Autophagy and Human Disease

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Review

Autophagy and Human Disease

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ABSTRACT

As a conserved cellular degradative pathway in eukaryotes, autophagy relieves cells from various types of stress. There are different forms of autophagy, and the ongoing studies of the molecular mechanisms and cellular functions of these processes are unraveling their significant roles in human health. Currently, the best-studied of these pathways is macroautophagy, which is linked to a range of human disease. For example, as part of the host immune defense mechanism, macroautophagy is activated to eliminate invasive pathogenic bacteria; however, in some cases bacteria subvert this process for their own replication. Autophagy also contributes to endogenous major histocompatibility complex class II antigen presentation, reflecting its role in adaptive immunity. In certain neurodegenerative diseases, which are associated with aggregation-prone proteins, macroautophagy plays a protective role in preventing or reducing cytotoxicity by clearance of the toxic proteins; however, the autophagy-dependent processing of some components correlates with the pathogenesis of certain myopathies. Finally, autophagy acts as a mechanism for tumor suppression, although some cancer cells use it as a cytoprotective mechanism. Thus, a fundamental paradox of autophagy is that it can act to promote both cell survival and cell death, depending on the specific conditions.

ABBREVIATIONS

3-MA, 3-methyladenine; AD, Alzheimer's disease; AT, alpha-1-antitrypsin; Atg, autophagy-related; Cdk, cyclin-dependent kinase; CKI, cyclin-kinase inhibitor; CMA, chaperone-mediated autophagy; ER, endoplasmic reticulum; GFP, green fluorescent protein; HD, Huntington's disease; LAMP, lysosome-associated membrane protein; LC3, microtubule associated protein 1 light chain 3; MHC, major histocompatibility complex; PKA, protein kinase A; PKB, protein kinase B; PtdIns, phosphatidylinositol; sIBM, sporadic inclusion body myositis; TOR, target of rapamycin

INTRODUCTION

Autophagy, a cellular process responding to stress, has been studied for decades but has received tremendous interest in the past few years.[1] When cells undergo stress conditions, such as nutrient limitation, heat, oxidative stress, and/or the accumulation of damaged or excess organelles and abnormal cellular components, autophagy is induced as a degradative pathway; the elimination of potentially toxic components coupled with the recycling of nutrients aids in cell survival.[2] Three general types of autophagic processes have been identified in eukaryotic cells, named macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).[1,3]

Both macro- and microautophagy involve membrane rearrangement to allow the sequestration of cytoplasm, but they differ in the site of sequestration. During macroautophagy, a double-membrane vesicle is formed de novo. The origin of the vesicle membrane is not fully known, but may include the endoplasmic reticulum (ER), Golgi complex and even mitochondria, but it appears to be distinct from the lysosome (or the yeast analog, the vacuole). This vesicle, termed an autophagosome, enwraps a portion of cytoplasm and subsequently delivers it to the lysosome/vacuole lumen by fusing with this organelle.[1] In contrast, during microautophagy, cytosolic components are directly taken up into the lysosome/vacuole lumen by invagination and scission (or a combination of protrusion and septation) of the organelle's limiting membrane. Unlike micro- and macroautophagy, CMA does not involve a similar type of membrane rearrangement, although it

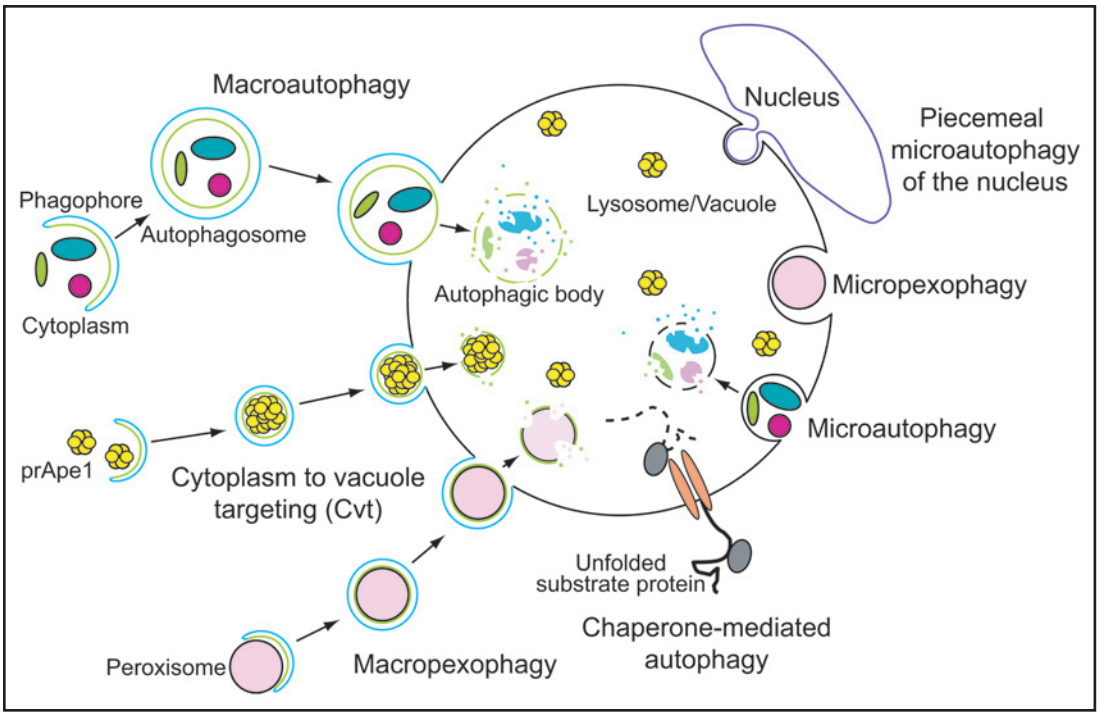

Figure 1. Schematic diagram of autophagy-related pathways. Three fundamentally different modes of autophagy are macroautophagy, microautophagy and chaperone-mediated autophagy. Specific autophagic pathways including macropexophagy, micropexophagy and piecemeal microautophagy of the nucleus allow the selective degradation of certain cargoes, such as peroxisomes, and portions of the nucleus. The biosynthetic delivery of the resident hydrolase aminopeptidase I into the yeast vacuole is mediated through the cytoplasm to vacuole targeting pathway, a type of specific autophagy. prApe1, precursor aminopeptidase I.

does depend on the lipid composition of the lysosome membrane.[4] CMA involves the translocation of unfolded proteins across the limiting membrane of the lysosome, and accordingly requires a molecular chaperone, Hsc70, both in the cytosol and within the lysosome lumen in addition to a lysosome-associated membrane protein (LAMP), LAMP-2a.[5]

In addition to the pathways mentioned above, several other morphologically similar processes have also been identified. For example, in certain conditions peroxisomes are targeted to the lysosome or vacuole for degradation by either a macro- or microautophagic-like mechanism, called macropexophagy or micropexophagy, respectively.[6] In addition, a specific microautophagic process termed piecemeal microautophagy of the nucleus has been identified in the yeast *Saccharomyces cerevisiae*. In this process, blebs of nuclear membrane and part of the nucleoplasm located in proximity to nucleus-vacuole junctions are pinched off into the vacuole and degraded.[7] Recent evidence also suggests that specific degradation of mitochondria, mitophagy, which can be used to remove damaged organelles, occurs through a microautophagic mechanism.[8,9] In both *S. cerevisiae* and *Pichia pastoris*, biosynthetic delivery of the vacuolar hydrolase precursor aminopeptidase I occurs via the cytoplasm to vacuole targeting pathway.[10,11] A schematic diagram of different autophagy-related pathways is shown in Figure 1. Interestingly, most of the autophagy-related pathways (with some notable exceptions such as CMA and piecemeal microautophagy of the nucleus) share most of the same molecular components.

In this review, we will focus on macroautophagy, hereafter referred to as autophagy. Autophagy is an evolutionarily conversed process that occurs in all eukaryotic cells.[1] Its role in helping lower eukaryotic organisms survive nutrient starvation conditions has been well studied and here we briefly highlight information on the machinery of autophagy based on studies in yeasts. In recent years, increasing lines of investigation indicate that autophagy has important functions in development, immunity, aging and pathophysiology in higher organisms including mammals.[2] These studies, which are the main focus of this review, reveal that autophagy can assume both life-saving and death-dealing roles.

AUTOPHAGY MACHINERY

Due to the advantage of yeast as a powerful genetic system, researchers have identified 31 autophagy-related (*ATG*) genes that are specifically involved in autophagy, pexophagy and the cytoplasm to vacuole targeting pathway.[1,12,13] The autophagy process can be dissected into a series of steps: induction, vesicle nucleation, cargo recognition (for specific types of autophagy) and packaging, vesicle expansion and completion, Atg protein cycling, vesicle fusion with the lysosome/vacuole, vesicle breakdown and nutrient recycling.[2,14] The Atg proteins can be categorized into different groups according to their functions at the various steps of the pathway. For example, the Atg1 kinase complex is involved in induction (although it also acts at later stages), Atg6 and Atg14 participate in vesicle nucleation, Atg11 and Atg19 are required for cargo recognition and packaging, the Atg8 and Atg12 ubiquitin-like conjugation systems are involved in vesicle formation, and Atg9, Atg23 and Atg27 participate in the protein retrieval step (Fig. 2).[15-17] Although the majority of the Atg proteins have probably been identified, their exact functions are largely unknown. Continued investigation of the molecular machinery in yeast will provide useful information that will be applicable for studying this process in other organisms. The process of autophagy in higher eukaryotes is essentially the same as that in yeast and mammalian orthologs of many of the yeast ATG genes have been identified.[13]

AUTOPHAGY AND HUMAN DISEASE

In the past several years, an increasing number of examples have emerged, which indicate that autophagy is involved in different aspects of human health. These studies point out both positive and negative effects of this process, depending on the specific disease and its level of progression.

