Autophagy in health and disease. 1. Regulation and significance of autophagy: an overview

Maryam Mehrpour,1,2 Audrey Esclatine,1,2 Isabelle Beau,1,2 and Patrice Codogno1,2

1Institut National de la Santé et de la Recherche Médicale U756 and 2Faculté de Pharmacie, Université Paris-Sud 11, Châtenay-Malabry, France

Submitted 16 November 2009; accepted in final form 14 January 2010

Mehrpour M, Esclatine A, Beau I, Codogno P. Autophagy in health and disease. 1. Regulation and significance of autophagy: an overview. Am J Physiol Cell Physiol 298: C776–C785, 2010. First published January 20, 2010; doi:10.1152/ajpcell.00507.2009.—Macroautophagy is a vacuolar degradation pathway that terminates in the lysosomal compartment after formation of a cytoplasmic vacuole or autophagosome that engulfs macromolecules and organelles. The identification of ATG (autophagy-related) genes that are involved in the formation of autophagosomes has greatly increased our knowledge of the molecular basis of macroautophagy, and its roles in cell function, which extend far beyond degradation and quality control of the cytoplasm. Macroautophagy, which plays a major role in tissue homeostasis, is now recognized as contributing to innate and adaptive immune responses. Recently, several mediators of apoptosis have been shown to control macroautophagy. Deciphering the cross talk between macroautophagy and apoptosis probably should help increase understanding of the role of macroautophagy in human disease and is likely to be of therapeutic importance.

macroautophagy; cell signaling; cell death; lysosomes; proteolysis

CELL HOmeOSTASIS DEPENDS ON the balance between the production and destruction of macromolecules and organelles. There are two major systems in eukaryotic cells that degrade cellular components: the ubiquitin proteasome system (UPS) and the lysosome. The UPS only degrades proteins, mainly short-lived proteins, which must be tagged by ubiquitin to be recognized by the proteasome (15). The lysosomal system is responsible for degrading macromolecules, including proteins, and for the turnover of organelles by autophagy (100). Recent evidence demonstrates cross talk and cooperation between the UPS and autophagy (63, 69, 106). The term “autophagy” was coined by Christian de Duve soon after his discovery of lysosomes (see Ref. 56 for a historical view of autophagy). The seminal discovery of ATG genes, originally in yeast and subsequently in multicellular organisms, has provided an important breakthrough in the understanding of macroautophagy and of its functions in physiology and disease (58, 105).

The term “autophagy” also embraces microautophagy and chaperone-mediated autophagy (CMA) (56). In contrast to macroautophagy, which starts with the formation of a vacuole, known as the autophagosome, which sequesters cytoplasmic components, microautophagy consists of the direct uptake of portions of the cytoplasm by the lysosomal membrane.

Macroautophagy and microautophagy are conserved from yeast to humans. These processes were originally described as mechanisms for bulk degradation. However, forms of macroautophagy and microautophagy selectively target organelles [mitophagy, pexophagy, ribophagy, ER-phagy (autophagy of the endoplasmic reticulum), piecemeal microautophagy of the nucleus], protein aggregates (aggrephagy), lipid droplets (lipophagy), and glycogen and microorganisms that invade the intracellular milieu (xenophagy) (4, 64, 151). Microautophagy is dependent on GTP hydrolysis and on calcium (150). However, the molecular regulation of microautophagy remains to be unraveled. Bulk microautophagy does not seem to be dependent on Atg proteins, whereas selective forms of microautophagy require different sets of Atg proteins (4, 64, 151).

CMA is a selective form of autophagy that has only been described in mammalian cells (20). Substrates for CMA contain a KFERQ-related motif in their amino acid sequence. This motif is recognized by the cytosolic constitutive chaperone hsc70 (heat shock cognate of the heat shock protein 70 family); this recognition allows for the lysosomal delivery of CMA substrates. The lysosomal membrane protein, LAMP-2A, serves as a receptor in the translocation of unfolded polypeptides across the lysosomal membrane. KFERQ-like motifs are found mainly in cytosolic proteins and are estimated to occur in ~30% of such proteins.

