Autophagy: Basic Principles and Relevance to Disease

Mondira Kundu[1] and Craig B. Thompson[2]

Department of Pathology and Laboratory Medicine[1] and Abramson Family Cancer Research Institute,[2] University of Pennsylvania, Philadelphia, Pennsylvania 19063; email: kundum@uphs.upenn.edu, craig@mail.med.upenn.edu

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Abstract

Autophagy is a process by which cytoplasmic components are sequestered in double membrane vesicles and degraded upon fusion with lysosomal compartments. In yeast, autophagy is activated in response to changes in the extracellular milieu. Depending upon the stimulus, autophagy can degrade cytoplasmic contents nonspecifically or can target the degradation of specific cellular components. Both of these have been adopted in higher eukaryotes and account for the expanding role of autophagy in various cellular processes, as well as contribute to the variation in cellular outcomes after induction of autophagy. In some cases, autophagy appears to be an adaptive response, whereas under other circumstances it is involved in cell death. In mammals, autophagy has been implicated in either the pathogenesis or response to a wide variety of diseases, including neurodegenerative disease, chronic bacterial and viral infections, atherosclerosis, and cancer. As the basic molecular pathways that regulate autophagy are elucidated, the relationship of autophagy to the pathogenesis of various disease states emerges.

INTRODUCTION

ATG: autophagy-related gene

Macroautophagy (hereafter referred to as autophagy) is a process by which cellular components are sequestered within the vesicular system and delivered to lysosomes for degradation and recycling of bioenergetic components. Although autophagy was first described in mammalian cells more than 50 years ago, it is only in the past decade that the molecular basis for this process has been illuminated. Elucidation of the molecular pathways that carry out autophagy was achieved largely through genetic approaches in yeast mutants defective in autophagy. Subsequently, it was determined that the basic steps involved in autophagy, and much of the molecular machinery, are conserved in mammals, including humans. Conceptually, autophagy can be broken down into seven discrete steps. These include induction or selection/packaging of cargo, nucleation, vesicle expansion, completion, fusion, degradation, and, finally, export of metabolic building blocks (**Figure 1**) (1). Over 20 different autophagy-related genes (ATGs) have been identified, and many of these are functionally conserved in higher eukaryotes (**Table 1**) (2). We first review the molecular basis for each step of autophagy, highlighting points at which defects have been associated with disease. The second part of this review discusses diseases in which autophagy plays a prominent role in pathogenesis.

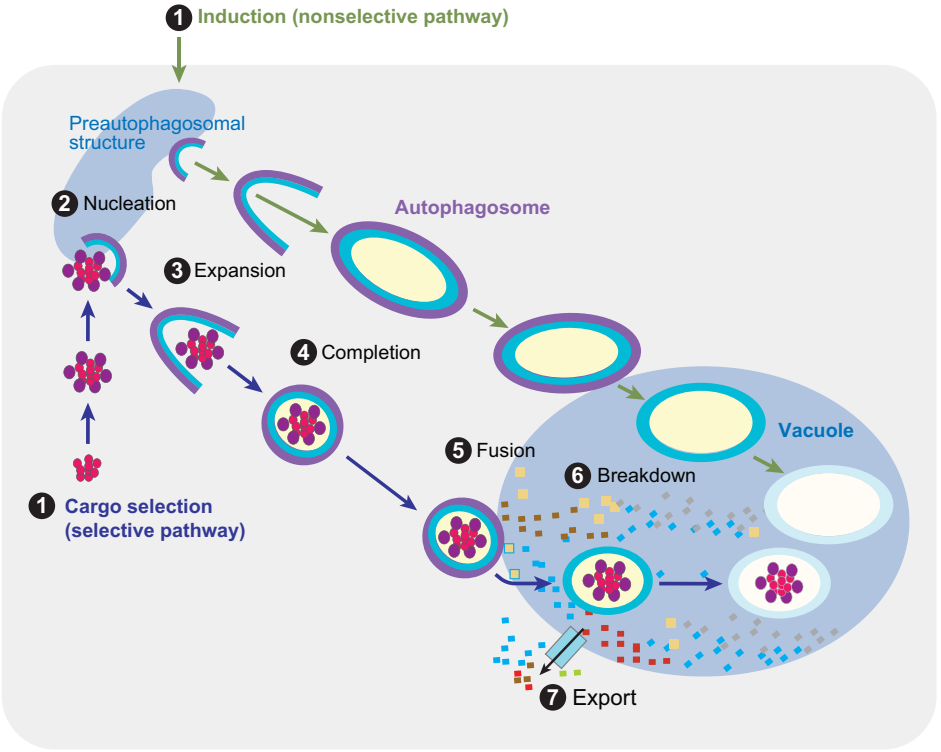

Figure 1

Selective and nonselective autophagic pathways. The signal to initiate autophagy comes either from changes in nutrient availability in nonselective autophagy or from the cargo itself, as in the cytoplasm-to-vacuole targeting pathway, a prototype of selective autophagy. Subsequent steps, including nucleation, expansion, completion, fusion, breakdown, and export, are common to both pathways.

Table 1 Genes involved in autophagy (A), CVT pathway (C), or pexophagy (P)

Yeast gene	Function	Pathway	Interactions	Human homolog ortholog	Chromosomal location
ATG1	Serine-threonine kinase; induction/membrane recycling	A, C, P	ATG13, ATG11, ATG17	*ULK1* *ULK2*	12q24.3 17p11.2
ATG2	Peripheral membrane protein; retrograde ATG9 transport (from PAS)	A, C, P	ATG9, ATG18	*C14orf103*	14q32.2
ATG3	E2-like; ATG8 conjugation	A, C, P	ATG7, ATG8, ATG12	*ATG3*	3q13.2
ATG4	Cysteine protease; cleavage of ATG8	A, C, P	ATG8	*ATG4A* *ATG4B* *ATG4C* *ATG4D*	Xq22.1–q22.3 2q37.3 1p31.3 19p13.2
ATG5	Conjugated to ATG12; formation/expansion	A, C, P	ATG12, ATG16	*ATG5*	6q21
ATG6	PI3P synthesis; formation/expansion	A, C, P	ATG14, VPS15, VPS34	*BECN1*	17q21
ATG7	E1-like; ATG8 and ATG5-ATG12 conjugation	A, C, P	ATG3, ATG8, ATG12	*ATG7*	3p25.3
ATG8	Ubiquitin-like protein conjugated to PE; formation/expansion	A, C, P	ATG3, ATG4, ATG7, ATG19	*MAP1LC3A* *MAP1LC3B* *GABARAP* *GABARAPL1*	20cen–q13 16q24.2 17p13.1 12p13.2
ATG9	Transmembrane protein shuttling between PAS and peripheral membranous structures (including mitochondria)	A, C, P	ATG2, ATG11, ATG18, ATG23	*ATG9A* *ATG9B*	2q35 7q36.1
ATG10	E2-like; ATG5-ATG12 conjugation	A, C, P	ATG12	*ATG10*	5q14.1–q14.2
ATG11	Cargo receptor/adaptor and anterograde (to PAS) ATG9 transport	C, P	ATG9, ATG17, ATG19, ATG20, ACTIN		
ATG12	Ubiquitin-like protein conjugated to ATG5; formation/expansion	A, C, P	ATG3, ATG5, ATG7, ATG10	*ATG12*	5q21–q22
ATG13	Modulates ATG1 activity; formation/expansion	A, C, P	ATG1, ATG17, Vac8		
ATG14	PI3P synthesis; formation/expansion	A, C, P	ATG6, VPS15, VPS34		
ATG15	Lipase-like; degradation of vesicle within vacuole (involved in other endosomal transport routes)	A, C,P			
ATG16	Forms homodimers and associates with ATG5-ATG12 complex; formation/expansion	A, C, P	ATG5, ATG12	*ATG16L1* *ATG16L2*	2q37.1 11q13.4
ATG17	Modulates ATG1 activity; regulates size of autophagosome	A, P	ATG1, ATG11, ATG12, ATG13, ATG24		

(*Continued*)

Table 1 (*Continued*)

Yeast gene	Function	Pathway	Interactions	Human homolog ortholog	Chromosomal location
ATG18	PI(3,5)P_2 binding protein involved in vacuole membrane recycling; PI3P binding protein involved in retrograde ATG9 recycling	A, C, P	ATG2, ATG9	*WIPI1* *WIPI2*	17q24 7p22.1
ATG19	Cargo receptor	C	PRAPE1, ATG8, ATG11		
ATG20	PI3P binding protein; formation/expansion in CVT and pexophagy	C, P	ATG24, ATG17		
ATG21	PI3P binding protein involved in lipidation and recruitment of ATG8 to PAS; vesicle formation/expansion	C, P			
ATG22	Vacuolar effluxer for amino acids (leucine, isoleucine, and tyrosine); functional redundancy with AVT3, AVT4	A		*AVT3* = *SLC36A1* *AVT4* = *SLC36A4*	5q33.1 11q21
ATG23	Peripheral membrane protein shuttles between PAS and peripheral membranous structures; cycling in CVT dependent on ATG1 kinase activity	A, C, P	ATG9		
ATG24	PI3P binding protein (aka SNX4); fusion with vacuole	C, P	ATG17, ATG20	*SNX30*	9q32
ATG25	Coiled-coil protein involved in completion of pexophagosome membrane or fusion with vacuole	P			
ATG26	UDP-glucose sterol glucosyltransferase required for efficient expansion of pexophagosome membranes	P			
ATG27	Type 1 transmembrane protein localized to mitochondria and Golgi; involved in anterograde ATG9 transport	C, P?	ATG9		
ATG28	Coiled-coil protein involved in completion of pexophagosome membrane or fusion with vacuole	P			

Table adapted with permission from Reference 2.