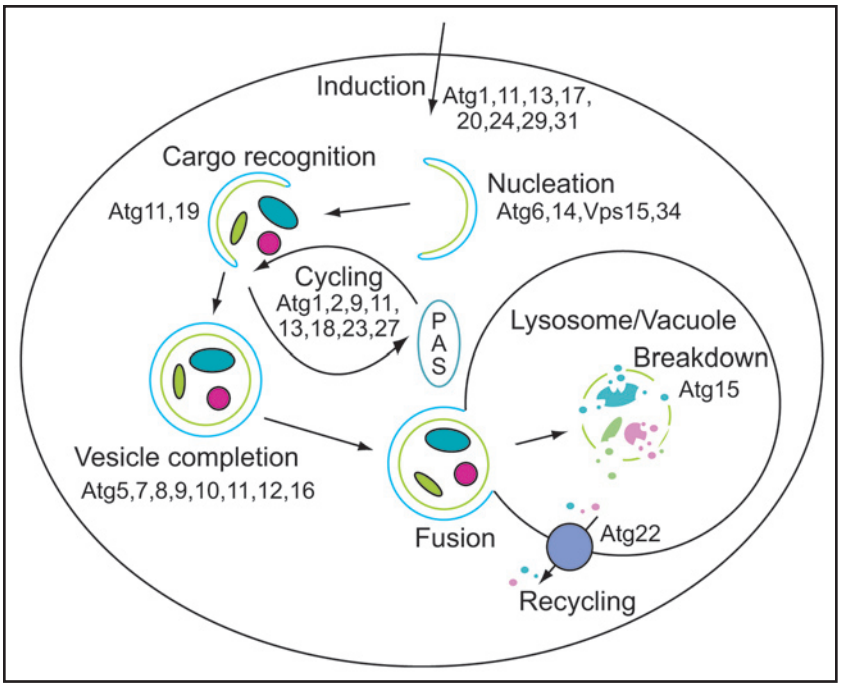

Figure 2. Schematic representation of the protein machinery involved in macroautophagy. The pathway can be dissected into several steps including induction, vesicle nucleation, cargo recognition, vesicle expansion and completion, cycling of Atg proteins, fusion, breakdown and recycling of macromolecules. The Atg proteins involved in each step are shown at the corresponding sites; the Atg1 complex may act at multiple steps of the pathway including induction and Atg protein cycling. PAS, phagophore assembly site; thought to be the organizing site for phagophore formation.

Autophagy and pathogen infection—bacteria. Bacteria are a major group of pathogens, which can cause a diversity of human diseases by infection. During their invasion, the bacteria promote entry into and replicate within the host, and spread into neighboring cells and tissues often resulting in cell death. In response to the threat of microbial invasion, the immune system has developed various mechanisms to eliminate these intruders. For example, invasive bacteria can be targeted into phagosomes through an endocytic/phagocytic pathway and delivered to the lysosome for destruction.[18] As a strategy against the host immune system, some bacteria are able to escape the phagosome and enter the cytosol to multiply, or in some cases modify the phagosome to prevent fusion with the lysosome, allowing them to replicate within the phagosomal compartment.[19] However, in response to these escapees, the host immune system may activate other defense mechanisms. Autophagy is one such mechanism that targets certain intracellular bacteria for sequestration within autophagosomes, ultimately delivering them to the lysosome where they are eliminated.

The autophagic process differs depending on the bacteria (Table 1). Autophagy targets bacteria that are either in the cytosol (*Streptococcus pyogenes*),[20] within immature phagosomes (*Mycobacterium tuberculosis*),[21] or in a damaged phagosome-like vacuole (*Salmonella enterica* serovar Typhimurium)[22] (Fig. 3A). In the case of Salmonella infection of macrophages (instead of epithelial cells) the bacteria may induce autophagy-dependent host cell death rather than be targeted to the lysosome for degradation.[23] This response is possibly also a host defense mechanism, which restricts bacterial growth and limits further infection of neighboring cells.[23]

In contrast to the above examples, some bacteria have evolved new strategies to escape from autophagy and propagate in the cytosol, or subvert the autophagy pathway and utilize it as a replicative niche. The different strategies for evading or subverting autophagy can be summarized as follows (Fig. 3B; Table 1):

Bacteria that evade autophagic sequestration. (1) *Listeria monocytogenes* and *Shigella flexneri*, can lyse the phagosome and escape into the cytosol, somehow circumventing autophagic surveillance, and replicate in the cytosol.[24-27] In both cases, mutants defective in mobility cannot escape the autophagy pathway and finally are killed via this defense mechanism.

Bacteria that induce autophagy and promote switching from a phagocytic to an autophagic pathway. (2) *Coxiella burnetii* and *Legionella pneumophila* subvert the autophagic machinery by delaying autophagosome maturation.[28-31] (3) *Brucella abortus*, *Porphyromonas gingivalis* and *Staphylococcus aureus* impair fusion of the autophagosome with the lysosome.[32,33] These latter bacteria reside in the autophagosome and utilize host nutrients, from the cytoplasmic materials sequestered by the autophagosome, to multiply. Upregulation of autophagic activity in host cells infected with these bacteria can actually enhance the bacterial infection.[28,31,33]

The mechanism(s) that allows autophagy to be triggered to recognize bacteria, or to be modified by bacteria for their own benefit during pathogen infection, is largely unknown, but is likely to involve bacterial proteins. The pathogenicity of bacteria mainly comes from the function of special secretion systems that secrete a set of effector proteins into host cells, which modulate host cellular processes to favor bacterial growth.[34] However, these effectors can also be signals triggering a host immune response, such as autophagy, to defend against them. For example, *Salmonella*-induced autophagy is dependent on a bacterial type III secretion system.[22,23] The bacterial effector protein SipB is able to induce autophagy in macrophages.[23] SipB is localized at mitochondria after bacterial infection, and disrupts the organelle's integrity, which probably serves as a signal to trigger the autophagic process.[23] With regard to Salmonella infection of non-phagocytic cells, the damaged Salmonella-containing vacuoles are possibly the signal for autophagy activation.[22]

A similar mechanism may apply for *Listeria monocytogenes* infection, in which autophagy induction depends on the perforation of the bacteria-containing vacuole;[35] however, once bacteria recruit actin and polymerization occurs, they can evade autophagic recognition in a process that is dependent on synthesis of bacterial phospholipases.[35] The *Shigella* surface protein VirG, which is involved in actin-dependent intracellular bacterial motility, induces autophagy through binding the host Atg5 protein,[25] whereas an effector protein, IcsB, secreted by Shigella, binds to VirG, thus inhibiting the interaction between Atg5 and VirG, and blocking the recognition of bacteria by the autophagic machinery.[25]

Factors released via the *Legionella pneumophila* type IV secretion system appear to be involved in the induction of autophagy and also delay autophagosome maturation.[28] The induction of autophagy by *S. aureus* is dependent on accessory gene regulator (*agr*), which is one of the virulence regulator systems that control the expression of bacterial virulence factors.[33] The above examples reflect an evolution of the strategies utilized by bacteria and their host cells during their ongoing battle for supremacy.

Table 1 **Autophagy controls the fate of intracellular bacteria**

Bacteria Name	Bacterial Strategy to Escape Immune System	Intracellular Bacterial Fate	Action of Autophagy on Bacteria	Characteristics of Autophagosome	Mechanism of Autophagy Induction
Autophagy targets bacteria for degradation					
Streptococcus pyogenes[20]	Lyse phagosome to enter cytosol	Killed by autophagy	Autophagosomes sequester cytosolic bacteria and fuse with lysosome	Big (5–10 μm), tightly enclose a cluster of bacteria	Surface protein?
Mycobacterium tuberculosis[21]	Block phagosome maturation	Killed by autophagy	Autophagy promotes phagosome maturation into phagolysosome	2–5 μm, cytosolic material can be seen inside	PtdIns(3)P, Immunity-Related p47 GTPase (IRG)
Salmonella enterica serovar Typhimurium[22,23]	Alter maturation of the phagosome-like compartment	A portion of bacterial population is removed by autophagy	Autophagosomes sequester damaged, bacteria-containing vacuole for degradation	1–2 μm	SipB, secreted by bacterial SPI-1 Type III secretion system
Bacteria escape autophagy or utilize autophagy for replication					
Listeria monocytogenes[26,27]	Lyse phagosome to enter cytosol	Killed by autophagy during early infection, but escape from autophagy and colonize in cytosol during late infection	Autophagy targets cytosolic bacteria before they recruit actin, and initiate polymerization	1 μm, enclose individual bacteria	Listeriolysin O-dependent induction and phospholipase-dependent evasion of autophagy
Shigella flexneri[24,25]	Lyse phagosome to enter cytosol	Escape from autophagy	Nonmotile mutant (Δ*icsB*) bacteria in cytosol are enwrapped by autophagosomes	~1 μm, enclose individual bacteria	Bacterial surface protein, VirG, inter acts with host Atg5, triggers autophagy in absence of IcsB
Coxiella burnetii[29,31]	Promotes entry into autophagosome and utilizes it as a replicative niche	Replicates in autophagosome	Phagosome fuses with autophagosome and forms replicative niche	Fusion of autophagosome with lysosome is delayed	No data
Brucella abortus[18,22]	Promotes entry into autophagosome and utilizes it as an intermediate step for reaching ER	Replicates in ER	Bacteria-containing early endosome is sequestered by autophagosome	Autophagosome is not matured and cannot fuse with lysosome	No data
Porphyromonas gingivalis[32]	Promotes entry into autophagosome and utilizes it as a replicative niche	Replicates in late autophagosome	Bacteria-containing early endosome is sequestered by autophagosome	Autophagosome is not matured and cannot fuse with lysosome	No data
Chlamydia trachomatis[123]	Uses autophagic nutrients	Replicates in special inclusions	Autophagosome sequesters chlamydial inclusions	No data	No data
Legionella pneumophila[28,30]	Perturbs autophagy to avoid/delay degradation	Replicates in early autophagosome	Autophagosome sequesters phagosome	Autophagosome maturation is much slower than normal, but finally fuses with lysosome	Factors released by bacterial type IV secretion system
Staphylococcus aureus[33]	Diverts into autophagosome, and utilizes it as a replicative niche	Replicates in autophagosome and escapes into cytoplasm	Autophagosome fuses with incomplete bacteria-containing vacuoles	Autophagosome fusion with lysosome is impaired	*agr*-dependent

Bacterially-induced autophagy displays certain differences in comparison with canonical, starvation-induced, bulk autophagy. First, induction in the former is mediated by bacterial proteins, which may directly or indirectly recruit autophagic components or activate the signaling pathway that regulates autophagy. The signaling pathway regulated by pathogens, however, remains unclear. Whether the bacteria can modulate classical signaling pathways, such as the class I phsophatidylinositol (PtdIns) 3-kinase-Akt pathway or the TSC1/2-Rheb-mTor pathway, which have been shown to regulate autophagic activity, or whether they can trigger other cellular signals that in turn activate autophagy, is one of the major unresolved questions in this field. Second, bacterially-induced autophagy is a specific process, as opposed to the typically random sequestration that occurs during starvation. For example, in Streptococcus-infected cells, the autophagosome membrane is closely apposed to the bacterial chain with the exclusion of most cytosol.[20] Motility-defective mutants from Shigella are similarly enwrapped closely by the autophagosome.[19] This phenomenon suggests that the bacteria may be specifically