CMA performs several general functions, such as the elimination of oxidized proteins and the removal of misfolded proteins, and also provides amino acids during prolonged periods of starvation. It is interesting to note that during starvation, cross talk occurs between macroautophagy and CMA (53, 90). When CMA is stimulated, macroautophagy is first induced and then declines. The molecular basis for this switch has not been identified. Prevention of the age-related decline of CMA is beneficial for the homeostasis of organs and function (163). This observation is indicative of the potential importance of CMA and macroautophagy, as we discuss below, as possible antiaging mechanisms. CMA is also involved in more specific functions, such as antigen presentation by major histocompatibility complex (MHC) class II molecules, neuronal survival, and kidney growth (20).

In this review we will first describe the molecular aspects of macroautophagy (hereinafter referred to as “autophagy”) and its regulation by summarizing what is known about the formation of autophagosomes and their maturation before they fuse with the lysosomal compartment. Many different stimuli can trigger an autophagic response, and several different signaling pathways modulate autophagy. Here, we will focus our attention on signaling pathways with identified targets in the molecular machinery of autophagy. Readers interested in more detailed information about the molecular aspects of autophagy and its regulation can consult several reviews published on these topics (44, 81, 95, 105). The second part of the review will be dedicated to the significance of autophagy. We will first...
discuss the role of basal and stimulated autophagy in cell homeostasis. Next we will describe the specific functions of autophagy in the innate immune response against pathogens (25, 153), in antigen presentation by MHC molecules (103), and in regulation of the inflammatory response (45, 57). In the last part of this review we will discuss the seemingly paradoxical role of autophagy in cell death (65, 135). Autophagy is a cytoprotective process but is also associated with cell death through its intertwined relationships with apoptosis and necrosis (17, 28, 82, 87).

Molecular Aspects of Autophagy and Regulation

Molecular aspects of autophagy. The autophagosome originates from an “isolation membrane” or phagophore of uncertain origin (138). Kistakis and colleagues (3) have recently proposed that autophagosomes are formed in a cup-shaped compartment containing high levels of phosphatidylinositol 3-phosphate (PtdIns3P), known as the omegasome (from its ω-like shape), which is dynamically connected to the endoplasmic reticulum (ER). Recent electron tomographic analyses have demonstrated that the ER and the phagophore are directly connected (43, 156). Autophagosome formation requires the activity of Atg proteins, which are implicated in four major steps: initiation, nucleation, cycling, and expansion/closure (Fig. 1). Readers interested in a detailed analysis of the role of Atg proteins should consult recent reviews (44, 81, 105). Atg proteins involved in the formation of autophagosomes are evolutionarily conserved from yeast to humans. However, yeast organisms have only one form of each Atg, whereas in mammals, some Atgs (for example, Atg1, Atg4, Atg8, and Atg18) have several isoforms and paralogs. The function(s) of the diverse forms of these Atgs remains to be investigated. Here we will use the nomenclature of mammalian Atgs and only refer to one isoform for the sake of clarity.

Autophagy is initiated by the ULK1 (ULK1 is the mammalian ortholog of the yeast Atg1) complex. This complex is formed by ULK1 Ser/Thr protein kinase, Atg13, and FIP200 (FIP200 is the mammalian homolog of the yeast Atg17) (36, 46, 51). In addition, a novel mammalian Atg13-binding protein, Atg101, which is not conserved in Saccharomyces cerevisiae, has been identified by two research teams (47, 97) (Fig. 2). The phosphorylation of Atg13 and FIP200 by ULK1 is an important step in triggering autophagy. However, the exact role of these phosphorylation steps in generating the autophagosome is not known. Moreover, ULK1 may have other substrates that are involved in the early stage of autophagy (12).

