoles as sites of replication (34).

Through its role in xenophagic clearance of pathogens, autophagy acts as a direct and critical effector of pattern recognition receptor (PRR) signaling following infection. PRRs are activated by pathogen-associated molecular patterns (PAMPs) and cellular stress signals called danger-associated molecular patterns (DAMPs), allowing recognition of specific pathogens or stress conditions associated with infection (17). The four main classes of PRRs are toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD) leucine-rich repeat containing receptors (NLRs), retinoic acid-inducible gene 1 protein (RIG-I)-like helicase receptors (RLRs), and C-type lectin receptors (CLRs). Autophagy induction downstream of TLR activation has been best characterized. TLR1, TLR2, TLR4, TLR5, and TLR6 mainly recognize bacterial PAMPs, whereas TLR3, TLR7, TLR8, and TLR9 primarily recognize viral molecules. Autophagy is induced downstream of both bacterial and viral TLRs. The mycobacterial lipoprotein LpqH activates autophagy downstream of TLR1 and TLR2 (19). The TLR4 ligand lipopolysaccharide (LPS), another bacterial molecule, induces autophagy and enhances autophagic degradation of *M. tuberculosis* (35). Activation of TLR3 with the synthetic double-stranded RNA analogue polyinosinic-polycytidylic acid or activation of TLR7 with single-stranded RNA stimulate formation of GFP-LC3 puncta and formation of LC3-II (Figure 2B) (36).

Our understanding of the signaling pathways connecting TLR activation to autophagy induction remains incomplete. Autophagy induction following TLR1/2 stimulation with LpqH is mediated by AMPK signaling (Figure 3C), and depletion or inhibition of AMPK decreases autophagosome formation following stimulation (19). Furthermore, Shi and Kehrl demonstrated an interaction between the TLR downstream signaling molecules MyD88 and TRIF and the autophagy protein Beclin 1, suggesting that Beclin 1 recruitment to TLR signaling components mediates autophagy induction. The interaction between Beclin 1 and MyD88 or TRIF is enhanced by TLR signaling and leads to disruption of the inhibitory

Bcl-2/Beclin 1 complex, similarly to BNIP3-dependent disruption of Bcl-2/Beclin 1 following hypoxia (Figures 3B, C) (18).

Autophagy machinery components also directly interact with PRRs and DAMPs. The NLRs NOD1 and NOD2 recruit the essential autophagy protein Atg16L1 to sites of bacterial entry at the plasma membrane, leading to AP formation at these sites (37). High mobility group box 1 (HMGB1) is a nuclear chromatin-associated protein that is released into the cytosol and extracellular space following oxidative stress or necrotic cell death, acting as a DAMP. Interestingly, HMGB1 was recently identified as a novel binding partner of Beclin 1. Following release into the cytosol, HMGB1 competitively binds Beclin 1, disrupting the inhibitory Bcl-2/Beclin 1 complex (38). Finally, the Atg12-Atg5 conjugate directly interacts with the RLRs RIG-I, MDA5, and MAVS. This result points to a potential non-canonical role for Atg12-Atg5 in infection, because in this case autophagy components negatively regulate RLR signaling and subsequent type I interferon (IFN) production, hence suppressing the antiviral immune response; in contrast, autophagy itself generally plays a positive role in antiviral immunity (39). Additional non-canonical roles of ATGs have emerged in recent years and are discussed in the sidebar.

SIDEBAR: Non-canonical functions of ATGs

Although ATGs are traditionally thought to function solely as autophagy mediators, several recent studies have identified autophagy-independent roles for ATGs. As described in the main text, the Atg12-Atg5 conjugate directly interacts with RLRs, suppressing the antiviral immune response (39). Interestingly, non-canonical ATG functions may be a common feature of the immune response. Following infection, the protozoa *Toxoplasma gondii* induces formation of a parasitophorous vacuole that acts as a replication site. Atg5 mediates recruitment of the IFNγ-inducible GTPase IIGP1 to the vacuole, leading to membrane disruption. Surprisingly, this does not involve AP formation, suggesting that this anti-parasitic role of Atg5 is autophagy-independent (147). Similarly, coronaviruses induce formation of double membrane vesicles (DMVs) that act as sites of replication. Non-lipidated LC3 decorates the DMV surface and is necessary for coronavirus infection; this process occurs independently of Atg7 (148).

ATGs may also have autophagy-independent roles outside the context of infection. Recently, a novel conjugation of Atg12 to Atg3 was uncovered. The Atg12-Atg3 complex does not affect classic macroautophagy but instead modulates mitochondrial homeostasis and cell death (149). Rubinstein et al. recently identified a novel proapoptotic role for free Atg12. Atg12 binds and negatively regulates antiapoptotic Bcl-2 family members via a BH3-like motif (150).

(Note to editor: Please insert sidebar near "Autophagy and the host response to infection" section discussing Atg12-Atg5 interaction with RIG-I, MDA5, and MAVS)

Abundant evidence points to a crucial role for autophagy adaptors, also known as cargo receptors, in targeting pathogens to autophagosomes. These adaptors include p62/ sequestosome 1 (SQSTM1), nuclear dot protein 52 kDa (NDP52), neighbor of BRCA1 gene 1 (NBR1), and Optineurin (OPTN), all of which bind both ubiquitin and LC3 family members such as LC3 and GABARAP-1, mediating recruitment of LC3-positive isolation membranes to ubiquitinated targets (Figure 4A) (40–44). Accordingly, ubiquitinated proteins decorate the surface of cytosolic bacteria and are one of the main signals recognized by autophagy adaptor proteins (Figure 4B) (45). Autophagic degradation of several pathogens including *S. typhimurium*, *L. monocytogenes*, *S. flexineri*, and the Sindbis virus is mediated by p62 (30, 31, 46, 47); NDP52 and OPTN have both been shown to promote autophagic degradation of *S. typhimurium* (43, 44).

In addition to these cargo receptors, other regulators of clearance have been identified that do not depend upon an ubiquitin signal. As described above, the essential autophagy protein Atg5 directly binds to VirG on the surface of *S. flexineri* (32). The adaptor Tecpr1 interacts with both Atg5 and WIPI-2 (the mammalian orthologue of Atg18), mediating targeting of early isolation membranes to *S. flexineri* (48). Recently, galectin 8 was identified as a novel adaptor that mediates autophagic degradation of *S. typhimurium*. Galectin 8 binds host glycans exposed on ruptured *Salmonella*-containing vesicles and recruits NDP52 to these sites; galectin-mediated NDP52 recruitment precedes and is independent of ubiquitin-mediated recruitment (Figure 4B) (49). Interestingly, galectin 8—as well as galectins 3 and 9—also bind host glycans on damaged endosomes and lysosomes in the absence of infection, suggesting that they act as general sensors of vesicle damage (49).

Autophagy plays other less direct roles in responding to infection in addition to its primary role in pathogen clearance. Autophagy acts as a topological inversion mechanism that engulfs and delivers cytosolic PAMPs to endosomal PRRs and to major histocompatibility complex class II (MHC II) compartments for antigen loading. The topological inversion function of autophagy is important for multiple components of the immune response including PRR activation in the innate immune response, activation of helper T cells in the adaptive immune response, and preventing autoimmunity (17). Evidence of the latter was demonstrated in thymus-specific *Atg5*-deficient mice. Presentation of self-derived antigens by MHC II complexes on thymic epithelial cells is essential for generation of self-tolerant T cells, and deletion of *Atg5* leads to altered T-cell selection and multiorgan inflammation (Table 2) (50). Finally, autophagy modulates the proinflammatory response. Secretion of the proinflammatory cytokines IL-1β and IL-18 is enhanced in *Atg16L1* and *Atg7* null macrophages, suggesting that basal autophagy suppresses the proinflammatory response (51). This is mediated at least in part by accumulation of damaged mitochondria in autophagy-deficient cells (discussed in detail below) and release of mitochondrial DNA into the cytosol, leading to increased signaling through the NALP3 (a NOD-like receptor family member) inflammasome (52). Interestingly, although basal autophagy suppresses IL-1β production, the opposite is true of autophagy induction. Dupont et al. recently identified a novel autophagy-dependent export pathway for extracellular secretion of IL-1β that requires Atg5, the mammalian Golgi reassembly stacking protein paralogue GRASP55 (GORASP2), and Rab8a, a regulator of exocytosis. Autophagy induction by starvation increases IL-1β secretion in response to multiple proinflammatory agonists, and inhibiting autophagic flux reduces IL-1β secretion (53). These data suggest that autophagy is uniquely able to both suppress proinflammatory signaling during basal conditions and upregulate proinflammatory signaling when induced during times of stress.

IL-1β lacks the signal sequence directing it to the conventional secretory pathway via the Golgi and ER. Interestingly, recent studies suggest that autophagy may play a general role in unconventional protein secretion. Autophagy is involved in unconventional secretion of Acyl-CoA binding protein (Acb1/AcbA) in yeast and Dictyostelium; this process is mediated by delivery of APs containing Acb1/AcbA to the plasma membrane rather than the lysosome (54). In mammalian cells, unconventional secretion of the DAMP HMGB1 and the cytokine IL-18 are increased following autophagy induction, similarly to IL-1β (53). Together, these data suggest that autophagy may play a more general role in secretion during the immune response.

Autophagy in Crohn's disease

Crohn's disease is a chronic form of inflammatory bowel disease that affects the intestines. Mechanisms driving Crohn's pathogenesis include an abnormal inflammatory response, abnormal Paneth cell granule secretion, and impaired intracellular bacterial clearance (55,

56). Autophagy has been implicated in the etiology of this disease by genome-wide association studies identifying disease-related polymorphisms in Atg16L1, IRGM (immunity-related GTPase family M protein), and NOD2 (Table 1) (57). Atg16L1 forms a complex with the Atg12-Atg5 conjugate and is necessary for proper autophagosome elongation (Figure 1B). IRGM and NOD2 are both involved in the autophagic response to infection. IRGM induces autophagy in response to interferon γ (IFNγ), leading to increased clearance of bacterial pathogens (58). As described above, the PRR NOD2 recruits Atg16L1 to bacterial entry sites, targeting bacteria for autophagic degradation (37).