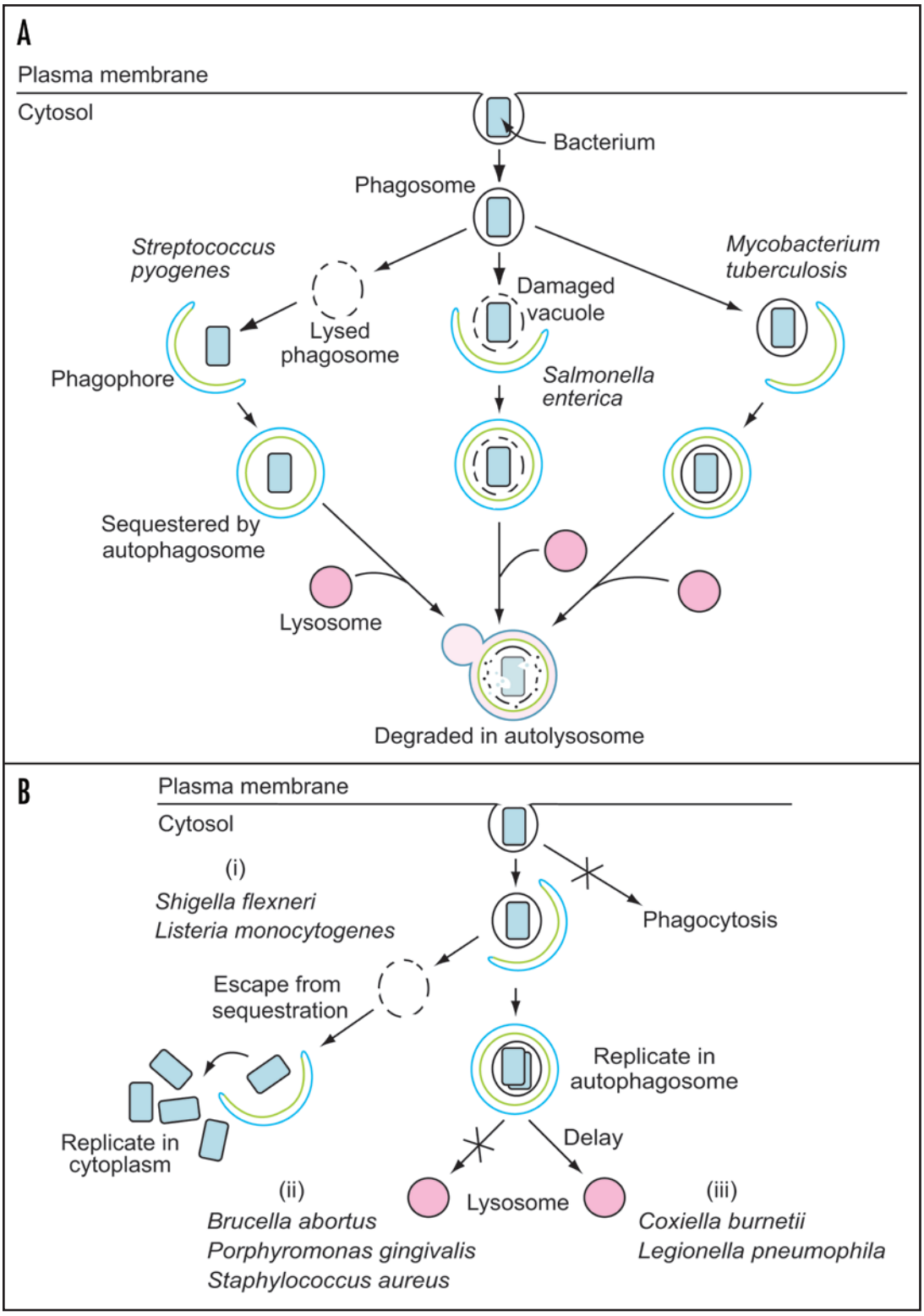

Figure 3. Diagram of the interactions between autophagy and different intracellular pathogens. (A) Intracellular bacteria are degraded by autophagy. *Streptococcus pyogenes* lyses the phagosome and escapes into the cytosol, where it is sequestered by an autophagosome and finally degraded in an autolysosome. *Mycobacterium tuberculosis* impairs the maturation of the phagosome and resides within it for replication. Induction of autophagy targets the bacteria-containing phagosome into the autophagy pathway, allowing degradation by fusion with a lysosome. A portion of the *Salmonella enterica* population damages the *Salmonella*-containing vacuole and is targeted by autophagy. (B) Bacteria subvert autophagy to escape into the cytosol or utilize autophagy as a replicative niche. (i) Bacteria subvert the sequestration step of autophagy. *Shigella flexneri* and *Listeria monocytogenes* can lyse the phagosome and escape into the cytosol. Both types of bacteria have the ability to escape from autophagy recognition and finally replicate in the cytosol. (ii) Bacteria promote entry into and block fusion of the autophagosome with the lysosome. *Brucella abortus*, *Porphyromonas gingivalis* and *Staphylococcus aureus* switch from a phagocytic to an autophagic pathway and replicate in autophagosomes that cannot fuse with the lysosome. (iii) Bacteria promote entry into and delay the fusion between the autophagosome and the lysosome. *Coxiella burnetii* and *Legionella pneumophila* can escape from phagocytosis and switch to the autophagy pathway. These bacteria modify the autophagosome and delay its fusion with the lysosome, thus allowing time to finish replication within the autophagosome.

recognized by autophagy and targeted for degradation as a cargo. This type of specific sequestration also suggests the possible involvement of interactions between autophagic components with bacterial surface proteins or polysaccharides. An example is that of the Shigella surface protein VirG that can bind Atg5, which is associated with the phagophore; however, the connection between cargo recognition and autophagosome formation is not clear in this case because Atg5 is thought to be present only on the outer surface of the forming vesicle that would not be in contact with the cargo. Studies of the interaction between autophagy and various bacteria will provide more insight into this and related questions. Third, the size of the autophagosomes surrounding the bacteria is generally larger than that of the normal autophagosomes formed in starvation conditions. For example, the autophagosomes formed during Streptococcus infection are 5–10 μm in diameter, much larger than the starvation- or rapamycin-induced autophagosomes, which are generally 1–2 μm.

Autophagy and pathogen infection—viruses. Viruses are another group of microbial pathogens. They infect their host by injecting their DNA or RNA into the host cytosol, and replicate within the cells. Upon virus infection, host cells may secret interferons (IFN), a type of cytokine, which can trigger cellular antiviral mechanisms to restrict viral replication. Recently IFN was shown to upregulate autophagy.[36] Type I IFN, such as α-IFN, can induce activation of PKR, an eIF2α kinase, to inhibit protein synthesis and restrict replication of the virus. PKR signaling promotes autophagy induction and autophagic degradation of the herpes simplex virus type 1 lacking the virulence factor, ICP34.5, which is an inhibitor of PKR function, in mammalian cells.[37,38] Type II IFN, IFN-γ, also can induce autophagy or autophagic cell death,[36] and can trigger autophagic destruction of intracellular mycobacteria.[21]

The antiviral role of autophagy in defense against viral infection is also supported by other data. For example, overexpression of Beclin 1 (the mammalian homolog of yeast Atg6/Vps30) in neurons inhibits Sindbis virus replication, which is the cause of fatal encephalitis.[39] In plants, autophagy is induced during tobacco mosaic virus infection, and *ATG* genes are required for the restriction of the hypersensitive response, a form of programmed cell death, at the infection sites, thus protecting uninfected tissues from undergoing death.[40]

On the other hand, as with certain bacteria, viruses have evolved strategies to block or utilize autophagy. The herpes simplex virus-encoded ICP34.5 factor blocks the PKR-dependent autophagy pathway, allowing the virus to evade degradation.[37,38] Some positive-strand RNA viruses, such as poliovirus, are able to induce autophagosome formation and appear to utilize autophagosomes as replication sites.[36,41] In this case, induction of autophagy increases the intracellular poliovirus and rhinovirus yields.[41] Viral proteins 2BC and 3A are involved in the induction of autophagosome formation, but it is not known yet at which stage, or how, the virus subverts the autophagy pathway. Finally, it is possible that autophagosome fusion with the plasma membrane may mediate non-lytic release of the poliovirus.[41]

Autophagy in antigen presentation. Besides its role in innate immunity, autophagy also plays an important role in the adaptive immune response, specifically in processing and presentation of major histocompatibility complex (MHC) class II antigens.[42] Adaptive immunity involves presentation on the cell surface of antigenic peptides by MHC class I and II molecules. This is followed by recognition by $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells, eliciting corresponding immune responses, such as killing infected cells or releasing cytokines to help activate cytotoxic cells.[43]

The traditional view is that endogenous antigens are processed by the proteasome, and the resulting antigenic peptides are translocated into the ER where they bind MHC class I molecules directing them for transport to the cell surface. In contrast, exogenously-derived antigens, such as those from phagocytosed pathogens, are internalized into the lysosomal system and presented on the plasma membrane in conjunction with MHC class II molecules.[44] It turns out, however, that the two systems are not so stringently separated and cross talk occurs between them. For example, biochemical evidence indicates that certain cytosolic and nuclear antigens are displayed by MHC class II molecules, and recent cell and molecular biological studies reveal that autophagy mediates the processing and presentation of endogenous antigens to MHC class II molecules.[42]

The first evidence connecting autophagy and the presentation of endogenous MHC class II antigens came from an analysis of the capacities of different antigen-presenting cells for the presentation of endogenous complement component 5.[45] A subsequent study of the endogenously-expressed model antigen neomycin phosphotransferase II (NeoR) revealed that cytosolic NeoR gains access to autophagosomes, is processed in endosomes or lysosomes, and then presented in conjunction with MHC class II molecules; inhibition of autophagy by 3-methyladenine (3-MA) or wortmannin impairs the NeoR presentation to MHC class II-restricted T cells.[46] The first reported pathogen-derived antigen to undergo endogenous MHC class II processing via autophagy was the Epstein-Barr virus nuclear antigen 1 (EBNA1).[47] This study also provides direct evidence that endogenous antigens are delivered for processing to lysosomes through autophagy; inhibition of autophagy with inhibitors or by siRNA-mediated knockdown of Atg12 blocks endogenous MHC class II presentation of EBNA1.[47] Chaperone-mediated autophagy may also contribute to endogenous MHC class II antigen presentation; modulating the protein level of LAMP-2a and Hsc70, essential components of the CMA pathway, result in alteration of the presentation of endogenous autoantigens through MHC class II molecules.[48]

The above studies suggest that certain endogenous self, model or viral antigens can be presented on MHC class II proteins after autophagy-mediated processing, but evaluation of the efficiency of autophagy and the extent of its participation in class II presentation is still limited. In a proteomic analysis of the constitutive MHC class II "ligandome" from human Awells cells, nearly 35% of the antigens came from intracellular sources, including cytosol and different organelles.[49] By subjecting the cells to starvation conditions to induce autophagic activity, researchers found that MHC class II presentation of intracellular antigens is significantly enhanced. These findings suggest that autophagy is a general pathway for delivering endogenous antigenic peptides into the lysosomal system for their subsequent presentation with MHC class II molecules.