The nucleation and assembly of the initial phagophore membrane are dependent on the Beclin 1: class III phosphatidylinositol 3-kinase (PI3K) complex. This complex consists of class III PI3K or hVps34 (hereinafter referred to as hVps34), its regulatory protein kinase p150 or hVps15, Beclin 1 (Atg6 in yeast), and the recently discovered mammalian homolog of Atg14 (49, 143) (Fig. 2). Beclin 1 is a platform protein that can interact with a range of cellular proteins (e.g., AMBRA 1, VMP1, MyD88, UVRAG) and viral proteins (e.g., ICP34.5, vBcl-2, and Nef) (11, 117, 139). The interaction of Beclin 1 with antiapoptotic proteins of the Bcl-2 family blocks the induction of autophagy by inhibiting the formation of PtdIns3P by hVps34 (75, 118). Thus, Bcl-2 is not only antiapoptotic but also an antiautophagic protein. PtdIns3P recruits WIPI-1 (the mammalian ortholog of the yeast Atg18) via its PtdIns3P-binding site (122). WIPI-1 probably acts in concert with Atg2 (Tassula Proikas-Cezanne, personal communication); however, the exact roles of WIPI-1 and Atg2 in the biogenesis of the autophagosome are not known. These proteins could constitute a platform to recruit other proteins or serve as a timer along with members of the myotubularin phosphoinositide 3-phosphatase family that degrade PtdIns3P (152) to control the levels of PtdIns3P. The precise mechanism that links the ULK1 complex to the activity of the Beclin 1:Atg14:hVps34 complex remains to be elucidated. The identification of ULK1 target(s) in the Beclin 1:Atg14:hVps34 complex would provide an important clue in efforts to understand the early steps in autophagosome formation.

The expansion and closure of the autophagosome are dependent on two ubiquitin-like conjugation systems: Atg12 and

![Fig. 1. Integrated view of mammalian autophagy. Autophagy is initiated by the nucleation of an “isolation membrane” or phagophore, which then elongates and closes on itself to form an autophagosome. In most cases, once the autophagosome has been formed it receives input from the endocytic pathway [early and late endosomes and multivesicular bodies (MVB)]. These steps are collectively termed “maturation.” The amphisomes that result from the fusion of autophagosomes with late endosomes/MVB are acidic, hydrolytic vacuoles.](http://ajpcell.physiology.org/)

AJP-Cell Physiol • VOL 298 • APRIL 2010 • www.ajpcell.org
LC3 (LC3 is the mammalian ortholog of the yeast Atg8) (105) (Fig. 2). Protein Atg12 conjugates with Atg5 in an Atg7- and Atg10-dependent manner, and the resulting complex is stabilized by Atg16. This complex is important in the stimulation and localization of the LC3 conjugation reaction. In this system the COOH terminus of LC3 is conjugated to the polar head of phosphatidylethanolamine (PE). The formation of LC3-PE (the conjugated form of LC3, also known as LC3-II) is dependent on Atg7 and Atg3. Atg4 primes LC3 for conjugation by exposing a glycine residue at the COOH terminus (the form of LC3 with this glycine exposed is also known as LC3-I). In contrast to other Atg proteins, LC3 is recruited to both the external and the inner surfaces of the expanding autophagosomal membrane (105, 110). After closure of the autophagosome, the LC3 located on the external surface is released from PE by Atg4, whereas the LC3-PE on the inner surface is transported to the lysosomal compartment to be degraded along with other cargos. Before completion of the autophagosomes, all Atg proteins assembled on the phagophore are recycled, with the exception of a fraction of LC3 that remains associated with the inner surface of the autophagosomal membrane. Atg9 is the only transmembrane Atg protein that cycles between peripheral pools and the phagophore. Its putative role is to carry lipids and/or serve as a platform for recruiting effectors to the phagophore (reviewed in Ref. 81). Defining the molecular events that control the formation and membrane recruitment of the Atg12 conjugate is important if we are to understand fully the expansion of the phagosomal membrane.

After being formed, the autophagosomes merge with endocytic compartments (early and late endosomes and multivesicular bodies may fuse with autophagosomes) before fusing with the lysosomal compartment (78, 127, 142). The direct fusion of autophagosomes and lysosomes is not what usually happens in mammalian cells (34, 35, 50). The term “amphisome” has been coined for the vacuole that results from the fusion of an autophagosome with an endosome (142) (Fig. 1). The term “autolysosome” is frequently used to refer to the last organelle in the process of autophagy. However, this terminology does not signify that only a subset of lysosomes is competent for macroautophagy; Seglen and coworkers (35) have shown that all lysosomes can receive input from the autophagic pathway. The maturation of autophagosomes is under the control of proteins involved in controlling membrane fusion in the intracellular transport (Rab GTPase, SNARE, and ESCRT proteins), and the function of acidic degradative compartments (v-ATPase, LAMP proteins, lysosomal carriers, and...
lysosomal hydrolases) (31, 33, 129). The protein Beclin 1 is also involved in the late stage of autophagy through its interaction with Rubicon and UVRAG (76, 93, 166). The Beclin 1:hVps34:UVRAG:Rubicon complex downregulates these trafficking events, whereas the Beclin 1:hVps34:UVRAG complex upregulates the maturation of autophagosomes and endocytic trafficking (93, 166). Thus, Beclin 1 regulates both the formation of autophagosomes (via interaction with Atg14) and the maturation of autophagosomes (via interaction with UVRAG and Rubicon).