Studies of *Atg16l1, Irgm1*, and *Nod2* knockout mice have provided critical insight into the mechanisms of Crohn's disease progression. *Atg16l1* hypomorphic mice have Paneth cell abnormalities and defects in granule secretion similar to that of Crohn's patients homozygous for the *ATG16L1* disease allele (Table 2) (55). Interestingly, Paneth cell abnormalities are not seen when *Atg16l1* hypomorphic mice are raised in gnotobiotic conditions. Administration of a specific strain of the enteric murine norovirus (MNV) capable of establishing persistent infection recapitulates Paneth cell abnormalities. Combined MNV infection and treatment with the inflammatory agent dextran sodium sulfate (DSS) induces intestinal injury and leads to further abnormalities characteristic of Crohn's disease including blunted intestinal villi and increased intestinal inflammation (59). Surprisingly, *Atg16l1* hypomorphic mice have equivalent rates of MNV replication as wild type mice, suggesting that defects in viral clearance do not underlie disease pathogenesis. Instead, intestinal damage is dependent upon TNFα, IFNγ, and the presence of commensal bacterial flora, suggesting that an abnormal proinflammatory response of *Atg16l1* hypomorphic mice—in the context of viral infection in combination with commensal bacteria—plays a major role in development of Crohn's-like abnormalities (59). Another study has demonstrated that macrophages from chimeric mice lacking Atg16L1 in hematopoietic cells have increased IL-1β production in response to LPS stimulation or infection with non-invasive enteric bacteria. These mice are highly sensitive to DSS-induced colitis, suggesting that increased proinflammatory cytokine secretion from hematopoietic cells may also promote intestinal damage in Atg16L1-dependent Crohn's (51).

Irgm1 null mice have impaired clearance of intracellular bacteria, lymphopenia, and defects in macrophage function (Table 2) (60, 61). Together with data demonstrating that IRGM is important for induction of autophagy and autophagic degradation of bacteria following infection (58), this suggests that autophagy defects underlie the impaired bacterial clearance seen in Crohn's patients. NOD2 disease variants are also associated with reduced autophagic clearance of bacteria (Table 2) (62). *Nod2* null mice have defects in responding to the bacterial PAMP muramyl dipeptide (MDP) and increased susceptibility to bacterial infection (63). Mutant mice expressing the disease-associated allele of NOD2 have increased proinflammatory cytokine secretion in response to NOD2 stimulation and increased sensitivity to DSS-induced intestinal inflammation (64). Interestingly, Atg16L1 directly interacts with NOD2, supporting a common role for the two proteins in Crohn's pathogenesis. As described above, NOD2 recruits Atg16L1 to sites of bacterial entry (37). Both NOD2 and Atg16L1 are necessary for autophagy induction in intestinal epithelial cells following NOD2 stimulation with MDP, and the disease-variant of Atg16L1 blocks MDP-mediated bacterial killing (65). Together, these data suggest that the defects in *Nod2* deficient or mutant mice are at least partly due to impaired autophagic clearance of bacteria.

Further direct support for the role of autophagy in Crohn's disease pathogenesis comes from *Atg5* and *Atg7* conditional knockout mice (Table 2). Mice lacking Atg5 or Atg7 in intestinal epithelial cells develop Paneth cell and granule secretion defects that phenocopy Atg16L1 hypomorphic mice and resemble abnormalities seen in Crohn's patients homozygous for the *ATG16L1* disease allele (55, 66). Overall, given the important role of autophagy in

xenophagic bacterial clearance, modulation of proinflammatory cytokine secretion, and contribution to extracellular secretion pathways, defects in autophagy may underlie multiple mechanisms that promote Crohn's pathogenesis.

AUTOPHAGY IN CELLULAR QUALITY CONTROL AND HOMEOSTASIS

Although autophagy was initially identified as a cellular response to stress, it has long been recognized that cells exhibit a basal level of autophagy independent of nutrient and stress status. The two principal degradation pathways in the cell are the ubiquitin-proteasome system (UPS) and autophagy. Whereas the UPS mainly degrades short-lived proteins, autophagy specializes in the removal of long-lived proteins, and, unlike the UPS, it is uniquely able to degrade whole organelles such as mitochondria, peroxisomes, and endoplasmic reticulum (ER) (67). The homeostatic role of autophagy involves both non-selective degradation that supports basal turnover of cytoplasmic components and selective degradation that specifically targets damaged or aggregated organelles and proteins. Selective autophagy serves an essential cellular quality control function in cells (67).

The generation of tissue-specific conditional knockout *Atg5* and *Atg7* mice first revealed the essential role for basal autophagy in the degradation of protein aggregates and damaged organelles (Table 2). *Atg7*-deficient hepatocytes and β cells, *Atg5*-deficient cardiomyocytes, and *Atg5*- or *Atg7*-deficient muscle cells accumulate abnormal mitochondria and ubiquitin-positive protein aggregates and inclusion bodies (8, 68–72). Ubiquitinated protein aggregates also accumulate in the brains of neuron-specific *Atg5* and *Atg7* knockout mice (73, 74). In addition to mitochondrial abnormalities, *Atg7* null hepatocytes accumulate peroxisomes, and *Atg7* null β cells have abnormally distended ER and Golgi (8, 69). Importantly, in *Atg5*-deficient neurons, diffuse ubiquitin positive proteins accumulate before protein aggregates are observed, supporting the idea that autophagy plays a crucial role in basal protein turnover in addition to degrading damaged or aggregated proteins (73).

As in xenophagy, autophagy adaptors target the autophagy machinery to specific substrates such as ubiquitinated proteins or depolarized and/or damaged mitochondria; p62 and NBR1, which bind both LC3 family members and ubiquitin and promote autophagic degradation of ubiquitinated substrates, also mediate the removal of protein aggregates (Figure 4A, C). Hence, autophagy-deficient cells accumulate ubiquitin-positive aggregates containing p62 and NBR1 (41, 42). Another ubiquitin-binding protein, HDAC6, is important for autophagic clearance of both protein aggregates and mitochondria. In contrast with other adaptors that interact with components of the autophagy machinery, HDAC6 recruits dynein microtubule motors and actin remodeling machinery to ubiquitinated substrates, promoting transport of ubiquitinated substrates to autophagosomes and leading to enhanced fusion of autophagosomes to lysosomes at these sites (Figure 4C) (75, 76).

Multiple adaptors have been identified that mediate specific degradation of mitochondria by autophagy, termed mitophagy (77). Nix, also known as BNIP3L, is a mitochondria-localized BH3-only protein that is upregulated during hypoxia. In response to mitochondrial stressors, Nix promotes mitochondrial depolarization and subsequent formation of reactive oxygen species (ROS), leading to autophagy induction via stress response pathways. Moreover, Nix binds LC3 family members, particularly GABARAP-L1, which mediates targeting of autophagosomes to depolarized mitochondria (Figure 5A, B) (78, 79). Another recently described mitochondrial protein, FUNDC1 (Figure 5A), interacts with LC3 following its hypoxia-induced dephosphorylation, acting as a mitophagy receptor following oxygen deprivation (80).

The most well described mitophagy pathway involves the proteins PINK1 (PTEN-induced putative kinase 1) and Parkin (Figure 5C). The mitochondrial resident protein PINK1 is constitutively degraded in healthy mitochondria; this is mediated by membrane potential-dependent cleavage of PINK1 by the inner mitochondrial membrane protease presenilin-associated rhomboid-like protein (PARL) (81). Following mitochondrial depolarization, PINK1 accumulates on the outer mitochondrial membrane (OMM) and recruits the E3 ubiquitin ligase Parkin. Parkin ubiquitinates multiple proteins on the OMM, leading to proteasomal degradation of OMM components followed by autophagosome recruitment and autophagic degradation of damaged mitochondria (82–87). Notably, other mitophagy adaptors also play a role in Parkin-mediated mitophagy. Because Nix promotes mitochondrial depolarization, it also promotes Parkin translocation (78). NBR1 and p62 colocalize with Parkin-positive mitochondria and mediate autophagosome targeting to damaged, ubiquitin positive mitochondria (82). Interestingly, on depolarized mitochondria, stabilized PINK1 also phosphorylates Miro, an adaptor protein that connects the motor protein kinesin to the OMM surface. Phosphorylation of Miro leads to its Parkin-dependent degradation and subsequently to mitochondrial arrest, suggesting that the PINK1-Parkin pathway serves as a mechanism to isolate damaged mitochondria in addition to promoting mitophagic degradation (88).

THE HOMEOSTATIC AND QUALITY CONTROL ROLES OF AUTOPHAGY PREVENT CELL INJURY AND DISEASE

Basal autophagy may be particularly important in post-mitotic cells such as neurons and myocytes, because, unlike dividing cells, they are unable to dilute out damaged proteins and organelles into daughter cells (1). Indeed, loss of autophagy in neurons or muscle generally leads to atrophy and cell death, as detailed below (70, 72–74). Highly secretory cells such as hepatocytes and β cells may also be more dependent upon intact autophagy since they produce large quantities of protein in the ER, leading to higher levels of misfolded proteins and aggregates (89). Below, we discuss the role of autophagy in these tissues and describe how impaired basal autophagy may contribute to diseases such as neurodegeneration, myopathy, liver disease, and diabetes.

Autophagy in neurodegenerative disease

Basal autophagy is essential for proper neuron function and generally plays a cytoprotective role, preventing buildup of protein aggregates and damaged mitochondria. Neuron-specific *Atg5* and *Atg7* knockout mice develop progressive motor defects accompanied by neurodegeneration and accumulation of polyubiquitinated proteins and aggregates (Table 2) (73, 74). Interestingly, Beclin 1 expression decreases with age in the human brain, suggesting that decreased autophagy may underlie the observed association between advanced age and increased incidence of neurodegenerative disease (90). Autophagy has been implicated in numerous neurodegenerative diseases; here, we focus on Parkinson's, Alzheimer's, and Huntington's disease.

Autophagy in Parkinson's disease—Parkinson's disease (PD) is caused by the degeneration of dopaminergic neurons in the substantia nigra. Characteristic features of PD include accumulation of autophagic vacuoles, abnormal mitochondrial function, and the presence of Lewy bodies—intracellular inclusions containing the protein α-synuclein and ubiquitin (77). Although most Parkinson's cases are sporadic, about 10% of cases are hereditary. Studies of familial inherited forms of PD have identified multiple disease-associated mutations, providing insight into the etiology of both hereditary and sporadic PD and implicating alterations in autophagy in disease pathogenesis (Table 1).