BASIC PRINCIPLES: YEAST TO MAMMALS

Autophagy occurs at a basal level under normal growth conditions and is involved in the degradation of damaged or aged organelles. Cellular stresses, including starvation, growth factor withdrawal, or accumulation of misfolded proteins, can dramatically induce

autophagy. However, the type of autophagy induced, selective or nonselective, may depend upon the stimulus. A key difference between selective and nonselective autophagy is that the signal for membrane biogenesis comes from the targeted cellular component in the former and from direct activation of the autophagic machinery in the latter. Membrane biogenesis occurs at a centralized location in yeast, termed the pre-autophagosomal structure (PAS); in higher eukaryotes, autophagy is initiated via isolation membranes distributed throughout the cytoplasm. Upon completion of the autophagic vesicle, it fuses with lysosomes (in yeast, termed the vacuole) and the contents are degraded. This section provides an overview of the basic pathways involved in regulating autophagy in yeast and in higher eukaryotes.

Induction

The target of rapamycin (TOR) kinase plays a critical role in coordinating nutrient availability and cell growth through a pathway that ultimately regulates protein translation and autophagy. When the supply of amino acids and other nutrients is ample, TOR is maintained in an active conformation and phosphorylates proteins that are directly or indirectly important for cellular growth (3). Under these conditions, vacuolar hydrolases are delivered to the yeast vacuole via the cytoplasm-to-vacuole targeting (CVT) pathway, which is considered a selective form of autophagy. Although the CVT pathway utilizes many of the same proteins required for autophagy, there are components unique to each process (**Table 1**). When nutrients are limiting, TOR is inactivated, which reduces overall protein translation and alters the repertoire of proteins translated to those required for cell survival. Autophagy is induced in an attempt to provide an alternate source of some basic bioenergetic building blocks, such as amino acids, required for cell survival under starvation conditions. Under these conditions, vacuolar hydrolases are included among the bulk cytoplasm delivered to the yeast vacuole via autophagy. TOR regulates the switch between autophagy and the CVT pathway by altering the interaction of the serine-threonine kinase ATG1 with other components of the induction complex, including ATG13, ATG17, and ATG11 (**Figure 2**) (4). Signaling through the starvation-induced elongation initiation factor 2α (eIF2α) kinase general control nonderepressible 2 (5), the AMP-activated kinase sucrose nonfermenting 1 (6), the cAMP-dependent protein kinase protein kinase A (PKA) (7), and RAS (8) pathways also regulates the induction of autophagy by altering the expression and/or activity of ATG1 and ATG13 in yeast (**Figure 3**). These regulatory kinases and the ATG1 component of the induction complex also play a conserved role in autophagy in higher eukaryotes (9).

The relevance of these signaling pathways to human disease is exemplified by the recent observation that a defect in autophagy is associated with the pathogenesis of pigmented lesions in patients with Carney complex (10). Carney complex is an inherited syndrome characterized by endocrine hyperactivity, spotty skin pigmentation (lentigines), and multiple neoplasias (including myxomas and primary pigmented nodular adrenocortical disease) (11). It is caused by heterozygous inactivating mutations in the gene encoding one of the negative regulatory subunits of PKA, *PRKAR1*, which leads to increased PKA activity. The accumulation of lipofuscin (a hallmark of decreased autophagy) and loss of heterozygosity at the *PRKAR1A* locus in the pigmented lesions are consistent with the defect in autophagy associated with *PRKAR1A* deficiency (10).

Cargo Selection and Packaging

Targeted degradation of cellular components presumably requires components of the basal autophagy machinery, as well as signals derived from the cargo selected for degradation, but very little is known about the signaling events regulating this process. The

PAS: pre-autophagosomal structure

TOR: target of rapamycin

CVT: cytoplasm to vacuole targeting

eIF2: elongation initiation factor 2

PKA: protein kinase A

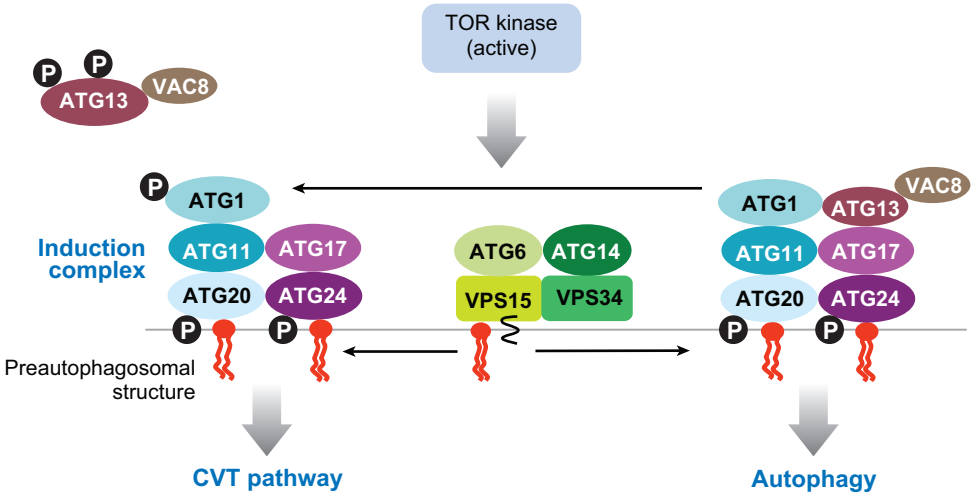

Figure 2

Target of rapamycin (TOR)-mediated regulation of the cytoplasm-to-vacuole targeting (CVT) pathway and autophagy. The TOR kinase regulates the switch between the nonselective and selective autophagy (CVT) pathway, at least in part by regulating the interaction between autophagy-related gene (ATG) 13 and the serine-threonine kinase ATG1. ATG13 is hyperphosphorylated in a TOR-dependent manner and in this form exhibits a reduced affinity for ATG1. Under starvation conditions, ATG13 becomes hypophosphorylated and can interact with ATG1. ATG1 and ATG13 are part of a putative larger complex that includes ATG11, ATG17, ATG20, and ATG24. This complex regulates the shift from the CVT pathway activation to autophagy upon starvation. Note, however, that the only components of this complex that appear to be conserved in higher eukaryotes are ATG1 and ATG20; this is perhaps not surprising because the CVT pathway has no direct metazoan counterpart. ATG6 forms a complex with the class III phosphoinositide 3-kinase complex, which includes vacuolar protein sorting-associated 34 (VPS34), ATG15, and ATG14.

CVT pathway is the prototype of selective autophagy in yeast but is not conserved in higher eukaryotes. The selective autophagy pathways relevant to higher eukaryotes are discussed in more detail below.

Mitophagy. Mitophagy, which refers to the autophagy of mitochondria, depends upon the presence of key regulators of autophagy as well as upon signals derived from mitochondria. In yeast, two mitochondrial proteins, UTH1 (12) and AUP1 (13), have been shown to play critical roles in mitophagy. UTH1 is a mitochondrial outer membrane protein involved in mitochondrial biogenesis and in the oxidative stress response that does not appear to be conserved in higher eukaryotes. AUP1 is a peripheral membrane protein located in the mitochondrial intermembrane space, and appears to be the unique yeast member of the highly conserved yet uncharacterized protein phosphatase 1K family of proteins, all of which contain a protein phosphatase 2C domain and are predicted to be mitochondrial (14). Further evidence that the signal for mitophagy can originate from the mitochondria itself comes from the observation that yeast mutants unable to maintain an electrical potential across the inner mitochondrial membrane exhibit an increase in mitophagy compared with wild-type cells, with preferential degradation of the damaged mitochondria (15). The exact relationship between AUP1, UTH1, and loss of mitochondrial membrane potential in signaling mitophagy (13, 14, 15) is difficult to assess because their roles in mitochondrial degradation were examined under different experimental conditions.