**Regulation of autophagy.** There have been several recent reviews of the regulation of autophagy by signaling pathways (16, 44, 95, 108). We focus here on targets of kinases that have been identified in the molecular machinery of autophagosome formation. Components of the ULK1 complex are targets of mammalian target of rapamycin (mTOR), whereas components of the Beclin 1 complex are targets of c-JUN NH2-terminal kinase 1 (JNK1), and death-associated kinase (DAPK) (Fig. 2). mTOR plays a major role in the regulation of autophagy because it integrates signals emitted by growth factors, amino acids, glucose, and energy status (2, 120, 130). However, autophagy can also be regulated independently of mTOR (134).

**Regulation of the ULK complex by mTOR.** The induction of autophagy by the inhibition of TOR under conditions of starvation is conserved from yeast to mammals (8, 111). The mTOR pathway involves two functional complexes: mTORC1 and mTORC2 (71). mTORC1, the rapamycin-sensitive mTOR complex 1, contains the mTOR catalytic subunit, raptor (regulatory associated protein of mTOR, a protein that acts as a scaffold for the mTOR-mediated phosphorylation of mTOR substrates), GβL, and PRAS40 (proline-rich Akt substrate of 40 kDa), and Deptor (DEP domain-containing mTOR-interacting protein). This complex regulates cell growth, metabolism (by integrating amino acid and growth factor signals), energy, and oxygen status. The other mTOR complex, mTORC2, which is less sensitive to rapamycin, includes mTOR, rictor (rapamycin-insensitive companion of mTOR), GβL, Sin1 (SAPK-interacting protein 1), PROTOR (protein observed with rictor), and Deptor. The mTORC2 complex regulates cytoskeletal organization, metabolism, and cell survival by phosphorylating the Ser473 of Akt/PKB (133). Phosphorylated Akt/PKB downregulates the activity of FoxO transcription factors (“Forkhead Box” with the second “O” denoting members related by sequence) (132). Interestingly, FoxO1 and FoxO3 have been shown to regulate autophagy by increasing the transcription of several genes involved in autophagy in hepatocytes and muscle cells, respectively (79, 88).

ULK1, Atg13, and FIP200 form a stable complex that signals to the autophagic machinery downstream of mTORC1 (13, 46, 51). Importantly, mTORC1 is incorporated into the ULK1:Atg13:FIP200 complex via ULK1 in a nutrient-dependent manner. mTOR phosphorylates both ULK1 and Atg13. Under starvation conditions or if the AMP-to-ATP ratio increases (conditions that activate AMP-dependent kinase) or in response to rapamycin treatment, mTORC1 dissociates from the ULK1 complex, resulting in the activation of ULK1. Activated ULK1 autophosphorylates and also phosphorylates Atg13 and FIP200 to initiate autophagy (13, 46, 51). In contrast, when activated by amino acids and growth factors, mTORC1 represses autophagy and favors cell growth by promoting translation via the phosphorylation of 70-kDa, polypeptide 1 ribosomal protein S6 kinase-1 (p70S6K) and phosphorylation of the inhibitor of translation initiation, 4E-BP1 (16, 108).