The first identified PD-associated polymorphism was in α-synuclein, the major component of Lewy bodies. PD-associated mutations in α-synuclein cause it to form amyloid like fibrils associated with disease progression. Transgenic mice overexpressing a disease-associated mutant A53T α-synuclein develop protein inclusions accompanied by neurodegeneration and motor defects, mimicking PD (91). Wild type and disease-associated mutant α-synuclein are degraded by macroautophagy, chaperone-mediated autophagy (CMA), and the proteasome (92–94). Mutant α-synuclein inhibits CMA by blocking the LAMP2A receptor, preventing protein translocation into the lysosome and subsequent degradation; the cell compensates for this defect in CMA by upregulating macroautophagy (92). Autophagy upregulation in A53T-expressing neurons in vitro leads to profound loss of mitochondrial mass accompanied by depletion of cellular ATP and cell death (95), and treatment with 3-MA or knockdown of Parkin or Beclin 1 partially protects against A53T-mediated cytotoxicity (95, 96). Overall, these data suggest that, in contrast to its usual cytoprotective role, the compensatory upregulation of macroautophagy—and increased mitophagy—in response to pathogenic α-synuclein is cytotoxic and may worsen progression of familial or sporadic forms Parkinson's disease caused by mutations in α-synuclein.

Interestingly, gene duplication of wild type α-synuclein has also been implicated in familial Parkinson's disease (97). Whereas mutant α-synuclein induces macroautophagy, increased expression of the wild type protein has the opposite effect. Overexpression of wild type α-synuclein in a human neuroblastoma cell line causes mislocalization of Atg9 and suppresses autophagosome biogenesis via inhibition of Rab1a, a GTPase involved in ER-Golgi trafficking (98). Transgenic mice overexpressing wild type α-synuclein accumulate α-synuclein aggregates and abnormal APs and lysosomes in neurons leading to neuron death and motor defects (99, 100). Lentiviral delivery of Beclin 1 to the brains of these mice reduces α-synuclein accumulation and disease pathology (100). Overall, these data suggest that imbalanced autophagy—either overactive or suppressed—can be detrimental to neuron survival in the context of α-synuclein pathology. Further research into α-synuclein pathology and alterations in autophagic flux in sporadic PD will be necessary to determine whether autophagy modulation might be beneficial or harmful in these contexts.

Mutations in PINK1 and Parkin cause an autosomal recessive form of early-onset PD (Table 1) (101, 102). As described above, PINK1 recruits Parkin to depolarized mitochondria, leading to ubiquitination of OMM proteins, autophagosome recruitment, and mitophagic degradation (Figure 5C) (82–87). In cell culture systems, PD-associated mutations in PINK1 or Parkin impair mitophagy via several mechanisms, including: 1) defects in targeting of Parkin to depolarized mitochondria, 2) disruption of Parkin's E3 ligase activity, or 3) formation of non-functional proteins that aggregate in intracellular inclusions (86, 103, 104). Fibroblasts isolated from patients with Parkin mutations have decreased mitochondrial complex I activity suggestive of impaired mitochondrial function; complex I activity defects are also seen in the substantia nigra of patients with sporadic PD (105, 106). Together, these data suggest that mitophagy defects may underlie PD pathogenesis.

Surprisingly, *Parkin* and *PINK1* knockout mice do not recapitulate PD (Table 2). Mice lacking Parkin or PINK1 have mitochondrial defects including decreased complex I activity, reduced respiration, and increased sensitivity to oxidative stress, but these defects occur in the absence of substantia nigra degeneration (107, 108). Aged *PINK1*–/– mice have progressive mitochondrial dysfunction, weight loss, and reduction in spontaneous locomotive activity similar to PD patients. However, they lack Lewy body accumulation, neurodegeneration, and decreased lifespan that are seen in PD patients (109). These data suggest that patients with *Parkin* or *PINK1* mutations have other genetic variations that modify neuronal sensitivity to decreased mitophagy. Alternatively, polymorphisms in *Parkin* and *PINK1* in patients may not strictly act as loss-of-function mutations; rather, they

may also exert dominant negative or dominant toxic effects. Supporting the latter hypothesis, transgenic mice overexpressing a truncated mutant human Parkin in dopaminergic neurons develop progressive motor defects accompanied by substantia nigra degeneration and α-synuclein accumulation, more closely mimicking PD progression (110). Overall, the alterations in Parkin and PINK1 in patients with hereditary PD suggest that a mitophagy deficit contributes to PD pathology, although it may not be the sole cause.

One of the most well-characterized mouse models of PD uses MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment. Administration of MPTP leads to Parkinson-like disease in both primates and mice. The toxic metabolite of MPTP, MPP+, accumulates in dopaminergic neurons, leading to neurodegeneration and motor defects similar to those seen in PD including rigidity, tremor, and akinesia (77). In MPP+ treated neurons, an increase in autophagosomes precedes cell death; both Atg5 or Atg7 knockdown as well as pretreatment with the early autophagy inhibitor 3-MA abrogate MPP+ cytotoxicity (96, 111), suggesting that overactive autophagy enhances MPP+-mediated cell death. However, because these studies have not measured autophagic flux, they are unable to distinguish between autophagy induction and a late block in autophagy in response to MPP+ (Figure 2). Additional studies indicate that MPTP treatment in mice or MPP+ treatment in cell culture leads to lysosomal membrane permeabilization and lysosomal depletion in neurons, presumably causing a block in autophagic flux and AP accumulation. Treatment with rapamycin restores lysosomal levels, enhances AP clearance, and blocks MPP+-mediated cell death in vitro and neurodegeneration in vivo (112). The mechanism by which rapamycin enhances lysosomal activity is unclear. Overall, similar to cardiac I/R, the results above suggest that autophagy protects against MPTP-induced cell death but that a block in autophagic flux and resulting lysosomal permeabilization is cytotoxic. As a result, inhibiting the initial steps of autophagy via 3-MA treatment or ATG knockdown reduces AP accumulation and is cytoprotective. Similarly, promoting autophagic flux via rapamycin treatment (by an unknown mechanism) reduces AP accumulation and also increases cell viability. Overall, given the conflicting results regarding the cytotoxic versus cytoprotective effects of autophagy in different models of PD (e.g. α-synuclein, MPTP, etc.), further research clarifying autophagic activity and flux in PD patients and model systems will be required to determine whether autophagy modulation is a viable therapy for this disease.

Autophagy in Alzheimer's disease—Alzheimer's disease (AD) is a form of dementia characterized by neuronal death in the cerebral cortex. The hallmarks of AD are the presence of intracellular neurofibrillary tangles containing hyperphosphorylated tau and presence of extracellular β-amyloid (Aβ) plaques generated by cleavage of amyloid precursor protein (APP) by γ-secretase (77). Accumulation of autophagosomes and abnormal mitochondria are seen in postmortem brains of AD patients, suggesting that autophagy plays a role in disease progression (113). Several studies suggest that defects in autophagic maturation may be a general feature of Alzheimer's pathology. AD-associated mutations in the transmembrane protein presenilin 1, that cause a form of familial early-onset AD, lead to defects in autophagic flux due to faulty targeting of the v-ATPase V0a1 subunit to the lysosome and resulting defects in lysosomal acidification (Table 1) (114). More broadly, immature forms of autophagic vacuoles such as isolation membranes (IMs) and autophagosomes (APs) rather than autolysosomes (ALs) (Figure 1) accumulate in AD brains. The accumulated autophagic vacuoles resemble those seen in primary cortical neurons following inhibition of lysosomal proteases or vinblastine treatment—both of which block autophagosome maturation—suggesting that autophagosome accumulation in AD brains is at least partly caused by a block in autophagic flux (113, 115, 116).

Other studies have demonstrated that autophagy plays a role in degradation of pathogenic APP and tau. In cells stably expressing wild type APP or an AD-associated mutant APP,

Beclin 1 knockdown leads to accumulation of APP and Aβ, whereas autophagy induction via Beclin 1 overexpression or rapamycin treatment decreases APP and Aβ levels (117, 118). Supporting this in vitro data, reduced Beclin 1 levels accompanied by increased APP and Aβ levels are seen in AD patients. Additionally, *Beclin1* heterozygous mice expressing AD-associated mutant APP have increased APP and Aβ aggregation and more severe neurodegeneration compared to wild type mice (118, 119). In keeping with the putative autophagic flux defect seen in brains of AD patients, APP overexpression itself leads to autophagosome accumulation in cell culture models; this is likely due to a maturation defect, although further studies more directly measuring autophagic flux (Figure 2C) remain to be done. Interestingly, both APP and γ-secretase accumulate in autophagosomes, suggesting that the autophagic compartment can also act as a source of pathogenic Aβ when autophagosome maturation is impaired (116). Pathogenic tau is also degraded by autophagy. Autophagy inhibition by 3-MA increases tau aggregation and toxicity, and autophagy induction with rapamycin decreases toxicity in cells overexpressing mutant tau (120, 121).

There is conflicting data about whether autophagy is cytoprotective or cytotoxic in AD. Autophagy inhibition has generally been found to increase tau toxicity, whereas rapamycin decreases toxicity (120, 121). Similarly, Atg7 knockdown increases Aβ toxicity in a neuroblastoma cell line, and *Beclin1* heterozygous mice have increased susceptibility to APP-induced neurodegeneration (118, 119, 122). However, another study indicates that inhibition of autophagy by 3-MA treatment or Beclin 1 knockdown decreases Aβ toxicity in human neuroblastoma and glioma cell lines (123). Once again, an intriguing possibility is that the cytoprotective versus cytotoxic effect of autophagy inhibition in AD models depends upon the level of autophagic flux and degree of lysosomal impairment in each system. In this case, autophagy inhibition will be cytoprotective in cells with profound impairment in autophagic maturation—in which there is expansion of the autophagosomal/lysosomal compartment and increased Aβ production—but cytotoxic in cells with less severe impairment in flux.

Although there is conflicting data about autophagy inhibition in AD models, autophagy induction using rapamycin has generally been found to play a cytoprotective role. In a mouse model of AD using transgenic mice expressing both pathogenic APP and tau, autophagy induction by rapamycin rescues cognitive deficits and decreases accumulation of Aβ and tau aggregates (117). One might speculate that rapamycin promotes both autophagy and lysosomal function in AD models, as is the case in MPTP models of PD, and is thus able to rescue autophagic flux defects. Overall, further research is required to determine the feasibility of autophagy modulation as a treatment for AD, including interrogating the changes in autophagic flux that occur during AD pathogenesis and dissecting the effects of specifically manipulating discrete stages of autophagy in AD patients.