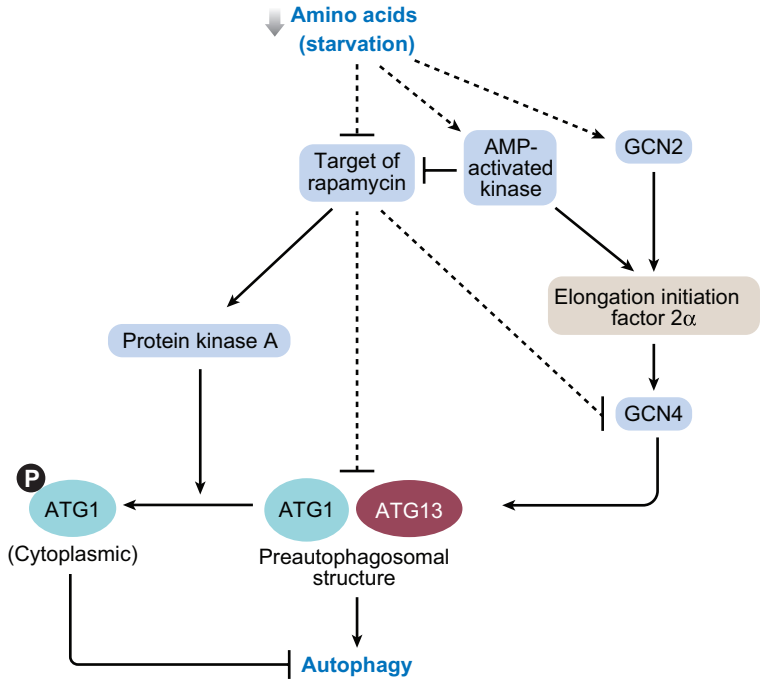

Figure 3

Multiple signaling pathways converge on the expression and activity of autophagy-related gene (ATG) 1 and ATG13 in yeast. Starvation alters the activity of several kinases, including target of rapamycin, protein kinase A, AMP-activated kinase, and general control nonderepressible (GCN) 2. Protein kinase A negatively regulates autophagy by phosphorylating ATG1, causing it to redistribute from the pre-autophagosomal structure to the cytoplasm. Phosphorylation of elongation initiation factor 2α by the starvation-induced kinase GCN2 activates the transcriptional regulator GCN4 and leads to increased expression of both ATG1 and ATG13. AMPK stimulates autophagy by inactivating target of rapamycin and phosphorylating elongation initiation factor 2α.

Genetic evidence that autophagy is involved in mitochondrial degradation in mammals comes from characterization of the *Atg7* conditional knockout mouse model (16). The defect in autophagy is associated with an accumulation of deformed mitochondria in Atg7-deficient hepatocytes, suggesting that autophagy is involved in turnover of damaged mitochondria in mammalian cells. In cell culture systems, mitochondrial autophagy in hepatocytes is inhibited by chemical inhibitors of autophagy (such as 3-methyladenine) and mitochondrial depolarization (such as cyclosporine) (17).

Pexophagy. The autophagy genes important for pexophagy (autophagy of peroxisomes) are highlighted in **Table 1**. In addition, degradation of peroxisomes is mediated by at least two peroxisome membrane proteins, PEX3 and PEX14, both of which are also required for peroxisome biogenesis and the import of class I peroxisomal membrane proteins (18). PEX3 and PEX14 are conserved in humans and are mutated in subsets of patients with Zellweger syndrome (19). However, the characteristic features are compatible with a defect in peroxisome biogenesis, as opposed to a defect in pexophagy; the roles of PEX3 and PEX14 in peroxisome degradation in higher eukaryotes have not been explored.

Degradation of ubiquitinated proteins. The degradation of long-lived proteins by

VPS: vacuolar protein sorting associated

autophagy has long been recognized (20); however, the exact mechanism by which this occurs has not been elucidated. Although it is possible that this lysosomal protein degradation reflects a default pathway for those proteins not specifically targeted by the ubiquitin-proteasome system, substrates normally degraded by the ubiquitin-proteasome pathway can be selectively degraded by autophagy under certain circumstances (21, 22). The following observations also support the hypothesis that there is cross-communication between the two major pathways of protein degradation: (*a*) Proteasome inhibition leads to the induction of compensatory autophagy (22a); (*b*) inactivation of the ubiquitin-activating enzyme E1 leads to an absence of ubiquitinated proteins in the lysosome and a defect in lysosome-mediated protein degradation (23); and (*c*) defects in autophagy lead to the accumulation of ubiquitinated protein deposits in the brains of mice and to neurodegeneration (24, 25). Another source of ubiquitinated proteins in the lysosome comes from transmembrane proteins, which are normally mono- or polyubiquitinated. Ubiquitination is critical for the proper sorting (recycling versus degradation) of these proteins following endocytosis, and, therefore, the accumulation of ubiquitinated proteins resulting from defects in autophagy may reflect a disruption in the normal degradation of misfolded, processed proteins that are diverted via autophagy from endosomal pathways to the lysosome. Potential linkers mediating autophagic degradation of ubiquitinated proteins include the autophagy-linked FYVE protein (26) and p62/Sequestosome 1 (27). Defects in this pathway have implications for certain neurodegenerative disorders.

Glycogen autophagy. Glycogen is one of two major types of glucose stores in yeast (the other is trehalose). It is a high molecular mass, branched polysaccharide of linear $\alpha(1,4)$-glucosyl chains with $\alpha(1,6)$ linkages. Glycogen is accumulated as a carbohydrate reserve under conditions of nutrient deprivation, with limitations in carbon, nitrogen, phosphorus, or sulfur, all acting as triggers for increased glycogen synthesis. The induction of autophagy is also critical for the maintenance of glycogen stores (6). In a systematic screen for genes affecting glycogen storage in yeast, ~10% of the mutants with aberrant glycogen levels were involved in vesicular trafficking or vacuolar function (28).

In mammals, the critical role of glycogen autophagy in allowing newborns to survive the immediate postnatal period of starvation, which demands massive liberation of free glucose, was highlighted by the death of Atg5- and Atg7-deficient mice shortly after birth from starvation (16, 29). Following glycogen autophagy, lysosomal glycogen is metabolized by acid α-glucosidase, which catalyzes the hydrolysis of α-(1,4) and α-(1,6) linkages (30). Cytosolic glycogen is metabolized to glucose in the cytosol by glycogen phosphorylase and debranching enzymes. Hepatic glycogen autophagy appears to be a selective and highly regulated process. In newborns, glycogen autophagy is stimulated by cAMP and cAMP-elevating enzymes such as glucagon and adrenalin and inhibited by cAMP-lowering drugs such as propanolol (31). Glycogen autophagy is also observed in newborn heart and diaphragm, two organs with abrupt increases in energy requirement at birth (32), and can be induced in muscle after strenuous exercise (33).

Membrane Biogenesis: Nucleation, Expansion, and Completion

Nucleation refers to the assembly of the PAS and depends upon the stepwise recruitment of specific autophagy proteins, beginning with ATG17 (34). ATG17 recruits ATG13 and ATG9 that together activate the class III phosphoinositide 3-kinase (PI3K) complex, which includes vacuolar protein sorting-associated (VPS) 34, ATG15, and ATG14. ATG6 (homolog of BECN1) associates with the PI3K complex. ATG13 interacts with ATG1, under conditions of nutrient limitation when TOR

is inactivated, and together with the PI3K complex recruits ATG18 [which contains a phosphatidylinositol-3-phosphate (PI3P) binding domain] and ATG2. ATG2 and ATG18 are involved in the retrograde transport of ATG9 to the periphery (35), a process thought to be involved in the recycling of membrane, and possibly other autophagy proteins. The PI3K complex is also required for the localization of the complex containing the ATG5/ATG12 conjugate and ATG16, as well as lipidated ATG8 (homolog of MAP1LC3) to the PAS. The covalent linking of ATG12 to ATG5 (ATG5/ATG12 conjugate) and phosphatidylethanolamine (PE) to ATG8 (ATG-PE, or lipidated ATG8) each depends upon the presence of intact ubiquitin-like conjugation systems involving ATG3, ATG4, ATG7, and/or ATG10 (1, 2). ATG8-PE associates with both the surfaces of the expanding double membrane autophagosome, unlike the complexes of ATG5/ATG12 and ATG16, which are found only on the cytosolic surface; both of these are critical for membrane expansion.

Vesicle completion refers to the fusion of the two extremities of the expanding isolation membrane. The molecular mechanisms involved in this process are not well understood but are independent of soluble N-ethylmaleimide sensitive factor attachment (SNARE) proteins, which are involved in many of the membrane fusion events in the endosomal pathway (2). This fusion event triggers the ATG4-mediated cleavage of ATG8-PE from its lipid moiety and the release of cleaved ATG8, and consequently ATG5/ATG12 and ATG16 complexes from the cytosolic surface of the completed autophagosome. The completed autophagosome is marked by the ATG8-PE associated with the internal membrane of the autophagosome, which was not accessible to cleavage by ATG4. The interaction of ATG4 with components of the yeast microtubule system, and also with ATG8, suggests that the microtubule system may be involved in the transport of autophagosomes during expansion and/or maturation (36).