**Regulation of the Beclin 1:hVps34 complex.** As discussed in the preceding sections, the trimer Beclin 1:hVps34:hVps15 can interact with different partners to control the formation and maturation of autophagosomes. Recently, the antiapoptotic protein Bcl-2, and antiapoptotic members of the Bcl-2 family such as Bcl-XL, have been shown to inhibit autophagy (30, 86, 119). Bcl-2/Bcl-XL binds Beclin 1 through a BH3 domain that mediates the docking of the latter in the BH3-binding groove. The constitutive Bcl-2/Bcl-XL:Beclin 1 interaction is disrupted by signals that promote autophagy. JNK-1 phosphorylates one threonine residue and two serine residues in the NH2-terminal loop of Bcl-2 to trigger its release from Beclin 1 in response to starvation or ceramide treatment (116, 154). In a reciprocal manner, the BH3 domain of Beclin 1 can be phosphorylated by DAPK, which has the effect of reducing its affinity for Bcl-XL (162). As discussed below, phosphorylation-independent mechanisms can also dissociate the Beclin 1:Bcl-2 interaction.

**The Significance of Autophagy**

**Basal and starvation-induced autophagy.** Autophagy occurs at a basal rate in most cells, where it acts as a cytoplasmic quality control mechanism to eliminate protein aggregates, damaged organelles, and other nonactive structures (60, 99). The elimination of maternal proteins in the egg after fertilization is probably responsible for the role of autophagy in preimplantation development (149). The physiological importance of basal autophagy in maintaining tissue homeostasis has been demonstrated in a large panel of organs including brain, liver, heart, striated muscle, intestine, pancreas, and adipose tissue (10, 27, 39, 52, 61, 62, 89, 104, 126, 140, 164) in murine Atg conditional knockout models. These studies have also revealed the role of autophagy in preventing the deposition of aggregation-prone proteins in the cytoplasm, and the contribution of autophagy to the elimination of ubiquitinated proteins. For example, an accumulation of polyubiquitinated proteins also occurs in neurons after conditional knockout of Atg5 or Atg7 in the mouse brain (39, 61), resulting in neurological abnormalities and neuronal death, suggesting that basal autophagy plays a crucial role in maintaining neural function and preventing neurodegeneration.

The antiaging role of autophagy probably depends, at least in part, on its quality control function that limits the production of reactive oxygen species (ROS) by the deposition of aggregation-prone proteins and damaged mitochondria (21). This is particularly important for cells with a low turnover rate, such as neurons, skeletal muscle fibers, and cardiac myocytes. While autophagic activity might be downregulated as a result of increasing age, the maintenance of autophagic activity by calorie restriction may help extend life span in various species from yeast to mammals (6, 21, 102). Studies in Caenorhabditis elegans show that several autophagy genes are required for life span extension during periods of nutrient shortage (38, 96). A recent study in rhesus monkeys shows that calorie restriction delays the onset of age-associated disorders and mortality (18). On the other hand, several studies show that inhibition of the mTOR pathway, which integrates nutrient sensing and con-
trols autophagy (see above), extends life span in eukaryotes ranging from yeast to mammals (41, 121, 147). These studies suggest that autophagy may contribute to counteracting the deleterious effects of aging, but this remains to be firmly demonstrated (42).

The quality control function of basal autophagy that results from limitation of the production of ROS, ER stress, harmful proteins, and damaged mitochondria also plays an important role in tumor suppression by controlling oncogenic pathways and DNA damage (91, 92). Interestingly, Beclin 1 (the human gene encoding for the Beclin 1 protein) is a haplo-insufficient tumor suppressor gene with frequent monoallelic deletions in breast, prostate, and ovarian cancers (1). Heterozygous deletion of beclin 1 in mice also increases the rate of spontaneous tumor development in various organs (124, 161).

Stimulation of autophagy during periods of starvation is an evolutionarily conserved stress response in eukaryotes (84, 99). The induction of autophagy at birth allows mammalian neonates to adapt to the starvation that results from the sudden termination of the placental nutrient supply (67). Under starvation conditions, autophagy is stimulated as a consequence of the decrease in the intracellular concentration of amino acids and the resulting inhibition of mTORC1 upstream from the ULK1 complex, and activation of JNK1 upstream of the Beclin 1:Atg14:hVps34 complex (see above and Fig. 2). The degradation of proteins and lipids allows the cell to adapt its metabolism to producing ATP and to respond to its energy needs (reviewed in Ref. 84). Amino acids produced by autophagic degradation are substrates for new protein synthesis and for mitochondrial oxidation through the tricarboxylic acid cycle, which contributes to generating ATP and various metabolites including acetyl-CoA. Lipid degradation by autophagy produces fatty acids, which also yield acetyl CoA after mitochondrial β-oxidation. Starvation-induced autophagy sustains apoptotic-deficient cell survival for several weeks after growth factor withdrawal by maintaining their bioenergetics (83). Moreover, in apoptotic-competent cells, starvation-induced autophagy is cytoprotective by blocking the induction of apoptosis upstream of mitochondrial events (9).