Autophagy in Huntington's disease—Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by a trinucleotide CAG (polyglutamine) expansion within the protein huntingtin (htt). HD causes death of striatal and cortical neurons accompanied by mitochondrial dysfunction and accumulation of mutant htt within inclusion bodies (77). Polyglutamine-expanded (PolyQ) htt is degraded by autophagy, suggesting that increased autophagy may provide a therapeutic benefit in HD. Mutant htt accumulates in autophagosomes (124, 125), and autophagy inhibition by Beclin 1 knockdown increases levels of polyQ-htt (90). In contrast, autophagy induction by rapamycin enhances clearance of a polyQ-htt fragment and reduces htt-dependent cell death (125).

Interestingly, in mouse and human cellular models of HD and cells from HD patients, autophagosome formation and clearance are normal but autophagic vacuoles contain little

cargo, suggesting that impaired cargo recognition and the resulting defect in engulfment of cytoplasmic components underlies the accumulation of dysfunctional mitochondria and protein aggregates seen in HD (126). The cargo recognition defect may be in part caused by sequestration of p62 by mutant htt (126). Given that the inefficient recognition of autophagic substrates may cause certain defects in Huntington's disease, either increasing the efficiency of autophagic cargo degradation or simply increasing overall levels of autophagy may be therapeutically useful in these patients. Indeed, treatment with rapamycin or a rapamycin analogue reduces htt aggregates and slows neurodegeneration in fly and mouse models of HD (127).

Autophagy in myopathy

Myopathy is defined as muscle weakness resulting from muscle fiber dysfunction. There are many forms of myopathy with distinct pathologic features including muscle cell degeneration, cellular changes leading to reduced contractile function, mitochondrial alterations leading to energy deficits, metabolic defects, and abnormal inflammation. Basal autophagy is essential for maintenance of proper muscle mass and function, and defects in autophagy are sufficient to cause myopathy in mice. Muscle-specific *Atg5* and *Atg7* knockout mice develop severe muscle atrophy and progressive muscle weakness accompanied by accumulation of abnormal mitochondria, accumulation of ubiquitinated proteins, and abnormal lysosome distribution (Table 2) (70, 72). Autophagy also protects against stress-induced myopathy; it is induced in muscle cells in response to muscle atrophy induced by starvation or denervation (128, 129), and mice lacking *Atg7* in myocytes have increased muscle loss in response to either of these stresses (70). The autophagic response to muscle atrophy is in part mediated by BNIP3 (Figure 3B) (130).

Direct evidence for the importance of autophagic flux for muscle maintenance in humans can be found in a group of myopathies characterized by lysosomal defects (Table 1). Pompe disease is caused by a defect in lysosomal α-glucosidase, leading to a defect in lysosomal glycogen degradation and severe myofibril loss. In a mouse model of Pompe disease, muscle-specific loss of α-glucosidase leads to muscle atrophy along with autophagy induction coupled to a defect in autophagosome-lysosome fusion. However, the loss of Atg5 in addition to α-glucosidase further increases the degree of muscle loss, suggesting that the block in AP-lysosome fusion is partial and that autophagy induction may still provide therapeutic benefit to these patients (72). Danon disease is caused by defects in the lysosomal protein LAMP2B, leading to cardiomyopathy and myopathy. The loss of LAMP2B function in human patients, as well as mice, results in the accumulation of autophagosomes due to impaired autophagosome-lysosome fusion (131). Other lysosomal myopathies include X-linked myopathy with excessive autophagy. In each of these diseases, defects in autophagic flux are associated with severe forms of myopathy.

Although autophagy defects can lead to myopathy, excessive autophagy may also promote muscle loss under certain conditions. High levels of oxidative stress contribute to muscle disease, and transgenic mice with muscle-specific expression of mutant G93A superoxide dismutase 1 (SOD1), one of the major antioxidant enzymes, develop progressive muscle atrophy and mitochondrial dysfunction associated with autophagy induction (132). In this context, muscle cell degeneration may be in part due to excessive autophagy; in vivo RNAi-mediated knockdown of LC3 via electrotransfer partially rescues muscle atrophy (132). Jumpy (myotubularin related protein 14, MTMR14) is a PI3P phosphatase that negatively regulates autophagy by reducing PI3K-dependent recruitment of WIPI-1 to autophagosomes (133). A R336Q mutation in Jumpy that renders it catalytically inactive was identified in a patient with centronuclear myopathy, a congenital myopathy in which nuclei are abnormally localized in skeletal muscle cells, leading to decreased contraction (Table 1). Mutant R336Q

Jumpy is unable to negatively regulate autophagy (133), suggesting that overactive autophagy may contribute to muscle dysfunction in this type of myopathy. Overall, these data suggest that homeostatic levels of autophagy in muscle are cytoprotective, but that either too much or too little can lead to muscle dysfunction.

Autophagy in liver disease

As in muscle, proper liver function requires basal autophagy. Hepatocytes may be particularly dependent upon basal autophagy due to their high biosynthetic activity and role in protein and carbohydrate storage. Mice lacking *Atg7* in the liver develop hepatomegaly and accumulate abnormal concentric membranous structures, abnormal organelles such as mitochondria and peroxisomes, and ubiquitin-positive protein inclusions (Table 2) (8). Liver-specific *Atg7* knockout mice also have altered lipid profiles including higher total triglyceride (TG) levels, increased TG accumulation following nutrient deprivation, and increased total cholesterol levels, suggesting that basal autophagy controls lipid metabolism (134).

One of the main causes of liver disease is chronic alcohol use. Interestingly, chronic alcohol consumption inhibits liver cell autophagy, suggesting that decreased autophagy may underlie the pathogenesis of alcoholic liver disease. Rats chronically fed alcohol exhibit decreased numbers of hepatic autophagic vacuoles and the accumulation of abnormal lysosomes in parallel with decreased degradation of long-lived proteins and accumulation of protein and lipid (135, 136). Consistent with an autophagy defect, patients with a form of alcoholic liver disease called alcoholic steatohepatitis accumulate protein aggregates called Mallory-Denk bodies (MDBs) primarily composed of keratin 8 and keratin 18 that are positive for ubiquitin and p62 (137). In a mouse model of MDB formation, autophagic vacuoles contain keratins 8 and 18 as well as ubiquitin, and rapamycin treatment decreases the number of MDBs (138), suggesting that autophagy is involved in clearance of these aggregates. Together, these data suggest that alcohol-mediated autophagy inhibition may promote the development of alcoholic liver disease. One potential mechanism by which alcohol inhibits autophagy is through the reduction of AMPK activity. As discussed above, AMPK induces autophagy in response to low cellular ATP (Figure 3A). Ethanol administration reduces hepatic AMPK activity in both mice and cell culture, and the AMPK activator AICAR protects against alcohol-induced fatty liver in rats, suggesting that autophagy induction via AMPK activation may be a viable therapy in alcoholic liver disease (139, 140).

Fatty liver disease, characterized by triglyceride accumulation in liver cells, can also occur in the absence of chronic alcohol consumption. The main risk factors for development of non-alcoholic fatty liver disease (NAFLD) are obesity and insulin resistance. As described above, basal autophagy regulates intracellular lipid content in hepatocytes. Following starvation, lipid droplets stored by hepatocytes are degraded by autophagy to supply free fatty acids for energy production; loss of Atg5 or 3-MA treatment leads to increased TG storage similar to that seen in fatty liver disease (134). Autophagy levels also modulate insulin signaling. *Atg5* and *Atg7* null MEFs have decreased insulin receptor signaling following insulin stimulation, and in vivo knockdown of *Atg7* in the liver of lean mice leads to severe insulin resistance (89). Together, these data suggest that decreases in hepatic autophagy can cause TG accumulation and insulin resistance and may contribute to fatty liver disease.

What mechanisms might underlie reduced autophagy in patients with fatty liver disease? Insulin is a major hormonal regulator of autophagy that suppresses autophagy induction, and obesity itself may elicit reductions in autophagy. In both genetic (*ob/ob*) and high fat diet (HFD)-induced models of obesity and insulin resistance, hepatic autophagy is decreased (89,

141). This may be partly due to hyperinsulinemia, although insulin depletion by ablation of pancreatic β cells does not restore autophagy levels (89). Instead, autophagy inhibition is partly mediated by an increase in calpain 2, which degrades Atg7, and overexpression of Atg7 in the liver of *ob/ob* mice partially rescues glucose tolerance and insulin sensitivity (89). Taken as a whole, the data suggest that alcohol- or obesity-mediated defects in autophagy contribute to progression of some forms of liver disease.

Autophagy in type 2 diabetes

As previously discussed, autophagy plays a key role in removing damaged mitochondria and ubiquitinated proteins and aggregates. Both of these processes—mitophagy and autophagic removal of damaged proteins—are implicated in β cell homeostasis and survival (69, 142). The removal of damaged or aggregated proteins via autophagy is especially important following endoplasmic reticulum (ER) dysfunction, known as ER stress, and β cells are particularly sensitive to ER stress because of their role in insulin secretion (69).

Direct evidence for the importance of autophagy in β cell function comes from β cell-specific A*tg7* knockout mice (*ATG7:RIP-Cre*) (Table 2). *ATG7:RIP-Cre* mice fed a high fat diet (HFD) phenocopy type 2 diabetes with progressive β cell degeneration, hyperglycemia, loss of insulin production, and cellular hypertrophy. The β cells of *ATG7:RIP-Cre* mice have characteristics associated with both autophagy defects and diabetes pathology, including accumulation of damaged mitochondria, reduced respiration, increased oxidative stress, and accumulation of ubiquitinated p62-positive aggregates (68, 69, 142). Ebato et al. demonstrated that autophagy maintains both β cell number and function (i.e. insulin secretion) in mice fed a high fat diet. In mice with intact autophagy, a compensatory increase in β cell mass is seen following HFD; this is not seen in mice lacking pancreatic Atg7 (68). Autophagy-deficient islet cells also have decreased insulin secretion. This defect can be partially rescued by antioxidant treatment, suggesting that increased ROS production by damaged mitochondria may play a role in suppressing secretion (142).