Fusion, Degradation, and Export

After the completed autophagosome is uncoated, it docks and fuses with the vacuolar membrane. This process is similar to that which mediates homotypic and heterotypic fusion events with late endosomes, multivesicular bodies, or Golgi; all of these processes use an identical fusion machinery. Components of this machinery include SNARE proteins, SEC18, SEC17, YPT7, the calcium-caffeine-zinc sensitivity protein 1/monensin sensitivity protein 1 complex, and the class C VPS protein complex that is also known as the homotypic vacuole fusion and vacuole protein sorting complex (37). In mammals, vesicle maturation occurs in a stepwise fashion and involves fusion of the completed autophagosome with endosomes, multivesicular bodies, and finally lysosomes (38).

Degradation of autophagic bodies is mediated by hydrolases, including the lipase ATG15, and vacuolar peptidases, including PEP4 (cathepsin D) and PRB1 (2). The final step in autophagy, which plays a critical role in the survival of yeast under starvation conditions, is the export of amino acids that results from autophagic degradation. This was highlighted by the identification of ATG22, AVT3, and AVT4 as partially redundant vacuolar effluxers that are involved in the efflux of leucine and other amino acids from the vacuole, and that allow the maintenance of protein synthesis and viability during nitrogen starvation (39).

PI3P: phosphatidylinositol-3-phosphate

ROLE OF AUTOPHAGY IN DISEASE

Autophagy has been implicated in the pathogenesis of various disease processes including cancer, neurodegeneration, and certain myopathies; it is also involved in the innate and adaptive immune response to various pathogens. Central to the discussion of autophagy in disease pathogenesis is its role in adapting to cellular stresses versus its role in contributing to cell death (40). In addition to

its undisputable role in cell survival in adverse conditions, autophagy can also be induced as part of a cell death response, and the accumulation of autophagosomes is characteristic of type II cell death (41). Although the observation that certain dying cells display morphological hallmarks of autophagy is clear, the question of whether autophagy causes cell death is still a subject of intense investigation. Ultimately, the fate of the cell may depend upon the strength and nature of the toxic stimulus, with mitochondria playing a central role in the process (42). For example, autophagy may sequester damaged cellular components and depolarized mitochondria, allowing the cell to survive weak insults; alternatively, if mitochondrial depolarization and release of proapoptotic factors exceed the autophagic capacity, apoptosis ensues. Degradation of mitochondria in the absence of apoptosis (or by inhibition of apoptosis at a postmitochondrial step using caspase inhibitors) may lead to ATP depletion and necrosis (40). Increased levels of certain autophagy proteins, including ATG5, BECN1/ATG6, and ATG1, have been associated with cell death (mostly by apoptosis) (43–45). The mechanism by which increased expression of autophagy genes leads to apoptotic cell death is not clear but may involve a positive feedback loop that results in the suppression of the AKT/TOR pathway (45, 46). At the molecular level, there are many points of cross-communication between apoptotic and autophagic pathways that involve BCL2 family members, p53, and p27, to name a few. Recent studies have also highlighted the importance of calpains in both autophagy and apoptosis (44, 47).

Cancer

The first indication that a defect in autophagy may contribute to tumorigenesis came from the realization that *BECN1/ATG6* may be a tumor suppressor. Monoallelic deletions of *BECN1* have been identified in 40% (9/22) of breast cancer cell lines, and the *BECN1* gene maps to a tumor-susceptibility locus on human chromosome 17q21 that is monoallelically deleted in up to 75% of ovarian cancers, 50% of breast cancers, and 40% of prostate cancers (48). These findings—together with the observation that ectopic BECN1 expression in MCF7 cells that have low endogenous levels of BECN1 can induce autophagy and restrict the growth of these tumor cells in a nude mouse model (49), and that *Becn1* haploinsufficiency in mice leads to an increased incidence of lymphomas and carcinomas of the lung and liver (50, 51)—have led to the hypothesis that *BECN1* (and by extension other genes involved in autophagy) may function as tumor suppressors and that defects in autophagy may promote tumorigenesis. Similarly, the PI3K/AKT/TOR pathway is frequently activated constitutively in response to mutations in genes encoding negative regulators such as TSC1/2 and PTEN, and is another mechanism by which autophagy may be inhibited in certain tumors (52).

There are a number of potential mechanisms by which a defect in autophagy could contribute to the pathogenesis of cancer (**Figure 4**). First, autophagy is involved in the removal of aged and damaged organelles, and defects in autophagy can lead to the accumulation of dysfunctional mitochondria; increased oxidative stress with damage to membranes, proteins, and DNA, the latter a potential source of genetic instability; and eventually to the outgrowth of a tumorigenic clone (53). Second, because autophagy can be part of a cell death pathway similar to apoptosis, a defect in autophagy could increase cell survival under certain circumstances. This is supported by the observation that deficiency of ATG1 in the context of a mosaic *Drosophila* (simulating cancerous or precancerous cells in a normal environment) confers a relative growth advantage (45), although the mechanism by which this occurs is not entirely clear. Central to both of these hypotheses is the role of autophagy in cell death and the relationship between autophagy and apoptosis. Further study of the *Drosophila* model may provide additional clues to how defects in autophagy may

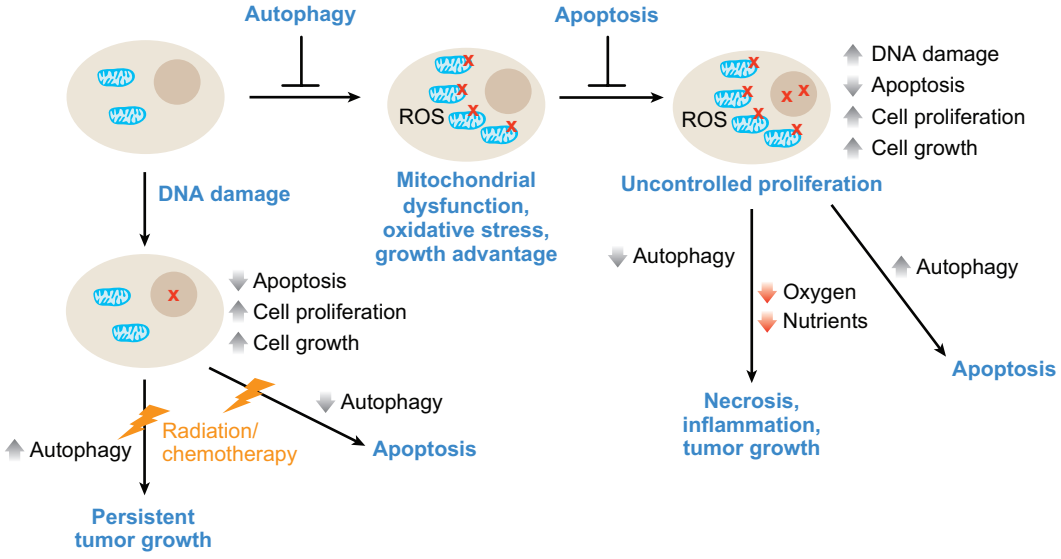

Figure 4

Autophagy and cancer. Defects in autophagy may contribute to the development of cancer by promoting the accumulation of damaged/aged mitochondria and/or conferring a selective growth advantage. Defects in autophagy and apoptosis may arise sequentially or simultaneously through mutations, leading to constitutive activation of the phosphoinositide 3-kinase/AKT/target of rapamycin pathway. Cancer cells harboring defects in autophagy and apoptosis are more susceptible than others to necrotic cell death in response to hypoxia/ischemia and/or nutrient deprivation. Induction of autophagy, especially in cells lacking p53 or p27, may promote apoptosis. By contrast, in other cancer cells, inhibition of autophagy may increase susceptibility to radiation or chemotherapy. ROS, reactive oxygen species.

contribute to the pathogenesis and growth of tumors.

In addition to blocking cell death, rapidly growing tumor cells must also increase nutrient uptake and angiogenesis to support the upregulation of metabolism necessary for unrestricted growth. Mutations that promote cell proliferation, the hypoxic tumor microenvironment, and perhaps mitochondrial malfunction may all contribute to the reliance on inefficient energy production by glycolysis (54). Interestingly, many of the same signals that enable unrestricted cell proliferation also inhibit autophagy, which is normally induced to sustain cells during nutrient limitation. The inhibition of autophagy that results from constitutive activation of such pathways, and uncoupling of the cellular response to nutrient availability and growth-regulatory signals, renders cells more susceptible to a metabolic catastrophe and necrotic cell death (55). At first glance, this may seem to be counterproductive for tumor growth; however, it appears that the inflammatory response to tumor necrosis may promote overall tumor burden (56).

Finally, in tumors that do not exhibit defects in autophagy, or in slower-growing tumors that do not outgrow their food supply, inhibition of autophagy may render cells more susceptible to chemotherapy- or radiation-induced stress (57, 58). By contrast, stimulation of autophagy in tumors with constitutive activation of the PI3K/AKT pathway can render them more susceptible to apoptosis, particularly in the absence of p53 or p27 (59, 60). Thus, the regulation of autophagy in tumor cells provides an exciting target for the development of new therapeutic strategies, although given the complexity of the signaling pathways involved in the regulation of tumor cell proliferation and death, the therapy will

likely have to be tailored to individual groups of tumors.