A recent study provides another direct assessment of autophagy and MHC class II presentation.[50] Constitutive autophagy occurs in MHC class II-positive human cells, including professional antigen-presenting cells as well as epithelial cells that have low endocytic capacity. In addition, autophagosomes fuse with MHC class II-loading compartments based on colocalization with GFP-tagged LC3, the homolog of yeast Atg8. This observation indicates that the late endosome is the convergence site of autophagy and MHC class II presentation pathways. Finally, the efficiency and functionality of autophagic processing of endogenous antigens was shown by targeting an LC3-fused cytosolic antigen, influenza matrix protein 1, to the autophagy pathway, which results in enhanced recognition by matrix protein 1-specific $CD4^+$ T cells.[50]

Due to this growing body of studies, the function of autophagy in adaptive immunity has become more evident. Thus, in addition to its role in providing nutrients or degrading unwanted materials, autophagy serves as a shuttle to transport cytosolic antigens to endosomes/lysosomes to facilitate processing and association with MHC class II molecules. This function of autophagy has clear immunological significance in connection with protection against pathogens and immune system surveillance. For example, antigens derived from long-lived proteins, such as viral antigen EBNA1, are not efficient substrates of the proteasome and accordingly cannot get processed in the cytosol for MHC class I presentation; as a result, EBNA1 cannot be recognized by cytotoxic T cells. As part of a constitutive surveillance system, autophagy is able to target these pathogenic antigens to the MHC class II presentation pathway and activate $CD4^+$ helper T cells to stimulate an immune response. On the other hand, when cells

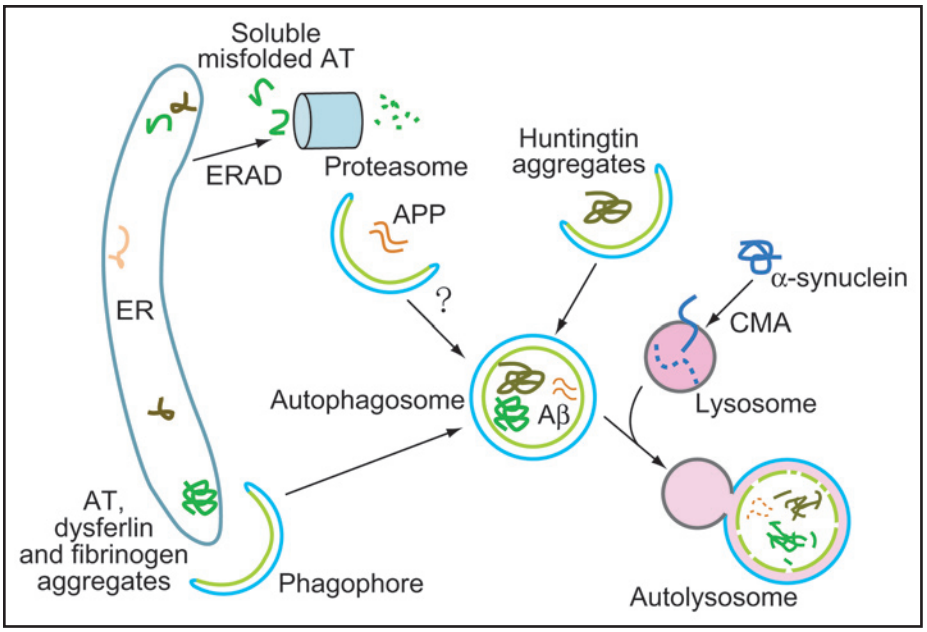

Figure 4. Clearance of protein aggregates through the autophagy pathway. Mutant alpha-1-antitrypsin (AT), fibrinogen or dysferlin form protein aggregates in the ER lumen. Autophagy is induced to target portions of the ER for degradation, relieving the stress that is associated with compromised ER function. Soluble form of these mutant proteins are transported back into the cytosol and degraded by the proteasome through the ER-associated degradation (ERAD) pathway. Mutant huntingtin forms protein aggregates in the cytosol that are degraded by autophagy. A portion of β-amyloid (Aβ) precursors (APP) are sequestered by autophagosomes and processed into Aβ peptides, which are degraded in autolysosomes in normal neurons. In Alzheimer's disease, the Aβ peptides accumulate in autophagosomes that are defective in fusion with lysosomes. The wild-type α-synuclein protein is degraded through chaperone-mediated autophagy (CMA). Mutant α-synuclein binds to the lysosome membrane receptor, but cannot be transported into the lysosome, thus blocking uptake of other proteins. Basal, constitutive autophagy reduces the level of these various cytosolic proteins, preventing them from accumulating and becoming toxic.

with low phagocytic potential respond to inflammatory cytokines, their MHC class II presentation is stimulated.[50] The presentation of self antigens through MHC class II molecules following autophagy may indicate a role in autoimmunity.

Autophagy and protein aggregation diseases. A link between autophagy and diseases associated with protein aggregation has been recognized and highlighted in recent years.[51] The common characteristics of the molecular mechanisms behind these types of diseases are: (1) a mutation leads to a change in protein conformation and misfolding; (2) misfolded proteins, accumulate to a high level and form aggregates or inclusion bodies; (3) the normal disposal pathways for these proteins are not able to degrade the altered and/or aggregated forms (Fig. 4).[52-55] Generally, the mutant proteins are problematic because they hinder normal cellular function when they accumulate to a high level. As a cellular adaptive response, degradation systems may be activated to eliminate the resulting abnormal inclusion bodies. The ubiquitin-proteasome system and autophagy are both activated by protein aggregates, but they are effective in the degradation of different forms of the substrates.[52,53]

One of the first lines of evidence suggesting a role of autophagy in diseases associated with aggregate-prone proteins came from the study of alpha-1-antitrypsin (AT) deficiency, which causes liver inflammation and carcinogenesis.[52] The normal AT protein is secreted from hepatic cells into the bloodstream where it inhibits a neutrophil protease. In contrast, a mutation in the gene encoding AT results in misfolding of the mutant protein and retention in the ER as an aggregated form.[53] In the liver cells from patients with AT deficiency, an increased number of autophagosomes is observed and degradation of the mutant AT aggregates, which accumulate in the ER, is specifically dependent on autophagy, whereas the soluble mutant proteins are subjected to ER-associated degradation (ERAD) by the proteasome.[53] Another ER storage disease, hypofibrinogenemia in which a mutant form of fibrinogen named Aguadilla γD forms aggregates in the hepatic ER, has symptoms and a phenotype very similar to AT deficiency.[54] Recently, it was reported that autophagy was responsible for degradation of a mutant form of dysferlin, the causative protein involved in limb girdle muscular dystrophy type 2B and Miyoshi myopathy.[56] The mutant dysferlin aggregates in the ER in muscle cells and induces autophagy, resulting in degradation in an autolysosome.

These data suggest a protective role of autophagy in relieving the cytotoxicity associated with abnormal protein aggregates in the ER.[57] The mechanism of autophagy induction by the ER-accumulated aggregates most likely involves ER stress signaling. When the capacity of the unfolded protein response and ERAD for the clearance of misfolded proteins in the ER is saturated or impaired, the accumulated abnormal proteins cause ER stress, which activates autophagy through the Ire1/Hac1, PERK/eIF2α or other signaling pathways (reviewed in ref. 58). The induced autophagy may specifically target the ER in a process termed reticulophagy,[59] and efficiently remove part of this abnormal organelle, presumably together with the accumulated protein aggregates to maintain organelle homeostasis.[60] In this context, autophagy serves as an "ERAD-like" mechanism and contributes to ER quality control.

With regard to diseases associated with cytosolic aggregate-prone proteins, the role of autophagy is most clearly seen with certain neurodegenerative diseases, such as some forms of Parkinson's disease, Huntington's disease (HD) and Alzheimer's disease (AD).[51,55] An accumulation of autophagosomes is observed in samples from the brains of patients, cell lines or mouse models of these diseases, which represents both an upregulation of autophagic activity and a deficiency in autophagosome-lysosome fusion.[4,61,62] For example, HD is caused by expansion of a polyglutamine tract in the huntingtin protein to an abnormally long sequence, which tends to aggregate and form inclusion bodies in the cytosol.[63] Both in vivo and in vitro experiments show that expression of exogenous mutant huntingtin results in the formation of cytosolic aggregates.[61,63] In addition autophagy is induced, and the cytosolic aggregates are degraded by autophagy. Stimulation of autophagy is able to reduce the mutant huntingtin aggregate formation and protect against the symptoms of neurodegeneration in fly and mouse models of HD.[64] Therefore, the pathogenesis may involve inadequate autophagic clearance of the mutant huntingtin protein.

In AD, the accumulation of aggregated tau protein in neurons and excessive extracellular deposit of β-amyloid (Aβ) are accompanied

by neuronal death.[62] Aβ peptide is a 40 to 42 amino acid cleavage product of the amyloid precursor protein. The generation of Aβ peptide occurs in intracellular compartments including the ER, Golgi complex, endosomes and lysosome. A recent study shows that autophagy provides another site for producing Aβ peptide.[62] Enrichment of Aβ in autophagosomes is observed in brains from AD patients and in the AD mouse model, and a marked accumulation of autophagosomes is also detected.[62] These observations suggest that inefficient autophagic degradation of the Aβ peptide leads to intracellular accumulation of the peptide and peptide aggregates, which may be toxic to neurons; intracellular Aβ can cause early symptoms of AD in a mouse model.[65] On the other hand, these studies imply a cytoprotective role of autophagy in degrading the Aβ peptides.

In contrast to AD and HD, Parkinson's disease involves the accumulation of mutant α-synuclein, which is normally degraded by CMA in its wild-type form.[4] Pathogenic α-synuclein can block uptake of the substrates of CMA by tight binding to the lysosomal membrane receptor, LAMP-2a, thus impairing the normal CMA pathway.[4] This leads to an upregulation of macroautophagy as a compensatory mechanism to degrade the accumulated α-synuclein aggregates.[66] A major question behind these different types of aggregate-prone diseases is what causes the inadequate or inefficient autophagic removal of the protein aggregates. Unraveling this question is critical to understand the pathogenesis of these diseases.

From current studies, it is now accepted that autophagy generally plays a protective role in diseases associated with aggregate-prone proteins by its degradative function in the clearance of the potentially toxic forms of these proteins. Inhibition of autophagy results in increased levels of pathogenic aggregate accumulation, whereas stimulation of autophagy can alleviate toxicity of the aggregate-prone proteins.[67] Treatment with rapamycin can enhance the autophagic clearance of huntingtin aggregates as well as general polyglutamine and polyalanine aggregates.[68] This suggests a promising avenue for therapeutic intervention for these types of diseases. However, it is important to note that the large aggregates that are often seen associated with these diseases may not be the critical species with regard to toxicity and neurodegeneration. Rather, it may be the monomeric or micro-aggregated forms of these neural proteins that are toxic.[69] Along these lines, basal autophagy plays a critical role in removing soluble, cytosolic proteins before they reach a toxic level. Mice with neuronally-restricted knockouts of autophagy genes develop symptoms of neurodegeneration even though they do not harbor the aggregate-prone versions of the neuropeptides typically associated with neurodegenerative diseases.[70,71] In addition, diffuse, ubiquitinated proteins accumulate before the appearance of aggregates, suggesting that these forms rather than aggregates are the immediate result of the autophagic block.