Data suggests that patients with type 2 diabetes have autophagy defects; patient samples have greatly increased numbers of autophagic vacuoles along with reduced LAMP2, suggesting that these patients have impaired lysosome activity and autophagosome maturation (143). The same holds true in multiple rodent models of diabetes. In a *Rab3A* −/− mouse model of diabetes, autophagosomes accumulate in islet β cells in conjunction with decreased LAMP2 (144). Increased autophagosomes are also seen by electron microscopy in *db/db* mice and ZDF rats, along with p62 accumulation in *db/db* mice and age-dependent accumulation of polyubiquitinated GFP-LC3-positive protein aggregates ZDF rats, again suggesting that autophagosomes accumulate due to a decrease in autophagic flux (68, 145, 146). Together, these data suggest that autophagy is essential for proper β cell function, particularly in response to a high fat diet. Autophagic flux is suppressed in diabetic patients and in multiple models of diabetes, suggesting that decreased autophagy contributes to the development of this disease.

CONCLUSION

Autophagy plays an essential role in the adaptive response to cell stress and in the maintenance of cellular homeostasis and quality control. Both functions of autophagy are important in disease: in responding to disease-induced cell stress and in preventing homeostatic imbalances that lead to cell injury and disease. On the whole, most evidence supports that autophagy has a beneficial role in human health and disease prevention. Defects in discrete steps in autophagy—either in induction, cargo recognition, or flux—are a common feature of multiple diseases including I/R, Crohn's disease, neurodegeneration, myopathy and diabetes; such deficiencies may drive or exacerbate disease progression

(Figure 6). However, as is the case in some rare forms of myopathy and PD, too much autophagy can also be pathogenic, leading to excessive self-digestion of cellular components. A more thorough understanding of the perturbations that occur in autophagy induction and autophagic flux in these diseases will be important for development of therapeutics that target autophagy. Autophagy inhibitors may be useful in treating diseases in which there is either overactive autophagy or a cytotoxic late block in autophagic flux. Similarly, treatments that induce autophagy may be beneficial in diseases where there is a block in autophagy initiation but will be harmful in diseases that have a severe block in autophagic maturation (Figure 6). Further research into the basic autophagic and lysosomal machinery and upstream signaling pathways will be necessary to identify novel targets for disease therapies that induce or inhibit autophagy at its different steps.

Acknowledgments

GLOSSARY

Macroautophagy	Catabolic pathway involving sequestration of cytoplasmic components into a double membrane structure called the autophagosome, and subsequent lysosomal degradation
Basal autophagy	Constitutive autophagic degradation that proceeds in the absence of any overt stress or stimulus and serves an important housekeeping mechanism
Xenophagy	Selective autophagic degradation of intracellular pathogens in a host cell
Mitophagy	Selective autophagic degradation of damaged or superfluous mitochondria
Pattern recognition receptor	Receptor activated by pathogen-specific molecules or damage signals following infection
Autophagy adaptor (cargo receptor)	Adaptor protein that mediates autophagosome targeting to cargo (e.g. mitochondria, aggregates), often via ubiquitin- and LC3-binding domains

LIST OF ACRONYMS USED

AP	autophagosome
ATG	autophagy gene
mTOR	mammalian target of rapamycin
3-MA	3-methyladenine
PRR	pattern recognition receptor
PAMP	pathogen-associate molecular pattern
DAMP	danger-associated molecular pattern
I/R	ischemia-reperfusion

PD	Parkinson's disease
AD	Alzheimer's disease
HD	Huntington's disease
NAFLD	non-alcoholic fatty liver disease

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SUMMARY POINTS

1. Autophagy is an essential pro-survival pathway in cells induced following diverse stresses such as starvation, hypoxia, infection, and oxidative stress.

2. Autophagy also maintains cellular homeostasis in the absence of stress by promoting basal (non-selective) turnover of cytoplasmic components including whole organelles.

3. Selective autophagy, including mitophagy and degradation of ubiquitinated substrates, is an essential cellular quality control mechanism that degrades damaged cellular components.

4. Specific autophagy adaptors, also known as cargo receptors, are important mediators or selective autophagy.

5. Alterations in autophagy in human disease include: 1) defects in autophagy induction, 2) defects in cargo recognition, 3) defects in autophagic maturation, and 4) overactive autophagy.

6. Autophagy induction (e.g. by rapamycin) may provide a therapeutic benefit in diseases with defects in autophagy induction, cargo recognition, or autophagic maturation.

7. Inhibiting the early steps in the autophagy pathway may be therapeutically useful in diseases with a severe block in autophagic maturation; in contrast, inhibiting either early or late steps in the autophagy trafficking process may be useful in diseases notable for "overactive" autophagy.

8. A more complete understanding of the autophagy alterations that occur in different diseases will be important for development of therapeutics that target autophagy.
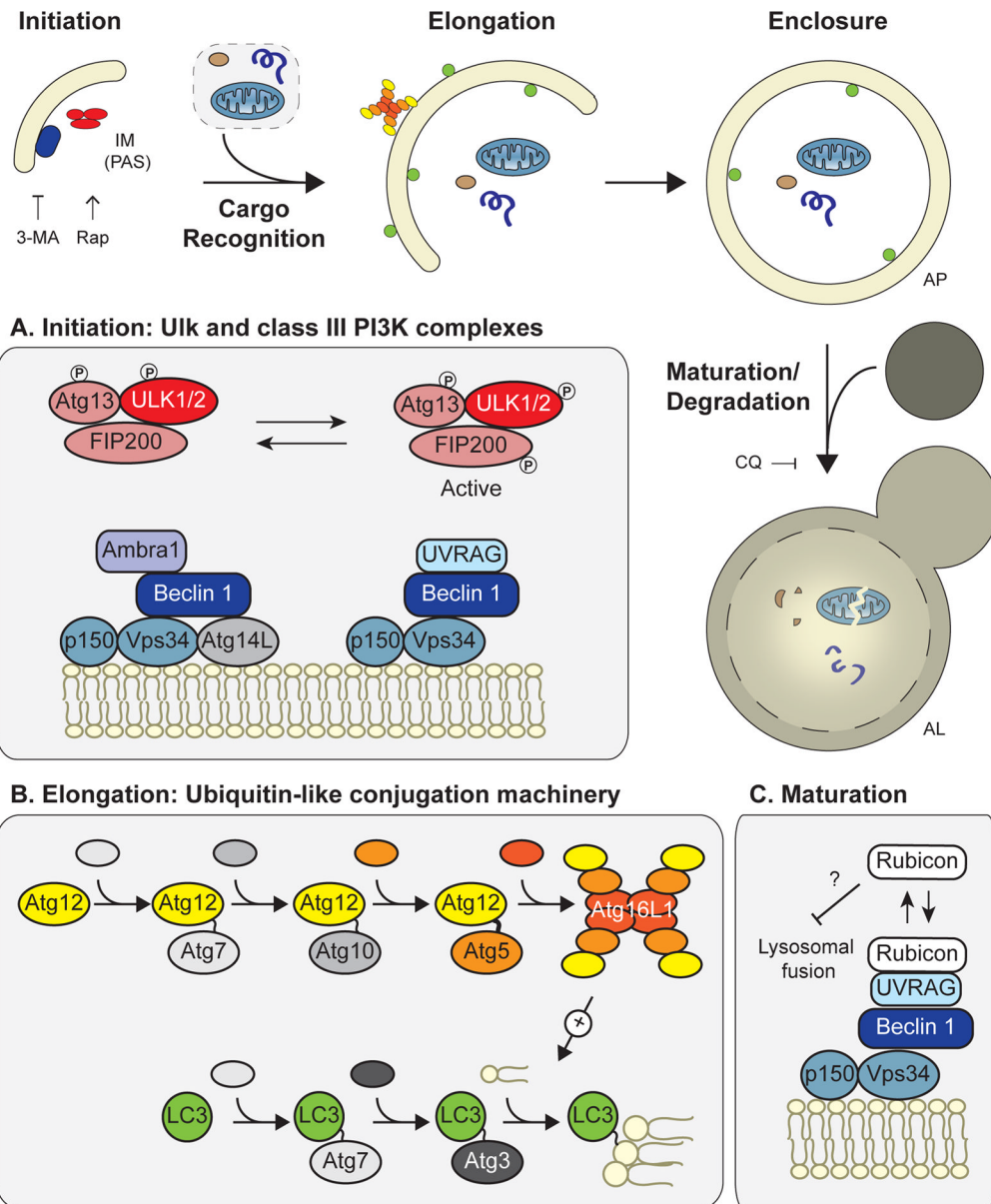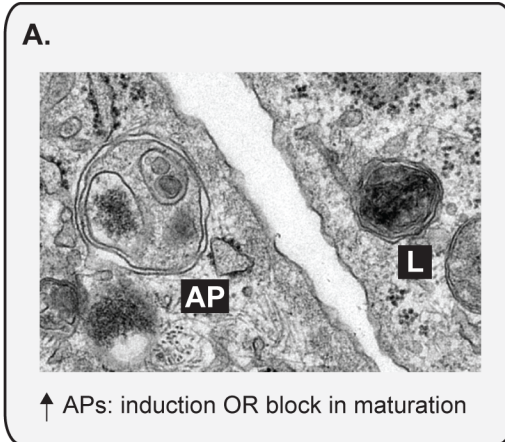
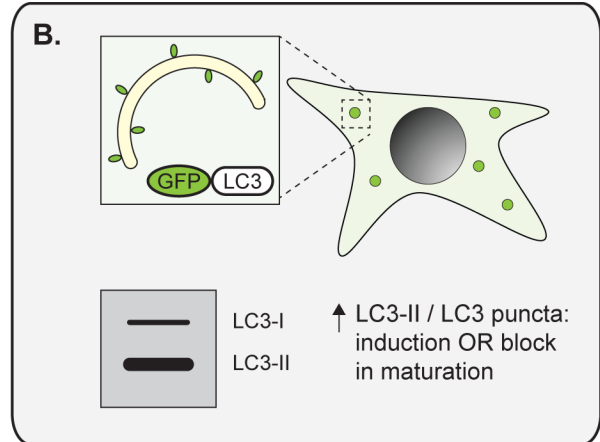**Figure 1. Overview of the mammalian autophagy machinery**