Neurodegeneration

Autophagic activity is essential for normal function of the nervous system. During development, autophagy participates in shaping the form and function of the nervous system. For example, substrate-specific autophagic degradation of synaptically localized proteins regulates synapse formation (61). In the fully developed animal, autophagy plays a critical role in neuronal homeostasis. Homeostasis in neurons involves massive synthesis and anterograde axonal transport of protein and membrane that must be balanced by a similar rate of retrograde transport and clearance by autophagy (62). The importance of autophagy in neuronal homeostasis is underscored by the finding that a deficiency of basal autophagy in the mouse brain (by conditional knockout of either *Atg5* or *Atg7*) results in neurodegeneration characterized by the accumulation of ubiquitinated protein aggregates (24, 25).

Autophagy was first implicated in human neurodegenerative disease on the basis of the presence of autophagic vacuoles containing disease-related proteins in brain regions affected by Alzheimer's disease (63). It has subsequently been documented in Huntington's and related CAG/polyglutamine (polyQ) diseases and in certain lysosomal storage disorders. The accumulation of autophagosomes in these conditions may be indicative of an adaptive response to stress or cell death, or a defect in autophagosome maturation. As autophagy in the setting of neurodegeneration has been investigated, it has become apparent that the role of autophagy may vary in different neurodegenerative diseases, as described below.

Autophagy as an adaptive response in neurodegeneration. A broad array of age-related neurodegenerative diseases is characterized by the accumulation and deposition of misfolded protein aggregates in affected brain regions, including Alzheimer's, Parkinson's, and the CAG/polyQ diseases (64). The central role that abnormal protein deposits play in pathogenesis was established by frequent identification of disease-causing mutations that promote aggregation and deposition of these same disease proteins. The role of autophagy as an adaptive, protective response in these proteopathies is supported by extensive evidence that these disease-related proteins (and protein aggregates) are degraded by autophagy. Significantly, with respect to the prospect of therapeutic intervention, genetic or chemical manipulation of autophagy can modify the disease phenotypes in animal models of disease (22a, 65).

Induction of autophagy has been seen in cell culture models of other conformational diseases, including spinocerebellar ataxia 7, Huntington's, Alzheimer's, and Parkinson's diseases (66–70). When combined with observations that rapamycin ameliorates the degenerative phenotype in fly and mouse models of polyQ disease, and that toxicity increases in autophagy-deficient backgrounds, these findings suggest that the induction of autophagy plays a protective role in neurodegeneration caused by toxic proteins (65; 22a). In the absence of toxic proteins, loss of autophagy also leads to neurodegeneration, with ubiquitin-positive pathology (24, 25). Although it remains to be determined how autophagy mitigates polyQ-induced degeneration, the presence of polyQ-expanded N-terminal fragments of androgen receptor within autophagic vacuoles (71), and evidence that lysosome-mediated degradation contributes to the turnover of some disease proteins in cell culture models (72–75) and in vivo (22a), suggests that autophagy may protect cells by clearing potentially noxious, aggregation-prone proteins. However, if this is true, it remains unclear why the physiologic autophagic response to expression of expanded polyQ is not sufficient to completely protect neurons from such proteotoxins, and why pharmacologic amplification of this response with rapamycin rescues the toxicity.

One possibility is that the accumulation of autophagic vacuoles in disease models reflects an abnormally prolonged rate of clearance of autophagolysosomes containing polyQ aggregates. Thus, polyQ proteins may be inefficiently cleared by autophagy owing to a block in processing autophagolysosomes, perhaps a consequence of polyQ toxicity, but this block may be overcome by inhibiting TOR. An additional unresolved issue is whether autophagy might play a role in driving the disease process. This question arises because of reports that autophagy may contribute to generating toxic fragments of disease-related proteins such as β-amyloid peptide and N-terminal huntingtin fragments (76, 77).

Autophagy and neuronal cell death. The idea that the accumulation of autophagic vacuoles in relevant neuronal subpopulations may in certain cases reflect cell death is supported by evidence that the death of neurons is often associated with autophagic features (78) and that autophagy contributes to cell death in several animal models of neurodegeneration associated with excitotoxic stress. In *Caenorhabditis elegans*, gain-of-function mutations in specific ion channel genes result in ion channel hyperactivity accompanied by intense degradation of cytoplasmic contents, membrane infolding, vacuole formation, and degeneration of a subset of neurons. This degeneration is partially suppressed in autophagy-deficient nematodes, compatible with a role for autophagy in excitotoxic neurodegeneration (79). Expression of a constitutively active mutant glutamate receptor in *Lurcher* mice causes cell death of virtually all cerebellar Purkinje cells from the direct activation of autophagy through a signaling complex that involves the mutant receptors (but not the wild-type receptor), a novel protein interacting with the GluRδ2 glutamate receptor via a PZD domain termed n-PIST, and Becn1. Although this model is also considered a model of neurodegeneration caused by excitotoxic stress, cell death can be dissociated from the degree of depolarization induced by the mutation (80).

Defects in autophagosome maturation in lysosomal storage disorders. In yeast and in mammals, defects in lysosome biogenesis and/or function can lead to inefficient fusion of autophagosomes with the lysosome and reduced degradation of autophagosomal contents. By extension, lysosomal storage disorders, which result from defects in genes that either directly or indirectly affect lysosomal function, should inhibit autophagosome maturation and lead to the accumulation of autophagic vacuoles in affected cells. This notion is supported by the observation that cathepsin D deficiency results in the accumulation of autophagic vacuoles and subunit c of mitochondrial ATP synthase in the brain of mice and of human patients with congenital neuronal ceroid lipofuscinoses (81). The mechanism by which this secondary defect in autophagy may contribute to neurodegeneration is not entirely clear, but recent studies suggested that inefficient autophagolysosomal recycling of mitochondria may generate fragmented, degenerated mitochondria with reduced mitochondrial Ca^{2+} buffering capacity and increased sensitivity to apoptosis (82).

Myopathies

In muscle cells, autophagy is induced in response to changes in the extracellular availability of nutrients, and also in response to changes in trophic input from presynaptic neurons (61, 83, 84). Accordingly, denervation of muscle results in myofibrillar disorganization accompanied by a prominent autophagic response that can lead to rapid muscle wasting. In normal muscle, however, this process is attenuated by a transcriptional program involving 29 genes, encoding ion channels, signaling molecules, and muscle structural proteins that is coordinated by RUNX1, a transcription factor best known for its role in normal hematopoiesis and in leukemogenesis (85). Because myofibrillar disorganization and

AVSFs: autophagic vacuole with sarcolemmal features

the accumulation of autophagic vacuoles are features seen in many congenital myopathies, autophagy may be involved in a final common pathway that leads to muscle wasting. With respect to their relationship with autophagy, myopathies can be broadly classified into three groups on the basis of (*a*) disruption of PI3P signaling and endosomal trafficking, (*b*) disruption of lysosomal degradation, and (*c*) accumulation of autophagic vacuoles with sarcolemmal features (AVSFs). In addition to the role of autophagy in myopathies, which is discussed in more detail below, literature is emerging on the topic of autophagy in myocardial ischemia, although it is not entirely clear whether its effects are beneficial, harmful, or both (43, 86).

Myopathies associated with defects in PI3P signaling and endosomal trafficking. X-linked myotubular myopathy and centronuclear myopathy share common pathological features, namely, the presence of hypotrophic myofibers with prominent internalized or centrally placed nuclei (87). These disorders are associated with defects in myotubularin and the novel phosphoinositide phosphatase hJUMPY, respectively, both of which dephosphorylate the product of the VPS34 PI3K, PI3P (88, 89). Although the roles of these phosphoinositide phosphates in the regulation of autophagy have not been directly examined, deficiency of PI3P phosphatases in yeast resulted in the accumulation of the phosphorylated substrate, especially in vacuolar membranes, leading to vacuolar protein-sorting defects, vacuolar fragmentation, and the misregulation of PI3P-specific effectors, including ATG24 (90). These preliminary studies suggest that this group of diseases may be associated with defects in endosomal processing, autophagy, and/or autophagic sequestration of glycogen, any of which could lead to the impaired muscle maintenance seen in these disorders.