Autophagy in immunity and inflammation. Autophagy plays a major role in innate and adaptive immunity. Moreover, autophagy controls the homeostasis of immune cells and contributes to the regulation of self-tolerance. The terms “immunophagy” and “xenophagy” have been introduced to highlight the importance of autophagy in immunity (24, 73). We will summarize some of the findings that illuminate the role of autophagy in immune response and inflammation. Readers interested in more detailed discussions can consult recent reviews on these topics (25, 45, 103, 153).

Autophagy contributes to innate immunity by protecting the cytosol from colonization by intracellular microbial pathogens. Many bacteria, such as group A Streptococcus, Rickettsia coronii, Staphylococcus aureus, or Mycobacterium tuberculosis, and also parasites, such as Toxoplasma gondii, are targeted by autophagy for elimination (25). The mechanism by which bacteria are targeted to autophagosomes was recently revealed by several studies showing that the protein p62/SQSTM1 acts as an adaptor between intracellular bacteria decorated with ubiquitinated proteins and LC3, as it does for ubiquitinated protein aggregates (157, 165). Moreover, the cytosolic protein NDP52 recognizes ubiquitin-coated Salmonella, and also binds LC3 (145). The specificity of p62/SQSTM1 and NDP52 toward different ubiquitinated structures remains to be investigated. In contrast to the numerous studies of bacteria and parasites, only two studies on single-stranded RNA viruses, the tobacco mosaic virus that infects plants, and the Sindbis virus, have demonstrated that viral replication can be successfully limited by autophagy (77, 80). Autophagy helps cells to rid themselves of intracellular microorganisms, and many pathogens have developed strategies to deal with or inhibit this process. For example, bacteria such as Shigella flexneri or Listeria monocytogenes possess “camouflage” proteins to avoid recognition by the autophagic machinery (112, 157). Two Herpesviruses express proteins that are able to block induction of autophagy: some viral homologs of Bcl-2 or ICP34.5 interact with Beclin 1, and a viral homolog of FLIP interacts with Atg3 (26, 72, 113).

Although autophagy acts as a cellular antimicrobial defense system in most models of infection studied so far, autophagy is also exploited by certain pathogens, such as poliovirus, the replication of which is increased when autophagy is stimulated (54). It has been reported recently that macrophages infected with human immunodeficiency virus (HIV-1) accumulate autophagosomes, in contrast to CD4 T-infected cells, in which autophagy is inhibited (32). Accumulation of these nondegradative autophagic vacuoles increase the yield of HIV (32, 68). HIV-1 blocks fusion of autophagosomes with lysosomes thereby avoiding degradation, possibly by the interaction of the viral protein Nef with Beclin 1 (68). A number of other innate immune processes are modulated by autophagy. Activation of Toll-like receptors and of the eIF2α kinase PKR, for example, induces autophagy and contributes to triggering the antimicrobial response and the de novo synthesis of proinflammatory molecules (22, 144).