Autophagy occurs in a series of distinct stages: 1) initiation of the isolation membrane, also known as the phagopore assembly site; 2) cargo recognition; 3) elongation; 4) enclosure of the double-membrane structure to form the autophagosome; and 5) maturation/degradation, in which the AP fuses to the lysosome to form an autolysosome, upon which its contents are degraded. The effects of autophagy inhibitors 3-MA and chloroquine and the autophagy inducer rapamycin are shown. (**A**) The Ulk and class III PI3K complexes are necessary for autophagy initiation. Beclin 1 forms two distinct autophagy-promoting complexes with the PI3K, Vps34. (**B**) The ubiquitin-like conjugation machinery attaches Atg12 to Atg5 and LC3 to the lipid phosphotidylethanolamine; this machinery mediates autophagosome elongation. Loss of any of these core components (e.g. Beclin 1, Atg5, etc.) leads to autophagy deficiency. (**C**) In addition to its role in autophagy induction, Beclin 1 promotes autophagic maturation via interactions with Rubicon. Abbreviations: IM, isolation

membrane; PAS, phagopore assembly site; AP, autophagosome; AL, autolysosome; 3-MA, 3-methyladenine; CQ, chloroquine; Rap, rapamycin; PI3K, phosphatidylinositol 3-kinase.
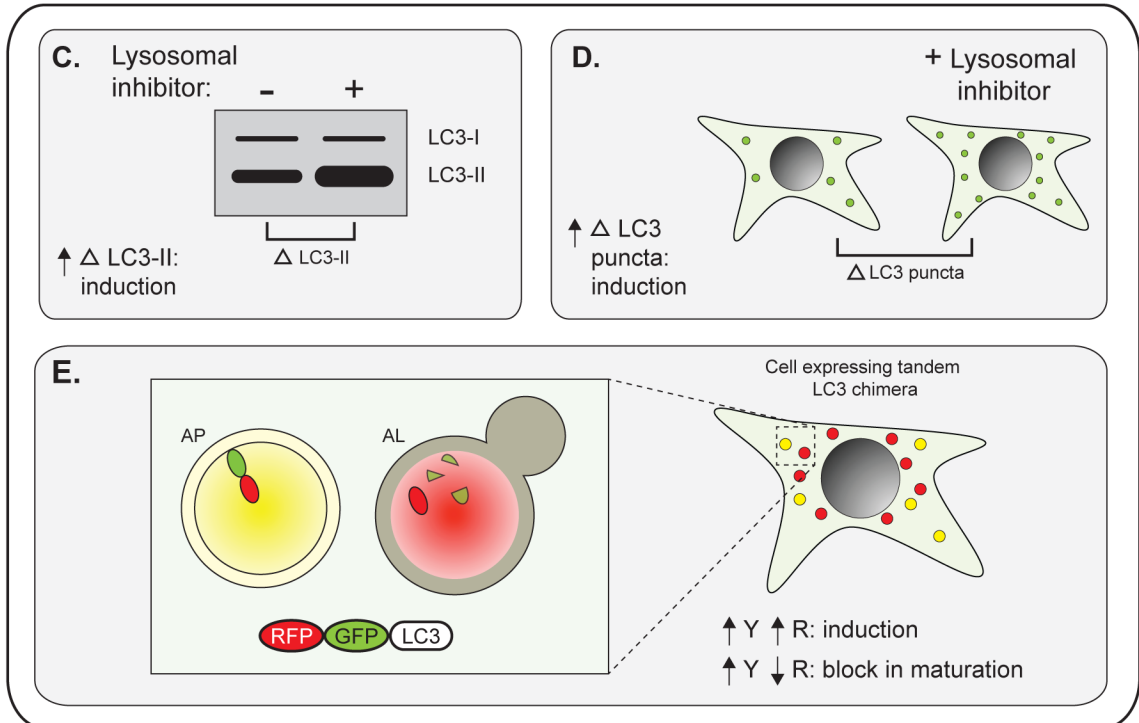
Ultrastructure

A.

↑ APs: induction OR block in maturation

Autophagosome Number

B.

LC3-I
LC3-II

↑ LC3-II / LC3 puncta: induction OR block in maturation

Autophagic Flux

C. Lysosomal inhibitor: −   +

LC3-I
LC3-II

↑ Δ LC3-II: induction   Δ LC3-II

D.   + Lysosomal inhibitor

↑ Δ LC3 puncta: induction

Δ LC3 puncta

E.

Cell expressing tandem LC3 chimera

AP   AL

RFP GFP LC3

↑ Y ↑ R: induction
↑ Y ↓ R: block in maturation

Figure 2. Methods for monitoring autophagy
Early studies implicating autophagy in disease primarily measured total autophagosome number, either (**A**) by identification of APs and ALs by electron microscopy or (**B**) by measuring total levels of lipidated LC3 (LC3-II) or number of LC3 puncta (which mark APs) in cells expressing GFP-tagged LC3 (GFP-LC3). Importantly, in both of these cases, an increase in APs can indicate either autophagy induction or a block in AP maturation. Autophagic flux assays more directly measure total autophagic activity. In the presence of lysosomal inhibitors, (**C**) increased accumulation of lipidated LC3 or (**D**) increased accumulation of GFP-LC3 puncta indicates autophagic induction. (**E**) In cells expressing tandem RFP-GFP-tagged LC3, APs are identified as yellow puncta and ALs are detected as red puncta following quenching of GFP fluorescence in the lysosome. An increase in both

signals indicates autophagic induction, whereas an increase in yellow with a decrease in red indicates a block in maturation. Abbreviations: AP, autophagosome; AL, autolysosome; LC3-I, non-lipidated LC3; LC3-II, lipidated LC3; Δ LC3-II, accumulation of lipidated LC3; Δ LC3 puncta, accumulation of GFP-LC3 puncta; Y, yellow; R, red.
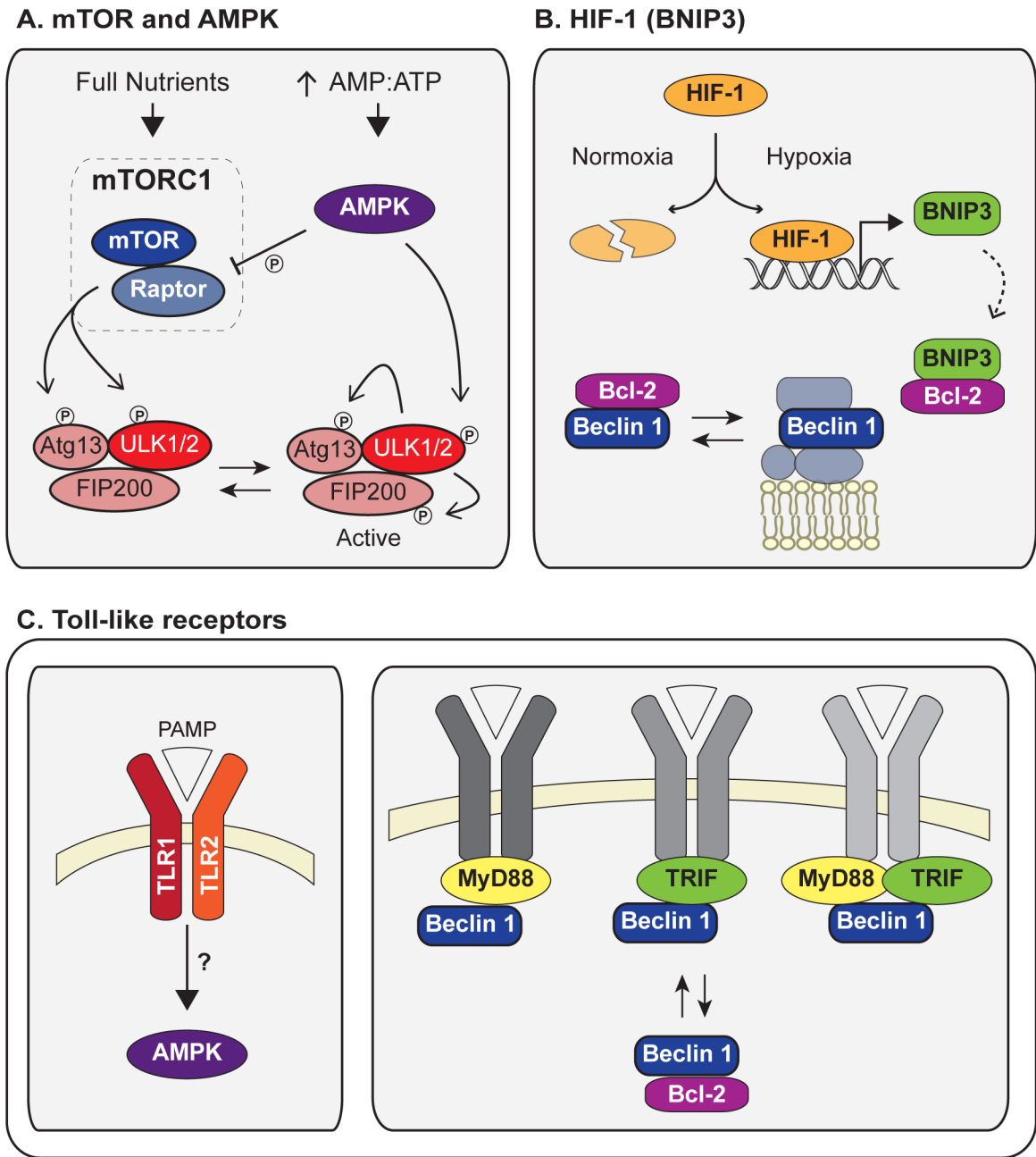
A. mTOR and AMPK