Myopathies associated with lysosomal deficiencies. Pompe's disease, or type II glycogenosis, is an autosomal recessive disorder characterized by the lack of lysosomal acid glucosidase (also known as acid maltase), which leads to the accumulation of undegraded glycogen inside and outside lysosomes, most prominently in the liver, heart, and skeletal muscles. There are three forms of the disease (infantile, childhood, and adult), which vary in age of onset of symptoms and severity of disease. The severity of symptoms, and degree of accumulation of Periodic Acid Schiff stain–positive, acid phosphatase-positive vacuoles in various tissues, correlates inversely with residual enzyme activity. When viewed by electron microscopy, these vacuoles contain cytoplasmic debris, electron dense bodies, myelin figures, and glycogen, although the glycogen deposition is more prominent outside the vacuole. Studies using myoblasts isolated from acid-α-glucosidase-deficient mice have revealed a marked expansion of vesicles of the endocytic and autophagic pathways, with large buildup of autophagosomes in type II muscles fibers compared with type I fibers. These observations, combined with the observation that starvation leads to an autophagic response in type II but not type I muscle fibers, have led to the following model: The failure of glycogen digestion results in a local starvation that stimulates a strong autophagic response in type II fibers, which in combination with the inability of the vesicles to fuse and release contents into lysosomes leads to the buildup of autophagosomes and disorganization of microtubules (91). This inability to adequately respond to starvation signals through autophagic breakdown of glycogen and other cytoplasmic constituents may account for the muscle wasting associated with the disease.

Myopathies associated with the accumulation of autophagic vacuoles with sarcolemmal features. Danon disease is an X-linked disorder characterized by mental retardation, myopathy, and cardiomyopathy resulting from a deficiency of LAMP2. LAMP2 is a lysosomal protein involved in

lysosome biogenesis. Patients typically die of cardiomyopathy, which is severe and life threatening; myopathy is mild and affects neck and shoulder girdle muscles, although distal muscles may also be involved. Heterozygous females are also affected and develop cardiomyopathy with later onset. Although Danon disease was initially termed lysosomal glycogen storage disease with normal acid maltase, because the pathological features superficially resembled Pompe's disease, the vacuoles that accumulate contain cytoplasmic debris and/or sarcoplasmic membranes and are known as AVSFs. These autophagic vacuoles are most prominent in skeletal muscle and ultimately lead to fiber splitting and degeneration. Patients with Danon disease harbor mutations in *LAMP2* that are predicted to truncate the protein and result in loss of transmembrane and cytoplasmic domains. As a result, LAMP2 is not detected in skeletal muscles of patients with Danon disease (92). Lamp2 deficiency in mice results in extensive accumulation of autophagic vacuoles in many tissues (93). AVSFs seen in patients with Danon disease represent autophagolysosomes surrounded by membranes with sarcolemmal features (positive for dystrophin-associated complex and acetylcholinesterase), and may represent abnormal trafficking of sarcolemmal proteins in response to defects in LAMP2-mediated fusion (94).

Other disorders characterized by the presence of AVSFs and autophagic vacuoles include X-linked recessive myopathy with excessive autophagy, X-linked congenital autophagic vacuolar myopathy, infantile autophagic vacuolar myopathy, and adult-onset autophagic vacuolar myopathy with multiorgan involvement (87). These disorders are distinguished from Danon disease by the deposition of complement (C5-b9) along the sarcolemma and the presence of multilayered basal lamina, which in some cases surrounds exocytosed material. The genes responsible for these disorders have not been identified, and the relationship of the autophagic vacuoles to the pathogenesis of the diseases is unclear.

Infectious Disease

Bacterial pathogens. The mammalian innate immune system is the first line of defense against invading bacterial pathogens. Although one of its functions is to stimulate an antigen-specific adaptive response, the peak of primary adaptive immunity does not occur until 5–7 days post infection. In the meantime, the innate immune system employs many strategies to control bacterial survival and replication. Induction of autophagy is among these host strategies and is particularly relevant for bacteria that enter an intracellular niche (95). As intracellular bacterial pathogens typically target macrophages or epithelial cells for entry, most of the studies examining the role of autophagy as a component of the host innate immune response focus on these cells. Bacteria may enter mammalian cells by phagocytosis or by facilitating their own uptake. During this process, bacterial ligands may trigger innate immune sensors such as the Toll-like receptors or Nod-like receptors to activate proinflammatory cytokines, such as interleukin-1β, which allow infected cells to communicate with other effectors of innate and adaptive immunity (96, 97). The role of these receptor networks in stimulating autophagy in mammalian cells has not been explored, although studies have suggested that VPS15 (involved in the regulation of the class III PI3K, VPS34) may be a regulator for the activation of the Imd NFκB immune signaling pathway in *Drosophila* (98).

Once inside the host cell, pathogens encounter cellular defenses within endosomes or phagosomes, although many bacteria have evolved ways to avoid these innate defenses by diverting normal vesicular trafficking or by escaping into the host cytosol. The predominant strategy in innate immunity to counteract intracellular bacterial pathogens appears to involve compartmentalization within membrane-bound vacoules,

GAS: *Group A Streptococcus*

including autophagosomes, which serve three main functions: (*a*) They provide an enclosed space within which the host cell can direct a potent antimicrobial attack such as the oxidative burst; (*b*) they contain bacteria within an environment that has low nutrient availability compared with the host cytosol; and (*c*) they facilitate major histocompatibility complex (MHC) class II antigen presentation by professional antigen presenting cells that stimulate antigen-specific adaptive immunity (99).

The relationship between cellular autophagy and the growth of various intracellular bacterial pathogens has been studied. These pathogens can be separated broadly into three groups on the basis of the role of autophagy in the replication cycle (**Figure 5**). Autophagy is involved in sequestering and eliminating intracellular (and specifically cytosolic) bacteria, including *Group A Streptococcus* (GAS), *Salmonella typhimurium*, *Shigella flexneri*, and *Listeria monocytogenes* (Group 1). Many pathogens, including *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Porphyromonas gingivalis*, establish replicative niches within phagosomes that are often arrested in maturation. These pathogens are grouped together because both bacterial growth and phagosome maturation can be influenced by manipulation of the autophagic pathway. Specifically, stimulation of autophagy in the host promotes degradation of *M. tuberculosis* (Group 2a), whereas inhibition of autophagy promotes degradation of *L. pneumophila* and *P. gingivalis* (Group 2b). Details on the role of autophagy in these organisms are provided in the section below. Other pathogens, including *Francisella tularensis* (100), *Coxiella burnetii* (101), and *Chlamydia* (102), have been found in association with autophagosomes; however, the role of autophagy in the growth of these pathogens has not been definitively established.

Group 1: sequestration of cytosolic bacteria by autophagy. GAS enters human epithelial cells via early endosomes and then escapes into the cytosol by the action of StreptolysinO. StreptolysinO is a member of a conserved family of cholesterol-dependent pore-forming cytolysins secreted by GAS. Once in the cytosol, GAS induces autophagy and becomes trapped in MAP1LC3/ATG8-positive autophagosomes, where it is subsequently killed (**Figure 5*a***). The importance of autophagy in the elimination of this pathogen is highlighted by the observation that GAS can survive and replicate in *Atg5*$^{-/-}$ animals (103). However, the mechanism by which GAS is recognized and sequestered in autophagosomes is not known.

S. typhimurium, the causative agent of food poisoning and typhoid fever, actively invade host cells, including macrophages and epithelial cells, and typically reside within a membrane-bound compartment known as the *Salmonella*-containing vacuole (SCV). The role of autophagy in macrophages compared with that in epithelial cells is very different. In macrophages, mitochondrial autophagy is triggered in response to mitochondrial damage caused by a bacterially encoded protein, which ultimately leads to cell death, whereas in epithelial cells, autophagy is involved in limiting bacterial growth. The *Salmonella* pathogenicity-island-1-encoded type three secretion system (TTSS) is known to damage eukaryotic cell membranes, including the SCV. In macrophages, this damage gives rise to an intracellular bacterial population that is removed by Synaptotagmin VII–dependent lysosomal fusion. In epithelial cells, most of the intracellular *S. typhimurium* remains within SCVs that are similar to phagosomes but arrested in maturation (with acquisition of RAB7 and LAMP1/2), and do not fuse with lysosomes (104). However, the TTSS-mediated damage gives rise to a population of bacteria within the cytosol (**Figure 5*a***), which are subsequently targeted by the ubiquitin-conjugating system, become associated with ubiquitinated proteins, and are sequestered by autophagosomes (105, 106). Autophagy-deficient epithelial cells are more permissive for intracellular growth by *S. typhimurium* than normal cells, supporting the notion that