Although autophagy protects cells from the toxicity caused by aggregate-prone proteins, its activity must be controlled at an appropriate level because excessive autophagy may result in cell death. The protective function may also be restricted to certain stages of the disease. For example, in the late stage of HD, the number and size of the aggregates increase to such an extent that they become inefficient even for autophagosome sequestration and are resistant to lysosomal degradation,[63] although as noted above, these may not be the toxic species. With regard to therapy, however, it is important to know the effects of autophagy in different stages of a certain disease and to avoid arbitrary treatment with autophagy inducers or inhibitors.

Autophagy, myopathies and lysosomal storage diseases. The lysosome is the major site of organelle and long-lived protein degradation. When the function of the lysosome is altered, excessive levels of undigested materials accumulate within the lysosome and ultimately become toxic to the cell. This phenotype is seen with certain lysosomal storage diseases, which are commonly manifested by cardio- and other myopathies.[72,73] Interestingly, autophagy often correlates with these diseases, which are grouped as autophagic vacuolar myopathies. These autophagic vacuoles have distinct morphological properties of sarcolemma, which characterize this group of diseases.[74] However, most studies in this field still rely primarily on morphological observations and it is not clear how these distinct autophagic vacuoles are formed or even whether the autophagic response is cytoprotective, or contributes to the disease.[72]

Danon's disease, one of the lysosomal glycogen storage diseases, is the best-studied example of autophagy-linked myopathies. Massive accumulation of autophagic vacuoles is present in cardiac and skeletal muscle cells of the patients.[75] The disease is caused by mutations in the lysosomal membrane protein LAMP-2b.[75] Studies of LAMP-2-deficient mice, which provide a mouse model of Danon's disease, suggest that fusion of autophagosomes with the endosome/lysosome is impaired.[76] It is not known how mutated LAMP-2b alters autophagy, but mutation or deletion of this lysosomal membrane protein may result in abnormal lysosomal membrane structure, which probably directly or indirectly hinders fusion between the autophagosome outer membrane and the lysosomal membrane.

Pompe disease, caused by acid α-glucosidase-deficiency and storage of glycogen in the lysosome, is another example of autophagic vacuolar myopathy, similar to Danon's disease.[77] Transcription of several autophagy genes, such as BECLIN 1, ATG12 and ATG8/MAP1LC3 are significantly upregulated in the muscle cells of acid α-glucosidase-knockout mice, suggesting induction of autophagy.[77] Recent studies indicate that it is the buildup of the big mass of autophagic vacuoles in the muscle fibers rather than lysosomal glycogen accumulation that disturbs normal muscle contraction.[77,78] Stimulation of autophagic activity could be the reason for the autophagic vacuole accumulation and this buildup may contribute more to disease symptoms than was previously thought. Other autophagic vacuolar myopathies, such as X-linked myopathy with excessive autophagy, and infantile autophagic vacuolar myopathy, have been reported to correlate with autophagy based only on morphological results.[74] On the other hand, lysosomal homeostasis is also critical in certain storage diseases. For example, in sialic acid storage disease, export of sialic acid is impaired due to mutations in the lysosomal membrane transporter molecule, sialin.[79,80] This disease also manifests accumulation of autophagosomes.[79,80]

Another example of autophagy associated with muscle disease is seen with sporadic inclusion body myositis (sIBM).[81,82] sIBM is considered to be the skeletal muscle equivalent of AD, and is marked by the accumulation of aggregate-prone proteins such as β-amyloid. Samples of degenerating muscle fibers from sIBM patients reveal the presence of Atg8/LC3-positive autophagosomes containing Aβ. Furthermore, some of these muscle fibers display MHC class II molecules and are surrounded by $CD4^+$ and $CD8^+$ T cells, suggesting that upregulated autophagy in sIBM may contribute to an autoimmune, inflammatory response that is characteristic of this disease.[81,82] The role of autophagy in lysosomal storage diseases and myopathies may be underestimated because of the limited animal or cell culture models available to study the pathophysiology. Unraveling the mechanism of how autophagy is altered in these diseases, as well as determining the direct consequences of autophagic dysfunction will help in the development of efficient therapies.

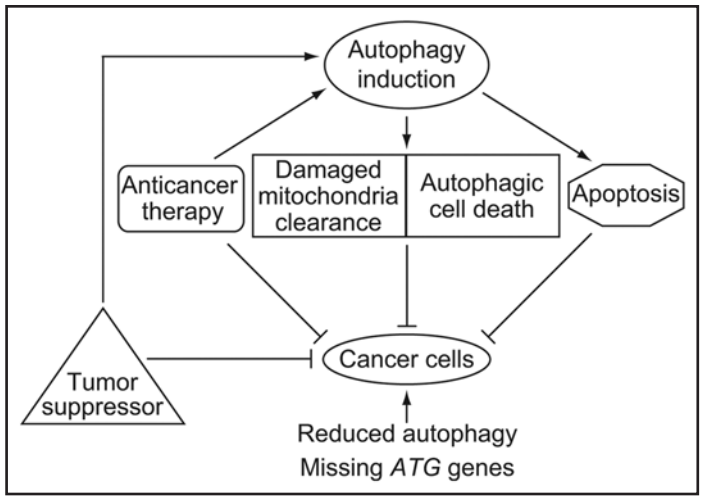

Figure 5. Diagram of the connections between autophagy and different tumor-suppressing processes. Possible mechanisms of the tumor suppressor function of autophagy include the clearance of damaged mitochondria by the autophagy pathway. This reduces the generation of reactive oxygen species and the rate of DNA mutation. In addition, excessive autophagy results in autophagic cell death, which may be triggered to help kill cancer cells. Links between autophagy and restriction of cancer cell growth are: (1) Certain tumor cells show reduced autophagy activity and mutations at some *ATG* gene loci; (2) Known tumor suppressor genes are involved in the signaling of autophagy induction; (3) Upregulation of autophagy activity is observed in different anti-cancer therapies; and (4) Induction of autophagy can promote apoptosis which inhibits tumorigenesis.

Autophagy and cancer. A correlation between autophagy and cancer was reported 20 years ago,[83,84] but progress on the elucidation of the possible molecular mechanisms was achieved only recently (reviewed in detail in refs 85–88). Although both positive and negative effects of autophagy are implicated in tumorigenesis, the balance has tilted more to its role as a tumor suppressor (Fig. 5). Evidence includes: (1) Reduced autophagic activity is observed in tumor cells;[83,84] (2) Human autophagy genes, *BECLIN 1*, *MAP1LC3* (the homolog of *ATG8* in yeast) and *ATG7/HsGSA7* (the homolog of *ATG7* in yeast) are absent in different cancers; for example, 40–75% of sporadic breast cancers and ovarian cancers have mutations at the *BECLIN 1* locus, and *ATG7* is mutated in certain lung cancers;[87] (3) Beclin $1^{+/-}$ mice show increased spontaneous tumorigenesis and hyperproliferation of mammary epithelial cells, which correlates with reduced autophagy. Stimulation of autophagy by forced expression of *BECLIN 1* in MCF7 *beclin* $1^{+/-}$ breast carcinoma cells inhibits tumorigenesis;[89-91] (4) Autophagy is induced in many anticancer therapies;[88] (5) Identification of autophagic cell death, a non-apoptotic programmed cell death mechanism associated with autophagosomes, depends on autophagy genes and lysosomal digestion;[92] inhibition of apoptosis by overepressing Bcl-X_L, downregulating caspase-8 activity, or by knockout of Bax and Bak causes cells to undergo autophagic cell death after death factor stimulation;[92-95] (6) A pro-apoptotic effect of Atg5 was recently proposed because overexpression of this protein inhibits tumor growth and stimulates apoptosis;[96] and (7) Tumor suppressor genes, such as PTEN (Phosphatase and Tensin homolog deleted on chromosome Ten), p53 and the Death Associated Protein Kinase protein family, are involved in signaling autophagy induction.[86,88,97] Similarly, oncogenic class I PtdIns 3-kinase/Akt signaling negatively regulates autophagy.[98]

The direct role of autophagy in oncogenesis, however, is not yet clear. Several models are proposed to account for the mechanism of autophagic tumor suppression such as the following: (1) Selective degradation of damaged mitochondria by autophagy reduces the production of reactive oxygen species and the subsequent increase in the DNA mutation rate, which is the basic cause of many cancers.[87] In this context, the protective role of autophagy contributes to the tumor suppression; (2) A deficiency in apoptosis generally leads to uncontrolled cell growth. In this condition, cytotoxic stimuli can induce autophagic cell death to kill tumor cells through inhibition of the mTOR pathway, or the class I PtdIns 3-kinase/Akt pathway, or by activation of the class III PtdIns 3-kinase/Beclin 1 pathway.[85,88] In this context, the self-killing effect of autophagy contributes to tumor suppression; (3) Autophagy proteins function as pro-apoptotic factors to trigger apoptosis.[96] For example, Atg5 is cleaved by calpain and the resulting amino-terminal part of Atg5 is capable of triggering apoptosis. The connections between autophagy and tumorigenesis are shown in Figure 5. These models indicate the promising potential of autophagy in cancer therapies. However, we must also be cautious in the interpretation of some of the data concerning the links between autophagy and cancer. For example, activation of the tumor suppressors PTEN and p53 is involved in the signaling of mTOR inhibition, which in turn can induce autophagy.[85] But mTOR also regulates cell growth and proliferation through its downstream translation effectors, p70S6 kinase (p70S6K) and 4E-BP1, which may contribute to the tumor suppression effect.[99] In some cases it is also not clear whether the decreased level of autophagic activity is the cause, or consequence, of tumor growth. Further study of the relationship between autophagy and cell proliferation will clarify the role of autophagy in cancer.

Although many lines of data point to a tumor suppressor role, autophagy also contributes to tumor survival, possibly through its cellular protective and nutrient recycling functions. In nutrient deprivation conditions, inhibition of autophagy by knockdown of *ATG* genes leads to apoptosis in HeLa cells, suggesting a cell survival role of autophagy.[100,101] Similarly, growth factor depletion induces autophagy in apoptosis-defective (*bax*/*bak*$^{-/-}$) cells, and results in prolonged cell survival in an autophagy gene-dependent manner.[102] Thus, the dual roles of autophagy in cytoprotection and programmed cell death mean that this process can act to protect or kill cancer cells, depending on the particular type of cancer, the progression of the disease and the treatment method being employed.[101] All of these factors need to be considered when contemplating the modulation of autophagy for therapeutic intervention.