B. HIF-1 (BNIP3)

C. Toll-like receptors

Figure 3. Major stress pathways that induce autophagy
(**A**) As part of the mTORC1 complex, active mTOR phosphorylates and inactivates Ulk1.
Starvation inactivates mTOR, leading to formation of an active Ulk1 complex in which Ulk1
phosphorylates Atg13 and FIP200. During conditions of low cellular energy, AMPK is
activated by high AMP:ATP and induces autophagy both by phosphorylation and activation
of Ulk1 and by inhibition of mTORC1 via phosphorylation of Raptor. (**B**) HIF-1 and BNIP3
induce autophagy following hypoxia. HIF-1 is stabilized under hypoxic conditions, leading
to increased BNIP3 transcription. BNIP3 binds Bcl-2 and disrupts its inhibitory interaction
with Beclin 1, leading to autophagy induction. (**C**) Following infection, activation of toll-
like receptors (TLRs) by pathogen-associated molecular patterns (PAMPs) leads to
autophagy induction. Induction downstream of TLR1/2 stimulation depends on AMPK

signaling. The downstream TLR signaling molecules MyD88 and TRIF interact with Beclin 1 following TLR activation and disrupt the inhibitory Bcl-2/Beclin 1 complex.
Abbreviations: PAMP, pathogen-associated molecular pattern.
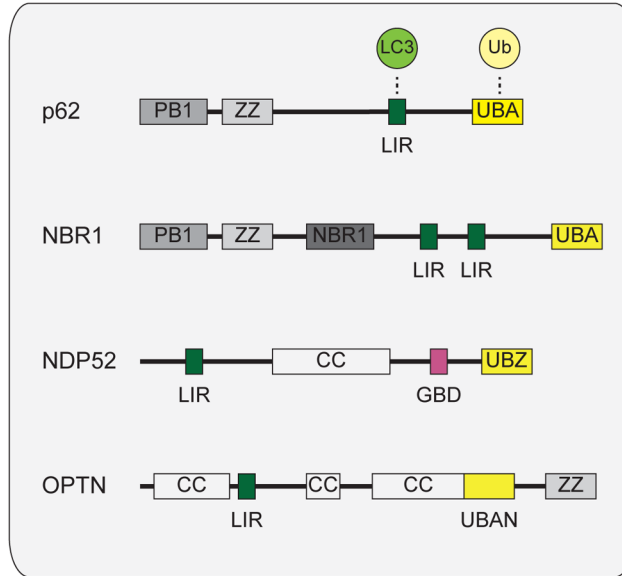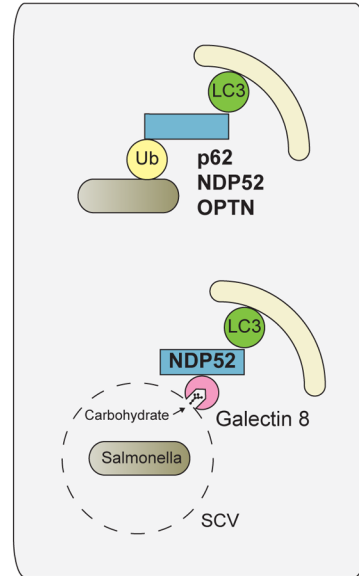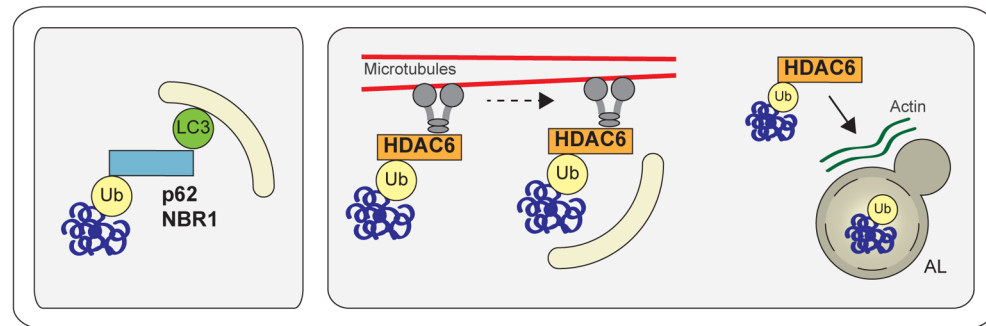
Figure 4. Degradation of ubiquitinated autophagy substrates
(**A**) Domain architecture of the autophagy cargo receptors p62, NBR1, NDP52, and Optineurin. These autophagy adaptors contain an ubiquitin-binding domain (UBA, UBZ, UBAN) and an LC3-interacting region (LIR), mediating recruitment of LC3-containing APs to ubiquitinated cargo (**B**) The adaptors p62, NDP52, and OPTN mediate degradation of ubiquitinated pathogens via ubiquitin-binding domain and LIR interactions. During *Salmonella* infection, Galectin 8 binds host carbohydrates exposed on ruptured *Salmonella*-containing vesicles and recruits NDP52 to these sites, mediating AP recruitment (**C**) p62 and NBR1 also mediate degradation of ubiquitinated protein aggregates via UBA and LIR interactions. HDAC6 is important for clearance of ubiquitinated aggregates and mitochondria. HDAC6 interacts with ubiquitin, dynein motors, and the actin remodeling machinery to promote dynein-mediated transport of ubiquitinated substrates to APs and enhanced AP-lysosome fusion at these sites. Abbreviations: OPTN, optineurin; Ub, ubiquitin; UBA/UBZ/UBAN, ubiquitin-binding domain; LIR, LC3-interacting region; SCV, *Salmonella*-containing vesicle
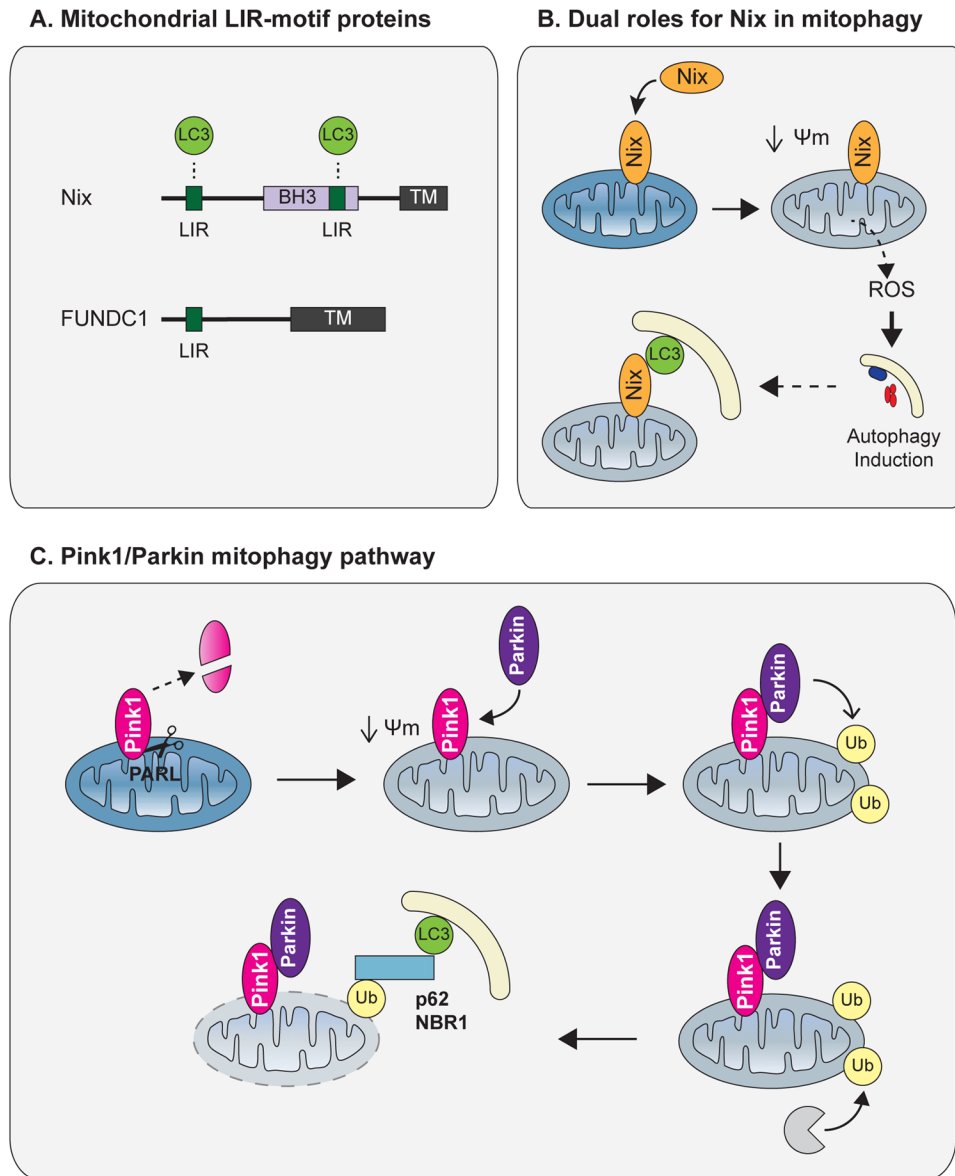
Figure 5. Mitophagy