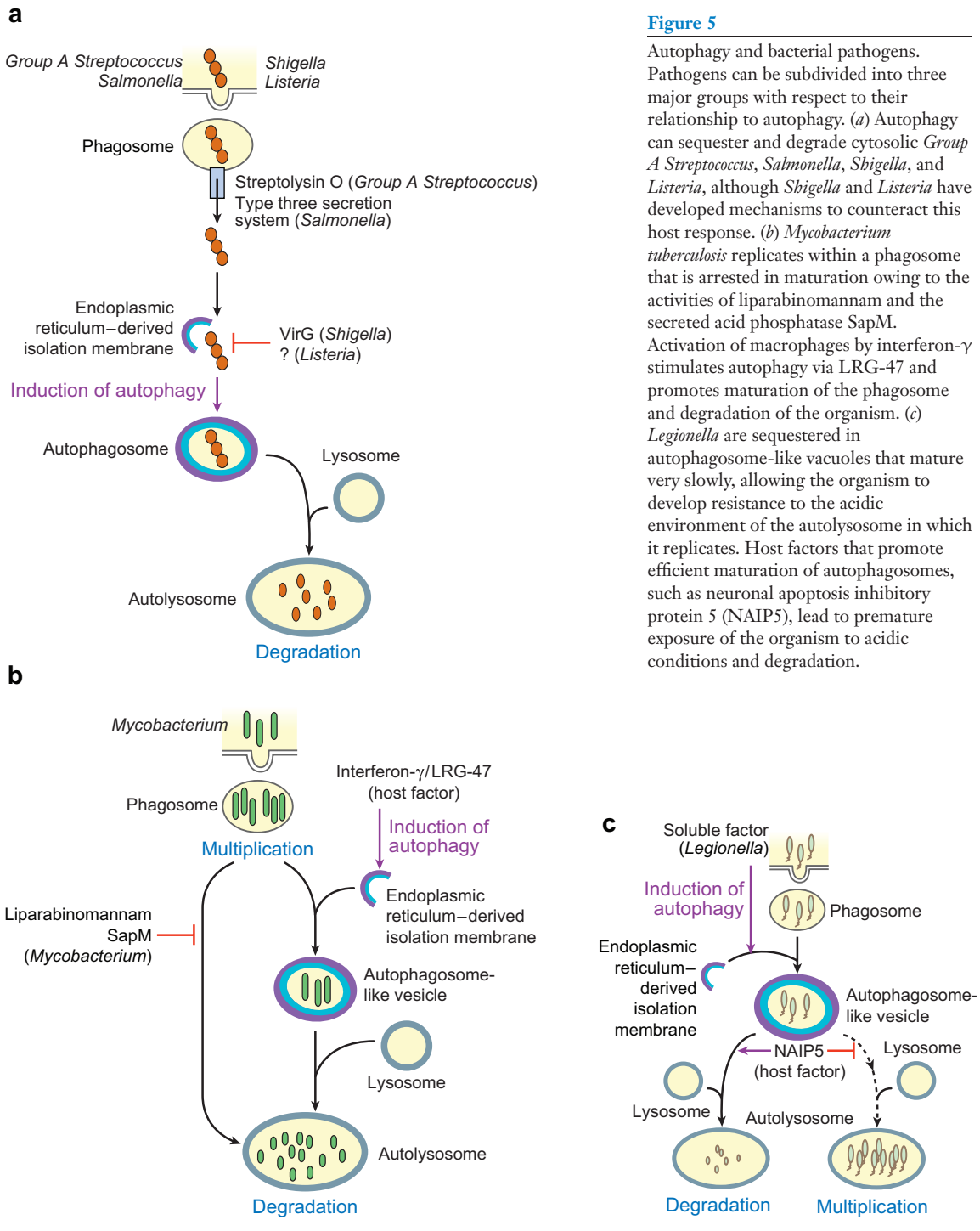

Figure 5

Autophagy and bacterial pathogens. Pathogens can be subdivided into three major groups with respect to their relationship to autophagy. (*a*) Autophagy can sequester and degrade cytosolic *Group A Streptococcus*, *Salmonella*, *Shigella*, and *Listeria*, although *Shigella* and *Listeria* have developed mechanisms to counteract this host response. (*b*) *Mycobacterium tuberculosis* replicates within a phagosome that is arrested in maturation owing to the activities of liparabinomannam and the secreted acid phosphatase SapM. Activation of macrophages by interferon-γ stimulates autophagy via LRG-47 and promotes maturation of the phagosome and degradation of the organism. (*c*) *Legionella* are sequestered in autophagosome-like vacuoles that mature very slowly, allowing the organism to develop resistance to the acidic environment of the autolysosome in which it replicates. Host factors that promote efficient maturation of autophagosomes, such as neuronal apoptosis inhibitory protein 5 (NAIP5), lead to premature exposure of the organism to acidic conditions and degradation.

IFN: interferon

autophagy contributes to host defense against bacterial colonization (105).

S. flexneri invade resident macrophages, escape from phagosomes into cytoplasm, multiply, and induce cell death. After being released from dead macrophages, *Shigella* enter surrounding enterocytes through the basolateral surface via activation of the TTSS. The intracellular *Shigella* can be sequestered in autophagosomes, a process triggered by the interaction of ATG5 with the bacterial VirG protein in *IcsB* mutants. In wild-type *Shigella*, however, secretion of IcsB, a component of TTSS, inhibits the innate autophagic response by binding VirG and preventing its interaction with ATG5 (**Figure 5*a***) (107).

The intracellular pathogen *L. monocytogenes* can also be a target for autophagy. Metabolic inhibition of cytoplasmic bacteria by treatment of macrophages with chloramphenicol after phagosome lysis prevents them from adapting to the intracellular niche and reveals a host mechanism that utilizes the autophagic pathway as a defense against invading pathogens. In chloramphenicol-treated macrophages, the bacteria are internalized from the cell cytoplasm into double membrane autophagic vacuoles that contain protein disulphide isomerase, a marker for the rough endoplasmic reticulum. The autophagic vacuoles appear to form by fusion of small cytoplasmic vesicles around the bacteria. This process is inhibited by autophagy-inhibiting drugs and accelerated under starvation conditions. Sequestration by autophagy provides a route for the removal of the organisms from the cytoplasm and degradation by the subsequent delivery to the endocytic pathway (108).

Group 2a: stimulation of autophagy promotes maturation of phagosomes and degradation of pathogens. *M. tuberculosis*, the causative agent of tuberculosis, is transmitted by aerosolized nasal droplets containing bacteria and penetrates to the alveoli of the respiratory tract of the uninfected individual. Alveolar macrophages ingest the *M. tuberculosis* bacilli and enclose them in phagosomes. These phagosomes are arrested in maturation at an early stage, expressing RAB5 but not RAB7, and also exhibit diminished acidification owing to disruption of the normal delivery of the VoH+ATPase subunits and lysosomal hydrolases. Both of these defects reflect the inhibition of PI3P-dependent trafficking by two *M. tuberculosis* products: liparabinomannam, a lipid that mimics mammalian phosphatidylinositol and prevents generation of PI3P, and the secreted acid phosphatase SapM. The bacilli survive and grow within phagosomes until the macrophages lyse, releasing mycobacteria into the surrounding lung tissue. Alternatively, activation of infected macrophages by interferon (IFN)-γ leads to the production of LRG-47, a p47 GTPase that induces autophagy. The mycobacteria-containing phagosomes then fuse with autophagosomes, resulting in acidification of the vesicle and subsequent degradation of the bacilli in lysosomes (**Figure 5*b***). Stimulation of autophagy in macrophages by amino acid starvation or treatment with rapamycin also promotes degradation of the bacteria (109).

Group 2b: autophagy is subverted for the establishment of a replicative niche. *L. pneumophila* invade pulmonary alveolar macrophages and multiply within susceptible hosts expressing reduced levels of neuronal apoptosis inhibitory protein 5, an endogenous caspase inhibitor thought to promote efficient autophagosome maturation by inhibition of caspase-3. Virulent *Legionella* produce a soluble factor that stimulates autophagy in macrophages. In permissive murine hosts, *Legionella* ingested by macrophages are sequestered in autophagosome-like vacuoles derived from the endoplasmic reticulum that mature very slowly and co-express ATG7 and ATG8. (The modification of ATG8 by ATG7 is normally so rapid that ATG7 is not detected on the surface of autophagosomes.) The delayed maturation of *Legionella*-containing vacuoles in permissive host allows

the organism to resist the acidification that occurs in the final stage of development, after fusion with lysosomes. Inhibition of autophagosome formation results in degradation of the *Legionella*-containing vacuole by the endosomal-lysosomal pathway. In nonpermissive hosts, efficient host autophagy results in rapid delivery to lysosomes and degradation of the organism (**Figure 5*c***) (110).

P. gingivalis is an important periodontal pathogen associated with adult periodontitis and a postulated contributing factor to atherosclerosis and cardiovascular disease. The survival of *P. gingivalis* depends upon the activation of autophagy and survival of the endothelial host cell. Initially, *P. gingivalis* adheres to the host cell surface and is internalized via lipid rafts and incorporation of the bacterium into early phagosomes. *P. gingivalis* activates autophagy, providing a replicative niche while suppressing apoptosis. The replicating vacuole contains host proteins delivered by autophagy, which are used by this asaccharolytic pathogen to survive and replicate within the host cell. Suppression of autophagy results in trafficking of internalized *P. gingivalis* to phagolysosomes and degradation of the organism (111).