Apart from being involved in the elimination of intracellular microorganisms, autophagy can also contribute to adaptive immunity. Lysosomes generate the antigenic peptides that are exposed on the cell surface in association with MHC class II (MHC II) for presentation to CD4-positive (CD4+) T cells. The general paradigm is that processed proteins are internalized from the extracellular space, but a high proportion of the peptides loaded on MHC II are derived from cytosolic and nuclear proteins. Autophagy can be involved in delivering Epstein-Barr virus nuclear antigen 1 (EBNA1), a viral cytosolic antigen, to the MHC class II loading compartment (114). Macropathogens can deliver antigens to MHC class II compartments, and this process is a constitutive and efficient pathway for MHC class II presentation of intracellular antigens in antigen-presenting cells, including dendritic and epithelial cells (137). In the context of a viral infection, autophagy can also contribute to antigen presentation by MHC class I to CD8+ T cells (29). Autophagy can also influence adaptive immunity by promoting the development, survival, and proliferation of B and T cells (98, 123). Autophagy is also involved in the homeostasis of immune cells. For example, thymic epithelial cells display an extraordinarily high level of autophagy (101), and autophagy can shape the T-cell repertoire during thymic selection (107). A thymic nude mice grafted with athymic−/− thymus develop multiple signs of autoimmunity within a few weeks (107). Autophagy is also an effector of Th1/Th2 polarization in resistance or susceptibility to intracellular pathogens. The T helper 1 (Th1) cytokines, such as...
IFN-γ, and TNF-α, induce autophagy, whereas the Th2 cytokines IL-4 and IL-13 inhibit autophagy and affect the ability of murine and human macrophages to control intracellular Mycobacterium tuberculosis (40).

Genome-wide association studies have shown that ATG16L1 and IRGM (IRGM belongs to the p47 immunity-related GTPase family), two autophagy genes essential for the elimination of intracellular pathogens, are associated with Crohn’s disease (CD), a chronic inflammatory bowel disease (94, 115, 128). The single-nucleotide polymorphism of the ATG16L1 gene corresponds to a substitution of threonine for alanine at position 300 (T300A). Human epithelial cells expressing the ATG16L1*T300A variant display reduced autophagic clearance of intracellular bacteria (66). The role of Atg16L1 and autophagy in bacterial clearance has been recently demonstrated (19, 70, 148). Moreover, Atg16L1 interacts with the host sensor Nod2 to trigger the autophagic response to invasive bacteria (148). Interestingly, Nod2 is another gene associated with CD (48).

The role of autophagy in CD is probably not limited to the sequestration and degradation of invading bacteria. Studies from Virgin and colleagues show that mice displaying hypomorphic expression of ATG16L1 in the intestine have morphological and functional abnormalities in Paneth cells similar to those observed in CD patients (10). Paneth cells are highly specialized intestinal cells localized in the crypt, which secrete lysozyme and other microbial peptides packaged in granules. Autophagy also regulates the endotoxin-induced inflammatory response (131): Mice with Atg16L1 or Atg5-deficient macrophages have a higher level of the proinflammatory cytokine interleukin-1β. IRGM is involved in the autophagy-mediated destruction of bacteria (141), and a single polymorphism similar to IRGM is strongly correlated with CD (94, 115). However, the precise role of IRGM in autophagy and in CD remains to be fully elucidated.

Autophagy and cell death. Autophagy provides protection against cell death by its ability to counteract the cell damage produced by toxic substances and energy depletion. However, autophagy can act at several different stages in the cell death cascade. During development, autophagy is required during the late stage of apoptosis for the clearance of dead cells by phagocytes (125). Autophagy exposes phosphatidylserine at the cell surface of dying cells, which is essential for the recognition and engulfment of apoptotic cells by phagocytes. In some situations, autophagy can act upstream of or in parallel to apoptosis to trigger cell death (28, 87, 135). For example, autophagy is required for salivary gland degradation during Drosophila development (7). Autophagy acts together with apoptosis to kill cells in various pathological situations (reviewed in Refs. 28, 87, and 135). It is not clear how autophagy induces apoptosis. The selective degradation of apoptotic brakes, such as antiapoptotic or antioxidant molecules, is one possibility (160). Another is that repeated cycles of autophagy contribute to apoptotic cell destruction by reducing cell volume (146). In an extreme form of this scenario, autophagy can be detrimental even in the absence of apoptosis. This type of cell death is referred to as type II cell death or autophagic cell death (type I cell death is apoptosis, and type III cell death is necrosis). Autophagic cell death is operative in mid gut cell death in Drosophila (23) and during differentiation in Dictyostelium discoideum (37). In the latter situation, inhibition of autophagy reveals necrotic cell death. In mammals, autophagic cell death reportedly occurs in a model of neurodegeneration (59); however, it is questionable whether it occurs in physiological settings (65, 135).