(**A**) Domain architecture of the mitophagy adaptors Nix and FUNDC1. Both proteins contain a transmembrane domain embedded in the outer mitochondrial membrane (OMM) as well as a LIR, which mediates recruitment of APs to mitochondria. (**B**). Following mitochondrial stress, Nix promotes mitochondrial depolarization and ROS formation, leading to autophagy induction. Nix also mediates targeting of APs to mitochondria via its LIR. (**C**) In healthy polarized mitochondria, PINK1 is constitutively degraded by the inner mitochondrial membrane protease PARL. Following depolarization, full length PINK1 accumulates on the OMM and recruits the E3 ligase Parkin. Parkin ubiquitinates multiple OMM proteins, leading to proteasomal degradation of OMM components and p62/NBR1-mediated AP recruitment to mitochondria. Abbreviations: TM, transmembrane domain; Ψ_m, mitochondrial membrane potential; ROS, reactive oxygen species.
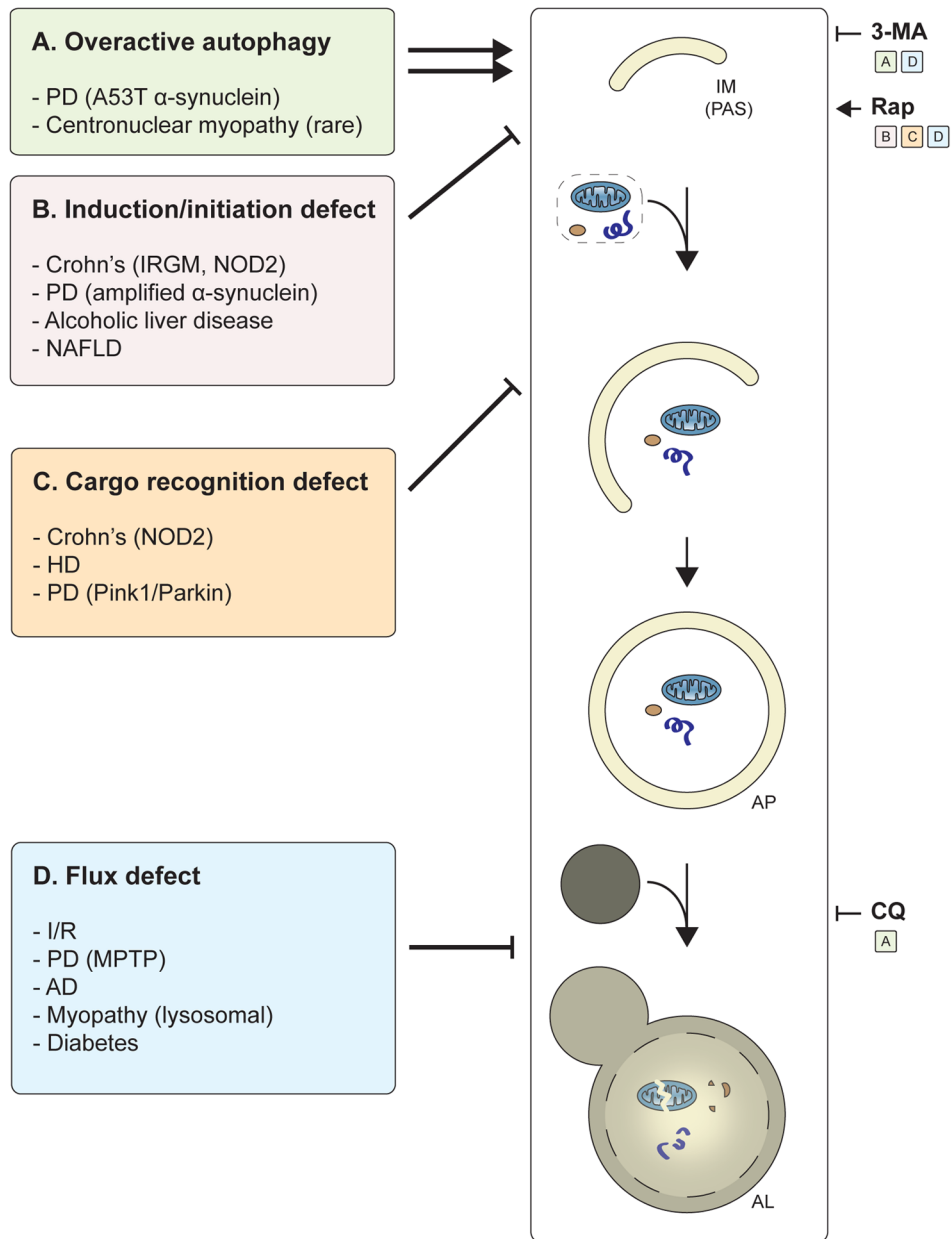
Figure 6. Summary of autophagy alterations in disease and potential treatments
The major autophagy alterations in human disease and examples of diseases for each class
are listed. (**A**) In diseases with "overactive" autophagy, inhibition of autophagy (via 3-MA
or CQ) may provide therapeutic benefit. In diseases that lead to a defect in (**B**) autophagy
induction/initiation or (**C**) cargo recognition, patients may benefit from drugs such as
rapamycin (Rap) that induce autophagy. (**D**) In diseases that lead to a block in autophagic
maturation, Rap treatment may partially restore flux and provide a cytoprotective effect.
Alternatively, in diseases with a severe block in autophagic maturation, blocking autophagy
at an early step (e.g., via 3-MA) may be therapeutically useful because they mitigate the
cytotoxic accumulation of autophagosomes and lysosomes.

Table 1

Human disease mutations that affect autophagy

Gene	Function in autophagy	Disease	Likely disease defect	Reference
Core autophagy component				
Atg16L1	Core autophagy component; essential for autophagosome elongation	Crohn's disease	Defect in bacterial clearance, increased proinflammatory cytokine secretion	Hampe, et al. 2006. *Nature genetics* 39: 207–11
Autophagy induction/initiation				
IRGM	Autophagy induction in response to IFNγ	Crohn's disease	Defect in bacterial clearance, abnormal lymphocytes and macrophages	Parkes, et al. 2007. *Nature genetics* 39: 830–32
Jumpy (MTMR14)	PI3P phosphatase, negative regulator of autophagy induction	Centronuclear myopathy	Overactive autophagy	Tosch, et al. 2006. *Human molecular genetics* 15: 3098
Cargo Recognition/ Autophagosome (AP) targeting				
NOD2	Autophagy induction in response to MDP; targeting of Atg16L1 to bacteria	Crohn's disease	Defect in bacterial clearance, increased proinflammatory cytokine secretion	Ogura, et al. 2001. *Nature* 411: 603–06
PINK1	Mitophagy	PD	Defect in mitochondrial clearance, accumulation of damaged mitochondria	Valente, et al. 2004. *Science* 304: 1158
Parkin	Mitophagy	PD	Defect in mitochondrial clearance, accumulation of damaged mitochondria	Kitada, et al. 1998. *Nature* 392: 605–08
Lysosome function				
presenilin 1	Targeting of v-ATPase V0a1 to the lysosome	AD	Defect in lysosomal acidification, accumulation of protein aggregates	Sherrington, et al. 1995. *Nature* 375: 754–60
lysosomal α-glucosidase	Glycogen breakdown in the lysosome	Pompe disease[a]	Lysosomal glycogen accumulation, defect in AP-lysosome fusion	Shen, et al. 1996. *Journal of Clinical Investigation* 98: 352
LAMP2B	Maintenance of lysosome function	Danon disease[b]	Defect in AP-lysosome fusion	Nishino, et al. 2000. *Nature* 406: 906–10

[a] glycogen storage disorder, characterized by muscle atrophy and cardiomyopathy

[b] X-linked hereditary disorder, characterized by cardiomyopathy and myopathy

Table 2

Disruption of autophagy in mouse models of disease

Genotype	Tissue	Phenotype	References
Beclin1^{+/−}	All	Spontaneous malignancies; Resistance to pressure-induced heart failure; Decreased infarct size during cardiac I/R	Qu, et al. 2003. *JCI* 112: 1809–20; Zhu, et al. 2007. *JCI* 117: 1782; Matsui, et al. 2007. *Circ Res* 100: 914–22
Atg5[f/f]*: MerCreMer*[a]	Cardiomyocytes	In adult mice: hypertrophy, contractile dysfunction, and accumulation of ubiquitinated proteins	Nakai, et al. 2007. *Nat Med* 13: 619–24
Atg5^{−/−}	Thymus (transplant)	Altered T-cell selection and multiorgan inflammation	Nedjic, et al. 2008. *Nature* 455: 396–400
Atg5[f/f]: villin-Cre	Intestinal epithelium	Paneth cell and granule secretion defects	Cadwell, et al. 2008. *Nature* 456: 259–63
Atg7[f/f]: villin-Cre	Intestinal epithelium	Paneth cell and granule secretion defects	Cadwell, et al. 2009. *Autophagy* 5: 250
Atg16L1[HM]	Intestinal epithelium	Paneth cell and granule secretion defects, increased susceptibility to DSS-induced colitis	Cadwell, et al. 2008. *Nature* 456: 259–63; Cadwell, et al. 2010. *Cell* 141: 1135–45
Atg5[f/f]*: nestin*-Cre	Neurons	Neurodegeneration and motor defects, accumulation of protein aggregates and inclusions	Hara, et al. 2006. *Nature* 441: 885–89
Atg7[f/f]*: nestin*-Cre	Neurons	Neurodegeneration and motor defects, accumulation of protein aggregates and inclusions	Komatsu, et al. 2006. *Nature* 441: 880–84
Becn1^{+/−} *APP*[b]	All	Increased APP/Aβ aggregation, severe neurodegeneration	Pickford, et al. 2008. *JCI* 118: 2190; Jaeger, et al. 2010. *PloS On*e 5: e11102
Parkin^{−/−}	All	Mitochondrial defects; no neurodegeneration	Palacino, 2004. *JBC* 279: 18614
Pink1^{−/−}	All	Age-dependent mitochondrial defects and loss of spontaneous motor activity; no neurodegeneration	Gispert, et al. 2009. *PloS One* 4: e5777
Slc6a3- Parkin- Q311X[c]	Dopaminergic neurons	Progressive motor defects, substantia nigra degeneration, α-synuclein accumulation	Lu, et al. 2009. *J Neurosci* 29: 1962–76
Atg5[f/f]*: HSA*-Cre	Muscle	Accumulation of abnormal mitochondria and protein aggregates and inclusions	Raben, et al. 2008. *Hum Mol Gen* 17: 3897
Atg5[f/f]*: HSA-Cre GAA KO*[d]	Muscle	Severe muscle atrophy, accumulation of abnormal mitochondria and protein aggregates and inclusions	Raben, et al. 2008. *Hum Mol Gen* 17: 3897
Atg7[f/f]*: MLC1f*-Cre	Muscle	Muscle atrophy, accumulation of abnormal mitochondria and protein aggregates and inclusions	Masiero, et al. 2009. *Cell Metabolism* 10: 507–15
Atg7[f/f]*: MX1*-Cre	Liver	Hepatomegaly; accumulation of abnormal membranous structures, mitochondria, and peroxisomes, and protein inclusions	Komatsu, et al. 2005. *JCB* 169: 425
Atg7[f/f]*: Alb*-Cre	Liver	Increased TG and cholesterol levels, increased TG accumulation following starvation	Singh, et al. 2009. *Nature* 458: 1131–35
Atg7[f/f]*: RIP*-Cre	β cells	β cell degeneration, loss of insulin production, accumulation of damaged mitochondria and protein aggregates	Ebato, et al. 2008. *Cell Metabolism* 8: 325–32; Jung, et al. 2008. *Cell Metabolism* 8: 318–24

[a] tamoxifen-inducible, cardiomyocyte specific

[b] Transgenic overexpression of human APP

[c] Transgenic overexpression of C-terminal truncated human Parkin

[d]Lysosomal alpha-glucosidase knockout, a mouse model of Pompe disease

Abbreviations: I/R, ischemia-reperfusion; TG, triglyceride