Viral pathogens. The role of the IFN signaling pathways in the innate immune response to viruses has long been recognized. The IFNs are a family of secreted cytokines that regulate many cellular functions including proliferation, cell survival, and immunomodulation, all aimed at inhibiting viral replication. The IFN signaling pathway intersects with autophagy at many points, most notably in the stimulation of autophagy by IFN-γ through activation of the double-stranded RNA-activated protein kinase (PKR) and phosphorylation of eIF2α. The importance of autophagy in viral elimination was first described in herpes simplex virus (HSV) infection and has been highlighted by the subversion of autophagy by viruses, including HSV, poliovirus and rhinovirus, and coronavirus. The relationship between cellular autophagy and replication of these viruses is discussed briefly in this section, in addition to the potential role of autophagy in Sindbis virus infection. Recent studies have also demonstrated that autophagy is involved in the recognition of cytosolic viral intermediates of single-stranded RNA viruses (including influenza virus, vesicular stomatitis virus, and sendai virus) by plasmacytoid dendritic cells, which are not typically infected but secrete IFN-α upon activation of Toll-like receptors in endosomes and lysosomes (112). However, in addition to its antiviral role, autophagy may be involved in the cell death response induced in bystander $CD4^+$ T cells in HIV infection, contributing to the depletion of $CD4^+$ cells and the onset of AIDS (113).

Herpes Simplex Virus-I inhibition of PKR-induced autophagy. PKR is among the family of kinases that phosphorylates eIF2α. It is activated by double-stranded RNA, which is a replication intermediate in the life cycle of many viruses. The importance of PKR in viral responses is highlighted by the fact that many viruses, including HSV-1, have evolved elaborate mechanisms to inhibit the PKR response, blocking the PKR response at virtually every step in the pathway, from activation through substrate phosphorylation (114). HSV-1 is a double-stranded DNA virus that encodes a well-characterized neurovirulence factor, HSV-1 ICP34.5, that plays a crucial role in the viral life cycle by inducing dephosphorylation of eIF2α, negating the effects of PKR. The importance of autophagy in controlling viral replication was highlighted by the observation that HSV-1 mutants that do not express ICP34.5 trigger autophagy via a PKR-dependent pathway in infected cells with concomitant degradation of the virus. It will be interesting to explore the role of autophagy in the life cycle of other viruses that target the PKR pathway.

Autophagy can be used to support viral replication. Poliovirus and rhinovirus are lytic nonenveloped viruses whose RNA genomes are translated, replicated, and

packaged within the cytoplasm of infected cells. Autophagosome-like vesicles containing MAP1LC3/ATG8 and LAMP1 accumulate in cells shortly after infection with poliovirus or rhinovirus (115, 116). This process is triggered by the co-expression of two poliovirus-encoded proteins, 2BC and 3A, and appears to play a critical role in the viral replication cycle, as inhibition of autophagy decreases both intracellular and extracellular viral load (116). Furthermore, MAP1LC3/ATG8 and the poliovirus capsid protein VP1 have been detected in extracellular structures adjacent to infected cells (117). These observations have led to the hypothesis that poliovirus and rhinovirus may induce the formation of autophagosome-like structures to serve as a scaffold for RNA replication, and lysosomal exocytosis of these autophagosome-like structures may provide a mechanism for the nonlytic release of the cytoplasmic virus.

Coronaviruses are enveloped single-stranded (positive-sense) RNA viruses that replicate entirely within the cytoplasm of cells. This group of viruses includes the causative agent of severe acute respiratory distress syndrome (SARS) and murine hepatitis virus (MHV). MHV RNA replication occurs on cytoplasmic double membrane vesicles derived from the autophagic pathway. The observation that extracellular viral yield is reduced (by approximately 1000-fold) in the absence of autophagy suggests that autophagy plays a vital role in the replication of MHV (118). Although MHV is often used as a model of coronavirus replication, the source of the double membrane vesicles used for the replication of SARS appears to be the endoplasmic reticulum and not an autophagosome-like structure (119).

Becn1-mediated suppression of Sindbis virus replication. Sindbis virus is a linear, single-stranded RNA virus that induces apoptosis in infected cells. This viral-induced apoptosis is critical for viral replication, and inhibition of apoptosis by Bcl2 protects neurons from virus-induced cell death, reducing viral replication in the central nervous system (120). Becn1 was identified as a Bcl2-interacting protein whose expression in the brains of mice resulted in lower levels of Sindbis virus replication than that of mice expressing a Becn1 mutant that did not interact with Bcl2. Although these results may support the idea that autophagy is involved in the innate immune response against Sindbis virus, one cannot discount other possibilities, particularly because overexpression of Becn1 mutants that do not interact with Bcl2 promotes cell death (121) and in this way may enhance viral replication. Furthermore, the presence of BECN1 appears to reduce susceptibility to apoptosis in *C. elegans* (122). Additional studies are required to further characterize autophagy in Sindbis virus replication.

Autophagy in Adaptive Immunity

T cells of the adaptive immune response monitor the protein degradation products of cells to detect pathogen-derived peptides. $CD4^+$ and $CD8^+$ T cells survey the output of the two main proteolytic machineries within cells, the proteasome and lysosome (123). $CD8^+$ T cell–stimulating epitopes are usually 8–9 amino acids in length, which originate from proteasomal degradation of cytosolic and nuclear proteins and are presented on MHC class I. By contrast, $CD4^+$ T cells are stimulated by longer peptides derived from lysosomal proteolysis and presented by MHC class II molecules. Because dendritic cells and macrophages are professional phagocytes and efficiently present extracellular antigens on MHC class II molecules, it was thought that the majority of MHC class II antigens originated from extracellular sources. However, purification of MHC class II–associated peptides from B cells revealed that a majority of the antigens were derived from intracellular sources (124). In the context of recent findings that both autophagy and chaperone-mediated autophagy contribute to MHC class

II antigen presentation, it is more likely that the degradation pathway of a given antigen, rather than its presence within or outside of the cell, dictates whether it is presented to $CD4^+$ or $CD8^+$ cells by MHC class II or I, respectively. Some antigens, such as mucin, may be presented by both MHC class I and class II molecules. This may reflect the involvement of both proteasomal and lysosomal pathways in the degradation of certain proteins, although this has not been shown. Alternatively, a phenomenon known as cross-priming, which involves transfer of endocytosed material to the cytosol via retrograde transport to the endoplasmic reticulum and subsequent digestion by the proteasome and access to the MHC class I processing pathway, may be involved. The observation that autophagy is involved in the processing of nuclear and cytosolic proteins for MHC class II presentation in dendritic cells, macrophages, and B cells is especially intriguing because, as discussed in the previous section, autophagy also plays an integral role in the reproductive cycles of many intracellular pathogens. It will be interesting to explore whether the role of autophagy in the reproduction cycle of different intracellular pathogens influences the ability of host cells to present antigens derived from these pathogens.

Given the role of autophagy in the presentation of cytosolic antigens, it is plausible that autophagy plays a role in the presentation of self-antigens during thymic development (and the induction of self-tolerance). Recent studies demonstrated that medullary thymic epithelial cells are involved in the presentation of self-antigens and express proteins typically not expressed in the thymus via the transcription factor AIRE (125), providing a source of proteins that could be presented in an autophagy-dependent manner. In support of this hypothesis, there is evidence of increased autophagy in thymic epithelial cells at birth (32). Although the role of autophagy in the development of self-tolerance remains to be explored, a genome-wide scan identified a Crohn's disease–associated short nucleotide polymorphism in ATG16L (126). Although this finding is preliminary, it may reflect an example of a defect in autophagy leading to a breakdown in self-tolerance and autoimmunity.

CONCLUSION

In the past few years, studies establishing autophagy as a vitally important biological process involved in a broad array of pathological conditions including cancer, neurodegeneration, myopathies, and infectious disease have emerged. Translation of our knowledge of the molecular basis of autophagy from yeast and mice to human will be the challenge for upcoming years, if we are to develop successful autophagy-based therapeutics for these diseases.

SUMMARY POINTS

1. Autophagy is a process by which cellular components are sequestered and delivered to lysosomes for degradation. It can be selective or nonselective.
2. Nonselective autophagy is often induced in response to changes in the availability of nutrients and/or trophic factors to maintain bioenergetic homeostasis.
3. Selective autophagy is often used in processes requiring cellular remodeling, but can also be induced in response to toxic stimuli for the sequestration of damaged components.
4. In certain instances, induction of autophagy may promote cell death.

5. Given the range of cellular functions in which autophagy plays a role, it is not surprising that autophagy has been implicated in a variety of disease processes, including cancer, neurodegeneration, myopathies, and infectious diseases.
6. Nevertheless, the role of autophagy in these different disease processes is complex and in general still poorly understood.

FUTURE ISSUES

1. The studies in yeast have provided an invaluable source of knowledge regarding the molecular basis of autophagy; future studies should aim at determining which pathways are conserved in higher eukaryotes and how this process is modified to adapt to the needs of multicellular organisms.
2. Further insight into the mechanism by which cellular components are selected for degradation and how the magnitude of the autophagic response is regulated should help demystify the relationship between autophagy and cell fate in response to various stimuli.
3. The relationship between autophagy and other intracellular trafficking pathways including the endosomal-lysosomal pathway and lysosome-related organelles should be further explored.
4. Further characterization of the role of autophagy in various cellular processes and in disease pathogenesis should aid the development of novel therapeutic targets and strategies.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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