The complex relationship between autophagy and cell death is beginning to be better understood as a result of the discovery of cross talk between the mediators of cell death and the molecular machinery of autophagy. As discussed in the previous section, the antiapoptotic protein Bcl-2 is also antiapoptagic as a consequence of its interaction with Beclin 1. Disassociation of the Beclin 1:Bcl-2:Bcl-XL complex may depend on the phosphorylation of Bcl-2 (see above), or the competitive displacement of the BH3-domain of Beclin 1 from Bcl-2/Bcl-XL by other BH3-containing proteins with proapoptotic properties, or BH3-mimetics (5, 30, 85, 86). Moreover, recent data show that DAPK, a proapoptotic kinase, induces dissociation of the Beclin 1:Bcl-2 complex by phosphorylating a threonine residue located in the BH3-domain of Beclin 1 (162). If Beclin 1 and Bcl-2 cannot reassociate, this leads to an unchecked autophagy that triggers cell death (119). Interestingly, the antiapoptotic protein FLIP, which blocks the activation of caspase 8 downstream of death receptors, is also an anti-autophagy molecule, which acts by blocking the formation of LC3-II via its interaction with Atg3 (72). Following on from this discovery, peptides derived from the Kaposi’s sarcoma-associated Herpesvirus form of FLIP can reduce tumorigenicity in lymphoma by stimulating autophagy and cell death (72). Beyond the potential therapeutic interest of substances that promote autophagy, these studies also show that interactions between apoptotic mediators and Atg proteins act as rheostats to help maintain autophagy at a level compatible with cell survival.

Another fascinating aspect of the interplay between autophagy and apoptosis involves the cleavage of Atg proteins and its consequence for cell death. It has recently been shown that Beclin 1 is a substrate for caspase 3 (85), and perhaps for other caspases (14). The cleavage of Beclin 1 by caspase 3 blocks the induction of autophagy and hence limits its antiapoptotic effect (85). The cleavage of Atg5 by calpains 1 and 2 can generate an NH2-terminal fragment that can be targeted to mitochondria, where it interferes with the antiapoptotic function of Bcl-XL (159). Interestingly, the calpain-dependent cleavage of Atg5 reduces the cell’s autophagic capacities (155). These studies show that Atg cleavage by proteases can limit the cytoprotective effect of autophagy in response to stress situations.

Conclusion and Prospects for the Future

Autophagy is now recognized as an important process in physiological and pathological situations. Studies in animal models, as well as observations and genetic analyses in patients, clearly implicate autophagy in numerous diseases, including cancer, neurodegenerative and muscle disease, hepatic disorders, cardiac failure, infectious and inflammatory diseases, diabetes, and obesity (74, 100). In this review, we have sought to identify breakthroughs in understanding the regulation and significance of autophagy in mammalian cells. However, we have not discussed some emerging aspects in this rapidly moving field of research. Such aspects include the following: First, in contrast to the situation observed in yeast, numerous mammalian Atg have isoforms or paralogs (81, 158),
the roles of which in autophagy remain to be determined. This variety of proteins may indicate tissue specificity of autophagy or may represent different steps of autophagosome formation and/or in selective responses to stimuli. Second, the discovery of noncanonical forms of autophagy, which use only a restricted number of Atg proteins to form a functional autophagosome, probably reflects the complexity and plasticity of the autophagic process (109, 136, 167). The specific stimuli that trigger these noncanonical forms of autophagy as well as the nature of their selective functions, if any, remain to be established. It should be kept in mind, though, that both canonical and noncanonical forms of autophagy display at least two common features: the formation of a double-membrane-bound autophagosome and the lysosomal degradation of the autophagic cargo. Finally, the discovery of the molecular regulation of selective forms of autophagy (55, 151) provides additional impetus for attempting to unravel the details of the regulation and function of the “self-eating” process which, as knowledge evolves, resembles an “à la carte” menu more than a fixed one.

GRANTS

Work in P. Codogno’s laboratory is supported by institutional funding from Institut National de la Santé et de la Recherche Médicale (INSERM), from Paris-Sud 11 University, and grants from the Institut National du Cancer (INCa)-Association pour la Recherche sur le Cancer (ARC), and Agence Nationale de la Recherche (ANR) to A. Esclatine.

REFERENCES

No conflicts of interest are declared by the authors.


REGULATION OF AUTOPHagy

C785