AUTOPHAGY AND THE CELL CYCLE

Eukaryotic cell growth and proliferation are controlled by a tightly regulated cellular process, the cell cycle. Through a series of events including DNA synthesis, chromosome duplication, mitosis and cell division, a somatic cell progresses through a cell cycle for propagation.[103] This process is divided into the M (mitosis) phase and interphase, which includes the G_1, S and G_2 phases; fully differentiated or compromised cells can exit the cell cycle at the G_1 phase and enter into G_0 to stop proliferation, and, if necessary, provide time for cellular repair mechanisms to take effect or for cell death to occur. Regulation of each step of the cycle is precise and complex, involving several groups of proteins called cyclins, cyclin-dependent kinases (Cdks), and cyclin-kinase inhibitors (CKIs).[104] When nutrients

are rich in the environment, the cell cycle progresses by sequential initiation of each phase under the regulation of different cyclins and Cdks; whereas when environmental conditions are not favorable for growth, such as nutrient deprivation, the cell cycle can be arrested by downregulating cyclins and Cdks, or activating CKIs. Accurate control of the cell cycle is critical for maintaining normal cell growth and development. Dysfunctional regulation of the cell cycle can lead to cell death and/or tumorigenesis.

Interestingly, cell cycle arrest is often accompanied by induction of autophagy, a key survival mechanism during stress conditions. An early study of peroxisome degradation in mammalian CHO-K1 cells revealed that amino acid starvation induced autophagic degradation of peroxisomes and arrested cells at the G_1 phase.[105] Autophagy inhibitors, 3-MA, okadaic acid and nocodazole, suppressed the G_1 arrest and increased the S phase or G_2/M phase cell population, suggesting an interrelationship between these two processes. Autophagy may also be regulated by cell cycle control. When cultured animal cells enter M phase and undergo mitosis, autophagy is inhibited even in amino acid-free conditions.[106] This mitotic inhibition of autophagy is not recovered until telophase-G_1 when the nuclear envelope seals. Cell cycle arrest caused by deficient function of Cdks or overexpression of CKIs is also able to induce autophagy. A conditional mutation in the yeast *CDC28* gene, encoding the catalytic subunit of the main cell cycle Cdk, results in G_1 and G_2/M arrest after incubation at non-permissive temperature, and the cells are enriched with autophagosomes.[107] Overexpression of the cdk2-cyclinE inhibitor, p27, induces autophagy and leads to cell death in malignant glioma cells.[108] p53, a transcription factor that regulates cell cycle arrest by stimulating CKI transcription, plays important roles in checkpoint monitoring, maintaining normal cell growth, and inducing apoptosis upon DNA damage and other cellular stress. Recently, it was reported that p53 can also upregulate autophagy.[109,110]

The above studies suggest that autophagy can be regulated by cell cycle control. This type of regulation makes sense considering that in most organisms during mitosis the nuclear envelope breaks down, exposing chromosomes to the cytoplasm; inhibition of autophagy may be necessary simply to prevent random sequestration and degradation of the DNA. The occurrence of autophagy in G_1 phase-arrested cells may reflect its physiological function of providing nutrients and energy to help cells survive starvation stress. When nutrients are limited, progression through the cell cycle is halted to avoid spending energy on proliferation; at this time, there may also be a signal to activate autophagy, allowing bulk degradation nutrient recycling for the synthesis of essential components needed to maintain viability until nutrient input is renewed. If the starvation condition persists, apoptosis is induced to initiate cell death, with possible contributions from autophagy.

In the context of anticancer therapy, CKIs are considered as anticancer agents due to their ability to inhibit cell growth. The CKI p27 shows a strong ability to suppress tumor cell growth accompanied by induction of autophagic cell death.[108] Certain anticancer drugs isolated from plants, such as plumbagin and triterpenoid B-group soyasaponins, are reported to stop the cell cycle by downregulation of Cdks and upregulation of CKIs in breast and colon cancer cells, respectively.[111-113] At the same time, these drugs also induce autophagic death of the cancer cells. These studies highlight the need to keep in mind the potential dual effects of autophagy during anticancer therapy: Autophagy can have a cellular protective effect that favors tumor growth, whereas it may conversely respond to cell cycle arrest and participate in a program of cell death.[101]

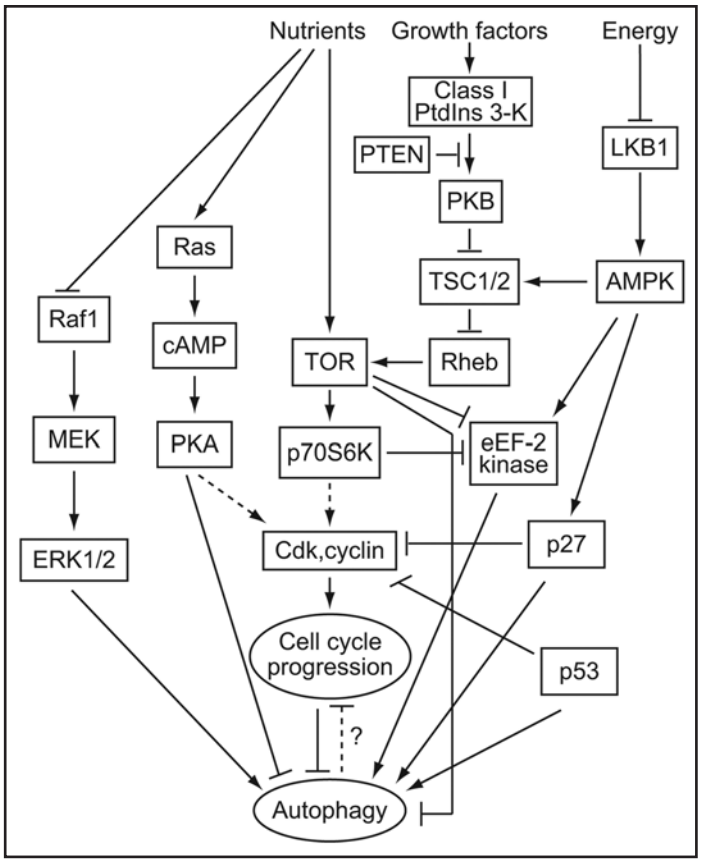

Figure 6. Schematic representation of signaling pathways regulating cell cycle progression and autophagy. Nutrient availability activates the TOR-p70S6K and Ras-cAMP-PKA pathways, which stimulate the transcription and translation of a range of proteins, ultimately activating cyclins and Cdks to promote cell cycle progression. At the same time, activation of these two pathways inhibits autophagy. The Raf1-MEK-ERK1/2 pathway stimulates autophagy and is inhibited by nutrients. Growth factors can trigger the class I PtdIns 3-kinase-PKB pathway, which positively regulates TOR activity. Energy stress activates the LKB1-AMPK pathway, stabilizing p27, which arrests the cell cycle and induces autophagy. The p53 tumor suppressor also inhibits cell cycle progression and induces autophagy. See the text for details.

A correlation between autophagy and the cell cycle is also suggested by the shared use of certain components of upstream signaling pathways, which transduce environmental signals into regulation of these two processes. For example, some classical signaling cascades that promote progression through the cell cycle are negative regulators of autophagy induction (Fig. 6). In mammalian cells, the two best-characterized signaling pathways that sense nutrient availability and control cell cycle progression are mediated through the TOR-p70S6K-eIF-4E and Ras-cAMP-protein kinase A (PKA) pathways.[114] When environmental nutrients are abundant, TOR kinase is active; this results in activation of p70S6K and eIF-4E, which further causes translation initiation of a range of proteins that may control cell cycle progression from G_1 to S phase and lead to cell growth. In parallel, the Ras-cAMP-PKA pathway also senses the nutritional status and regulates the cell's entry into the G_0 phase.[114] Both of these pathways are also involved in the negative regulation of autophagy; inhibition of TOR or Ras-PKA induces autophagy.[115,116]

Amino acids inhibit Raf1 activity, which mediates Raf1-Mitogen-activated protein (MAP) kinase or ERK kinase (MEK)-extracellular signal-regulated kinases (ERK)1/2 –induced autophagy (Fig. 6).[117]

It is not known whether this pathway is involved in cell cycle control, but in colon cancer cells treated with soybean B-group triterpenoid saponins, the Raf1-MEK-ERK1/2 pathway is upregulated; this results in autophagic induction, a decrease in the level of certain Cdks, an increase in some CKIs, and cell arrest at S phase.[112] In addition to nutrients, cells also sense hormones/growth factors and trigger special signaling pathways to promote cell growth. One example of hormone-responsive signaling is seen with the class I PtdIns 3-kinase-protein kinase B (PKB)/Akt pathway. Upon activation, PKB can regulate different downstream effectors that affect cell viability, as well as negatively regulate autophagy.[85] The effects of PtdIns 3-kinase-PKB on cell growth and autophagy may be mediated through TOR. Activation of PKB inhibits tuberous sclerosis complex 1/2 (TSC1/2); TSC1/2 negatively regulates the Ras homology enriched in brain (Rheb) small GTPase, which positively regulates TOR activity.[118] Growth factor withdrawal from apoptosis-defective (*bax*/*bak*$^{-/-}$) cells results in G_0-G_1 arrest and induction of autophagy, which is required for the survival of these cells.[102]

Energy status also controls both the cell cycle and autophagy. A recent study shows that in conditions of energy stress, accumulation of AMP activates the LKB1-AMPK pathway, which inhibits TOR by activating TSC1/2.[119] In addition, AMPK stabilizes the p27 protein and inhibits cell cycle progression in breast cancer cells. Autophagy is induced by stable, active p27, whereas knockdown of p27 leads to induction of apoptotic cell death.[120] Thus, under metabolic/energy stress, p27 is stabilized to arrest the cell cycle, while directing the induction of autophagy for cell survival.

Although regulation of autophagy and the cell cycle share many components of their upstream signaling cascades, the direct correlation between these two processes is still not clear. They may be two parallel readouts of the same signaling pathway, such as TOR, or it is possible that one is downstream of the other. It is not clear yet which is the case because direct inhibition of cell cycle progression through modulating cyclin, Cdk and CKI induces autophagy, suggesting that autophagy is controlled by cell cycle regulation. On the other hand, inhibition of autophagy by 3-MA and other inhibitors results in relief of cell cycle arrest under starvation conditions. A recent study shows that Atg1 specifically inhibits p70S6K activity, which regulates cell growth, in Drosophila and mammalian cells.[121] Mutation or knockdown of Atg1 results in activation of p70S6K and suppresses the developmental delay and cell growth defect of TOR mutant Drosophila. This result provides a signaling connection between autophagy and cell growth downstream of TOR and implies that autophagy signaling can also regulate cell cycle progression. Therefore, further experiments will be needed to determine the relationship between autophagy and the cell cycle. In addition, it seems likely that an understanding of the direct upstream modulators of the autophagy pathway will help us understand the potential interactions between the regulatory mechanisms that control the cell cycle and autophagy.

CONCLUSION

The significance of autophagy in human health has been revealed by a growing number of studies. In regard to its role in promoting and/or maintaining health, autophagy targets invasive bacteria and viruses for destruction, plays a role in the presentation of endogenous antigens on MHC class II molecules, prevents and/or relieves cytotoxicity caused by aggregate-prone proteins, suppresses tumor growth by promoting type II programmed cell death, removes damaged and aberrant proteins and organelles for lysosomal degradation, and recycles cellular macromolecules. On the other hand, autophagy also has negative effects on human health. Autophagy is utilized by certain pathogens for replication, it can hinder muscle function due to the accumulation of autophagosomes in muscle tissues and potentially by participating in an autoimmune inflammatory response, and it can function as a cytoprotective mechanism for cancer cells allowing them to resist anti-cancer treatment. The critical point is that the tremendous degradative capacity of autophagy allows this process to function in both cell survival and cell death. Additional information on the mechanism and regulation will be necessary to allow practical modulation of autophagy for therapeutic purposes.

References

1. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. Cell Death Differ 2005; 12:1542-52.
2. Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 2004; 6:463-77.
3. Wang C-W, Klionsky DJ. The molecular mechanism of autophagy. Mol Med 2003; 9:65-76.
4. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. Science 2004; 305:1292-5.
5. Massey AC, Zhang C, Cuervo AM. Chaperone-mediated autophagy in aging and disease. Curr Top Dev Biol 2006; 73:205-35.
6. Dunn WA, Jr., Cregg JM, Kiel JAKW, van der Klei IJ, Oku M, Sakai Y, Sibirny AA, Stasyk OV, Veenhuis M. Pexophagy: the selective autophagy of peroxisomes. Autophagy 2005; 1:75-83.
7. Roberts P, Moshitch-Moshkovitz S, Kvam E, O'Toole E, Winey M, Goldfarb DS. Piecemeal microautophagy of nucleus in *Saccharomyces cerevisiae*. Mol Biol Cell 2003; 14:129-41.
8. Kissova I, Salin B, Schaeffer J, Bhatia S, Manon S, Camougrand N. Selective and non-selective autophagic degradation of mitochondria in yeast. Autophagy 2007; 3:329-36.
9. Zhang Y, Qi H, Taylor R, Xu W, Liu LF, Jin S. The role of autophagy in mitochondria maintenance: characterization of mitochondrial functions in autophagy-deficient *S. cerevisiae* strains. Autophagy 2007; 3:337-46.
10. Farre JC, Vidal J, Subramani S. A cytoplasm to vacuole targeting pathway in *P. pastoris*. Autophagy 2007; 3:230-4.
11. Nair U, Klionsky DJ. Molecular mechanisms and regulation of specific and nonspecific autophagy pathways in yeast. J Biol Chem 2005; 280:41785-8.
12. Kabeya Y, Kawamata T, Suzuki K, Ohsumi Y. Cis1/Atg31 is required for autophagosome formation in *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 2007; 356:405-10.
13. Klionsky DJ, Cregg JM, Dunn WA, Jr., Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, Ohsumi Y. A unified nomenclature for yeast autophagy-related genes. Dev Cell 2003; 5:539-45.
14. Yang Z, Huang J, Geng J, Nair U, Klionsky DJ. Atg22 recycles amino acids to link the degradative and recycling functions of autophagy. Mol Biol Cell 2006; 17:5094-104.
15. Legakis JE, Yen W-L, Klionsky DJ. A cycling protein complex required for selective autophagy. Autophagy 2007; 3:in press.
16. Reggiori F, Tucker KA, Stromhaug PE, Klionsky DJ. The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. Dev Cell 2004; 6:79-90.
17. Yen W-L, Legakis JE, Nair U, Klionsky DJ. Atg27 is required for autophagy-dependent cycling of Atg9. Mol Biol Cell 2007; 18:581-93.
18. Dorn BR, Dunn WA, Jr., Progulske-Fox A. Bacterial interactions with the autophagic pathway. Cell Microbiol 2002; 4:1-10.
19. Ogawa M, Sasakawa C. Bacterial evasion of the autophagic defense system. Curr Opin Microbiol 2006; 9:62-8.
20. Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, Tsuda K, Hamada S, Yoshimori T. Autophagy defends cells against invading group A *Streptococcus*. Science 2004; 306:1037-40.
21. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. Cell 2004; 119:753-66.
22. Birmingham CL, Smith AC, Bakowski MA, Yoshimori T, Brumell JH. Autophagy controls *Salmonella* infection in response to damage to the Salmonella-containing vacuole. J Biol Chem 2006; 281:11374-83.
23. Hernandez LD, Pypaert M, Flavell RA, Galan JE. A *Salmonella* protein causes macrophage cell death by inducing autophagy. J Cell Biol 2003; 163:1123-31.
24. Ogawa M, Sasakawa C. *Shigella* and autophagy. Autophagy 2006; 2:171-4.
25. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular *Shigella* from autophagy. Science 2005; 307:727-31.
26. Rich KA, Burkett C, Webster P. Cytoplasmic bacteria can be targets for autophagy. Cell Microbiol 2003; 5:455-68.
27. Webster P. Cytoplasmic bacteria and the autophagic pathway. Autophagy 2006; 2:159-61.

28. Amer AO, Swanson MS. Autophagy is an immediate macrophage response to *Legionella pneumophila*. Cell Microbiol 2005; 7:765-78.
29. Colombo MI, Gutierrez MG, Romano PS. The two faces of autophagy: *Coxiella* and *Mycobacterium*. Autophagy 2006; 2:162-4.
30. Dubuisson JF, Swanson MS. Mouse infection by *Legionella*, a model to analyze autophagy. Autophagy 2006; 2:179-82.
31. Gutierrez MG, Vazquez CL, Munafo DB, Zoppino FC, Beron W, Rabinovitch M, Colombo MI. Autophagy induction favours the generation and maturation of the *Coxiella*-replicative vacuoles. Cell Microbiol 2005; 7:981-93.
32. Belanger M, Rodrigues PH, Dunn WA, Jr., Progulske-Fox A. Autophagy: a highway for *Porphyromonas gingivalis* in endothelial cells. Autophagy 2006; 2:165-70.
33. Schnaith A, Kashkar H, Leggio S, Addicks K, Kronke M, Krut O. *Staphylococcus aureus* subvert autophagy for induction of caspase-independent host cell death. J Biol Chem 2006.
34. Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev 1998; 62:379-433.
35. Py BF, Lipinski MM, Yuan J. Autophagy limits *Listeria monocytogenes* intracellular growth in the early phase of primary infection. Autophagy 2007; 3:117-25.
36. Espert L, Codogno P, Biard-Piechaczyk M. Involvement of autophagy in viral infections: antiviral function and subversion by viruses. J Mol Med 2007, in press.
37. Tallóczy Z, Jiang W, Virgin HW, IV, Leib DA, Scheuner D, Kaufman RJ, Eskelinen E-L, Levine B. Regulation of starvation- and virus-induced autophagy by the eIF2αkinase signaling pathway. Proc Natl Acad Sci USA 2002; 99:190-5.
38. Tallóczy Z, Virgin HW, IV, Levine B. PKR-dependent autophagic degradation of herpes simplex virus type 1. Autophagy 2006; 2:24-9.
39. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, Herman B, Levine B. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol 1998; 72:8586-96.
40. Liu Y, Schiff M, Czymmek K, Talloczy Z, Levine B, Dinesh-Kumar SP. Autophagy regulates programmed cell death during the plant innate immune response. Cell 2005; 121:567-77.
41. Jackson WT, Giddings TH, Jr., Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, Kirkegaard K. Subversion of cellular autophagosomal machinery by RNA viruses. PLoS Biol 2005; 3:e156.
42. Schmid D, Dengjel J, Schoor O, Stevanovic S, Munz C. Autophagy in innate and adaptive immunity against intracellular pathogens. J Mol Med 2006; 84:194-202.
43. Castellino F, Germain RN. Cooperation between CD4+ and CD8+ T cells: when, where, and how. Annu Rev Immunol 2006; 24:519-40.
44. Strawbridge AB, Blum JS. Autophagy in MHC class II antigen processing. Curr Opin Immunol 2007; 19:87-92.
45. Brazil MI, Weiss S, Stockinger B. Excessive degradation of intracellular protein in macrophages prevents presentation in the context of major histocompatibility complex class II molecules. Eur J Immunol 1997; 27:1506-14.
46. Nimmerjahn F, Milosevic S, Behrends U, Jaffee EM, Pardoll DM, Bornkamm GW, Mautner J. Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. Eur J Immunol 2003; 33:1250-9.
47. Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, Munz C. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. Science 2005; 307:593-6.
48. Zhou D, Li P, Lin Y, Lott JM, Hislop AD, Canaday DH, Brutkiewicz RR, Blum JS. Lamp-2a facilitates MHC class II presentation of cytoplasmic antigens. Immunity 2005; 22:571-81.
49. Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Muller M, Kreymborg K, Altenberend F, Brandenburg J, Kalbacher H, Brock R, Driessen C, Rammensee HG, Stevanovic S. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc Natl Acad Sci USA 2005; 102:7922-7.
50. Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. Immunity 2007; 26:79-92.
51. Rubinsztein DC, DiFiglia M, Heintz N, Nixon RA, Qin ZH, Ravikumar B, Stefanis L, Tolkovsky A. Autophagy and its possible roles in nervous system diseases, damage and repair. Autophagy 2005; 1:11-22.
52. Teckman JH, Perlmutter DH. Retention of mutant α_1-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. Am J Physiol Gastrointest Liver Physiol 2000; 279:G961-74.
53. Perlmutter DH. The role of autophagy in alpha-1-antitrypsin deficiency: a specific cellular response in genetic diseases associated with aggregation-prone proteins. Autophagy 2006; 2:258-63.
54. Kruse KB, Dear A, Kaltenbrun ER, Crum BE, George PM, Brennan SO, McCracken AA. Mutant fibrinogen cleared from the endoplasmic reticulum via endoplasmic reticulum-associated protein degradation and autophagy: an explanation for liver disease. Am J Pathol 2006; 168:1299-308.
55. Nixon RA. Autophagy in neurodegenerative disease: friend, foe or turncoat? Trends Neurosci 2006; 29:528-35.
56. Fujita E, Kouroku Y, Isoai A, Kumagai H, Misutani A, Matsuda C, Hayashi YK, Momoi T. Two endoplasmic reticulum-associated degradation (ERAD) systems for the novel variant of the mutant dysferlin: ubiquitin/proteasome ERAD(I) and autophagy/lysosome ERAD(II). Hum Mol Genet 2007; 16:618-29.
57. Kamimoto T, Shoji S, Hidvegi T, Mizushima N, Umebayashi K, Perlmutter DH, Yoshimori T. Intracellular inclusions containing mutant α_1-antitrypsin Z are propagated in the absence of autophagic activity. J Biol Chem 2006; 281:4467-76.
58. Yorimitsu T, Klionsky DJ. Eating the endoplasmic reticulum: quality control by autophagy. Trends Cell Biol 2007; In press.
59. Klionsky DJ, Cuervo AM, Dunn WA, Jr., Levine B, van der Klei IJ, Seglen PO. How shall I eat thee? Autophagy 2007; In press.
60. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. PLoS biology 2006; 4:e423.
61. Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet 2002; 11:1107-17.
62. Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, Mohan PS, Mercken M, Farmery MR, Tjernberg LO, Jiang Y, Duff K, Uchiyama Y, Naslund J, Mathews PM, Cataldo AM, Nixon RA. Macroautophagy—a novel β-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol 2005; 171:87-98.
63. Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, DiFiglia M. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. J Neurosci 2000; 20:7268-78.
64. Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 2004; 36:585-95.
65. Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal Aβ causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. Neuron 2005; 45:675-88.
66. Massey AC, Kaushik S, Sovak G, Kiffin R, Cuervo AM. Consequences of the selective blockage of chaperone-mediated autophagy. Proc Natl Acad Sci USA 2006; 103:5805-10.
67. Cuervo AM. Autophagy: in sickness and in health. Trends Cell Biol 2004; 14:70-7.
68. Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, Schmitt I, Wullner U, Evert BO, O'Kane CJ, Rubinsztein DC. Rapamycin alleviates toxicity of different aggregate-prone proteins. Hum Mol Genet 2006; 15:433-42.
69. Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ. Potential therapeutic applications of autophagy. Nat Rev Drug Discov 2007; 6:304-12.
70. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 2006; 441:885-9.
71. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 2006; 441:880-4.
72. Terman A, Brunk UT. Autophagy in cardiac myocyte homeostasis, aging, and pathology. Cardiovasc Res 2005; 68:355-65.
73. Eskelinen E-L. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. Mol Aspects Med 2006; 27:495-502.
74. Nishino I. Autophagic vacuolar myopathy. Semin Pediatr Neurol 2006; 13:90-5.
75. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y, Sue CM, Yamamoto A, Murakami N, Shanske S, Byrne E, Bonilla E, Nonaka I, DiMauro S, Hirano M. Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). Nature 2000; 406:906-10.
76. Tanaka Y, Guhde G, Suter A, Eskelinen E-L, Hartmann D, Lullmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. Nature 2000; 406:902-6.
77. Fukuda T, Roberts A, Ahearn M, Zaal K, Ralston E, Plotz PH, Raben N. Autophagy and lysosomes in Pompe disease. Autophagy 2006; 2:318-20.
78. Fukuda T, Ewan L, Bauer M, Mattaliano RJ, Zaal K, Ralston E, Plotz PH, Raben N. Dysfunction of endocytic and autophagic pathways in a lysosomal storage disease. Ann Neurol 2006; 59:700-8.
79. Verheijen FW, Verbeek E, Aula N, Beerens CE, Havelaar AC, Joosse M, Peltonen L, Aula P, Galjaard H, van der Spek PJ, Mancini GM. A new gene, encoding an anion transporter, is mutated in sialic acid storage diseases. Nat Genet 1999; 23:462-5.
80. Town M, Jean G, Cherqui S, Attard M, Forestier L, Whitmore SA, Callen DF, Gribouval O, Broyer M, Bates GP, van't Hoff W, Antignac C. A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. Nat Genet 1998; 18:319-24.
81. Lunemann JD, Schmidt J, Dalakas MC, Münz C. Macroautophagy as a pathomechanism in sporadic inclusion body myositis. Autophagy 2007; 3.
82. Lunemann JD, Schmidt J, Schmid D, Barthel K, Wrede A, Dalakas MC, Münz C. β-amyloid is a substrate of autophagy in sporadic inclusion body myositis. Annals Neurol 2007; in press.
83. Schwarze PE, Seglen PO. Reduced autophagic activity, improved protein balance and enhanced in vitro survival of hepatocytes isolated from carcinogen-treated rats. Exp Cell Res 1985; 157:15-28.
84. Knecht E, Hernandez-Yago J, Grisolia S. Regulation of lysosomal autophagy in transformed and non-transformed mouse fibroblasts under several growth conditions. Exp Cell Res 1984; 154:224-32.
85. Botti J, Djavaheri-Mergny M, Pilatte Y, Codogno P. Autophagy signaling and the cogwheels of cancer. Autophagy 2006; 2:67-73.
86. Gozuacik D, Kimchi A. DAPk protein family and cancer. Autophagy 2006; 2:74-9.
87. Jin S. Autophagy, mitochondrial quality control, and oncogenesis. Autophagy 2006; 2:80-4.
88. Kondo Y, Kondo S. Autophagy and cancer therapy. Autophagy 2006; 2:85-90.
89. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of autophagy and inhibition of tumorigenesis by *beclin 1*. Nature 1999; 402:672-6.

90. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen E-L, Mizushima N, Ohsumi Y, Cattoretti G, Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003; 112:1809-20.
91. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Natl Acad Sci USA 2003; 100:15077-82.
92. Tsujimoto Y, Shimizu S. Another way to die: autophagic programmed cell death. Cell Death Differ 2005; 12:1528-34.
93. Shao Y, Gao Z, Marks PA, Jiang X. Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. Proc Natl Acad Sci USA 2004; 101:18030-5.
94. Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, Tsujimoto Y. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004; 6:1221-8.
95. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH, Lenardo MJ. Regulation of an *ATG7-beclin 1* program of autophagic cell death by caspase-8. Science 2004; 304:1500-2.
96. Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, Brunner T, Simon HU. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat Cell Biol 2006; 8:1124-32.
97. Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, Gasco M, Garrone O, Crook T, Ryan KM. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell 2006; 126:121-34.
98. Petiot A, Ogier-Denis E, Blommaart EF, Meijer AJ, Codogno P. Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. J Biol Chem 2000; 275:992-8.
99. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene 2004; 23:3151-71.
100. Boya P, Gonzalez-Polo RA, Casares N, Perfettini JL, Dessen P, Larochette N, Metivier D, Meley D, Souquere S, Yoshimori T, Pierron G, Codogno P, Kroemer G. Inhibition of macroautophagy triggers apoptosis. Mol Cell Biol 2005; 25:1025-40.
101. Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. Science 2004; 306:990-5.
102. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005; 120:237-48.
103. Cross F, Roberts J, Weintraub H. Simple and complex cell cycles. Annu Rev Cell Biol 1989; 5:341-96.
104. Roberts JM, Koff A, Polyak K, Firpo E, Collins S, Ohtsubo M, Massague J. Cyclins, Cdks, and cyclin kinase inhibitors. Cold Spring Harbor symposia on quantitative biology 1994; 59:31-8.
105. Arai K, Ohkuma S, Matsukawa T, Kato S. A simple estimation of peroxisomal degradation with green fluorescent protein - an application for cell cycle analysis. FEBS Lett 2001; 507:181-6.
106. Eskelinen E-L, Prescott AR, Cooper J, Brachmann SM, Wang L, Tang X, Backer JM, Lucocq JM. Inhibition of autophagy in mitotic animal cells. Traffic 2002; 3:878-93.
107. Motizuki M, Yokota S, Tsurugi K. Autophagic death after cell cycle arrest at the restrictive temperature in temperature-sensitive cell division cycle and secretory mutants of the yeast *Saccharomyces cerevisiae*. Eur J Cell Biol 1995; 68:275-87.
108. Komata T, Kanzawa T, Takeuchi H, Germano IM, Schreiber M, Kondo Y, Kondo S. Antitumour effect of cyclin-dependent kinase inhibitors (p16(INK4A), p18(INK4C), p19(INK4D), p21(WAF1/CIP1) and p27(KIP1)) on malignant glioma cells. Br J Cancer 2003; 88:1277-80.
109. Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. Proc Natl Acad Sci USA 2005; 102:8204-9.
110. Jin S. p53, Autophagy and tumor suppression. Autophagy 2005; 1:171-3.
111. Ellington AA, Berhow M, Singletary KW. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. Carcinogenesis 2005; 26:159-67.
112. Ellington AA, Berhow MA, Singletary KW. Inhibition of Akt signaling and enhanced ERK1/2 activity are involved in induction of macroautophagy by triterpenoid B-group soyasaponins in colon cancer cells. Carcinogenesis 2006; 27:298-306.
113. Kuo PL, Hsu YL, Cho CY. Plumbagin induces G2-M arrest and autophagy by inhibiting the AKT/mammalian target of rapamycin pathway in breast cancer cells. Molec Cancer Therapeut 2006; 5:3209-21.
114. Cutler NS, Heitman J, Cardenas ME. TOR kinase homologs function in a signal transduction pathway that is conserved from yeast to mammals. Mol Cell Endocrinol 1999; 155:135-42.
115. Schmelzle T, Beck T, Martin DE, Hall MN. Activation of the RAS/cyclic AMP pathway suppresses a TOR deficiency in yeast. Mol Cell Biol 2004; 24:338-51.
116. Noda T, Ohsumi Y. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J Biol Chem 1998; 273:3963-6.
117. Pattingre S, Bauvy C, Codogno P. Amino acids interfere with the ERK1/2-dependent control of macroautophagy by controlling the activation of Raf-1 in human colon cancer HT-29 cells. J Biol Chem 2003; 278:16667-74.
118. Avruch J, Hara K, Lin Y, Liu M, Long X, Ortiz-Vega S, Yonezawa K. Insulin and amino-acid regulation of mTOR signaling and kinase activity through the Rheb GTPase. Oncogene 2006; 25:6361-72.
119. Høyer-Hansen M, Jaattela M. AMP-activated protein kinase: A universal regulator of autophagy? Autophagy 2007; 3.
120. Liang J, Shao SH, Xu ZX, Hennessy B, Ding Z, Larrea M, Kondo S, Dumont DJ, Gutterman JU, Walker CL, Slingerland JM, Mills GB. The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. Nat Cell Biol 2007; 9:218-24.
121. Lee SB, Kim S, Lee J, Park J, Lee G, Kim Y, Kim JM, Chung J. ATG1, an autophagy regulator, inhibits cell growth by negatively regulating S6 kinase. EMBO Rep 2007; 8:360-5.
122. Pizarro-Cerda J, Meresse S, Parton RG, van der Goot G, Sola-Landa A, Lopez-Goni I, Moreno E, Gorvel JP. *Brucella abortus* transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. Infect Immun 1998; 66:5711-24.
123. Al-Younes HM, Brinkmann V, Meyer TF. Interaction of *Chlamydia trachomatis* serovar L2 with the host autophagic pathway. Infect Immun 2004; 72:4751-62.