Part I
Introduction to Autophagy

Autophagy in Immunity and Infection. Edited by Vojo Deretic

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1
Overview of Autophagy

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1.1
Overview of Autophagy

Just as cells must manufacture necessary components for proper function, so must they break down damaged or unnecessary organelles and other cellular constituents. In order to maintain this balance, the cells employ two primary degradative pathways – the proteasome, which is responsible for the breakdown of most short-lived proteins, and autophagy, a process induced by nutrient limitation and cellular stress, which governs the degradation of the majority of long-lived proteins, protein aggregates and whole organelles. It enables cells to survive stress from the external environment, such as nutrient deprivation, as well as internal stresses like accumulation of damaged organelles and pathogen invasion. Autophagy is induced by starvation in all eukaryotic systems examined, including several species of fungi, plants, slime mold, nematodes, fruit flies, mice, rats and humans [1]. By degrading superfluous intracellular components and reusing the breakdown products, these organisms are able to survive periods of scarce nutrients [1, 2]. Along these lines, autophagy aids in maintenance of homeostasis in cellular differentiation, tissue remodeling, growth control [3, 4] and a type of programmed cell death separate from apoptosis [5–7]. There exist multiple types of autophagy, which differ mainly in the site of cargo sequestration and in the type of cargo. These include micro- and macroautophagy, chaperone-mediated autophagy, micro- and macropexophagy, piecemeal microautophagy of the nucleus, and the cytoplasm-to-vacuole targeting (Cvt) pathway (Fig. 1.1) (reviewed in Ref. [8]). This chapter will focus on the process known as macroautophagy, which will be referred to as autophagy from this point on.

Autophagy is induced during certain developmental states, in response to various hormones, under conditions of nutrient deprivation or by other types of stress. This process involves the sequestration of bulk cytoplasm within a cytosolic double-membrane vesicle termed the autophagosome, which ultimately fuses with the lysosome (or the vacuole in yeast). Fusion results in the release

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of the inner vesicle, now termed an autophagic body, into the lysosome lumen. Within the lysosome the engulfed material is degraded and the products are recycled. Autophagy has been implicated in a number of human diseases and conditions, including cancer, neurodegenerative disorders, certain myopathies, aging and defense against pathogens. The potential ability to control autophagy for therapeutic intervention will require a better understanding of the mechanistic details of this degradative process.

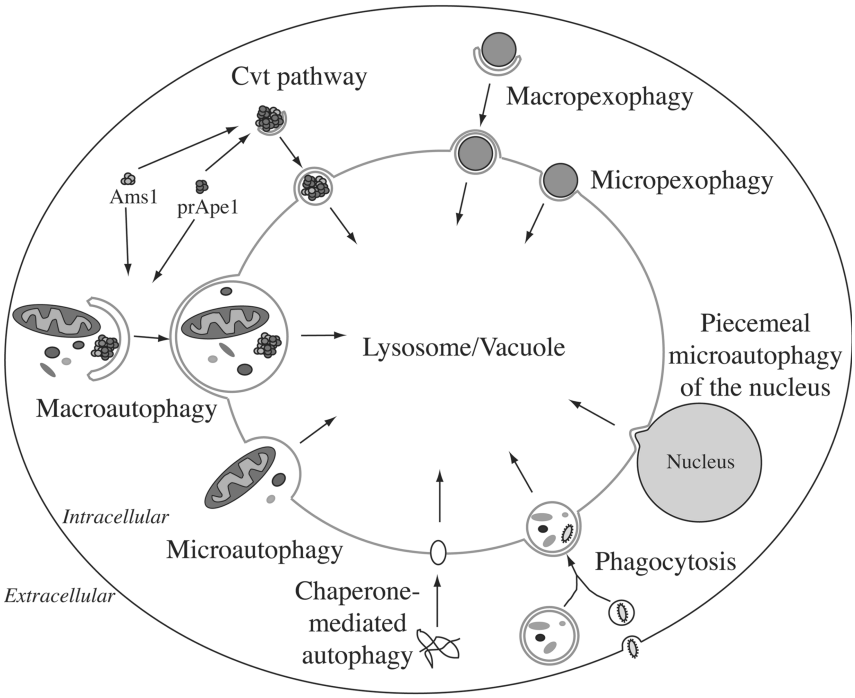

Fig. 1.1 Schematic representation of various transport routes to the lysosome/vacuole. There exist a number of pathways by which substrates are delivered to the lysosome/vacuole. Some of the sequestration events occur at the organelle membrane, these are denoted by the prefix "micro". In other cases, the enclosure of the substrate occurs spatially away from the lysosome/vacuole membrane. These pathways begin with the prefix "macro". Macro- and microautophagy are nonspecific degradation pathways, which include a variety of cargoes, depending on the organism and the particular stress conditions or stage of development. Selective degradation of peroxisomes, small parts of the nucleus or foreign pathogens occurs via macropexophagy, micropexophagy, piecemeal microautophagy of the nucleus or phagocytosis, respectively. Chaperone-mediated autophagy is a receptor-driven degradative pathway that is a secondary response to starvation conditions. The biosynthetic Cvt pathway is a method of delivery for at least two vacuolar hydrolases. (This figure also appears with the color plates).

1.2 The Discovery of Autophagy

The first description of autophagy was published almost 50 years ago. For nearly four decades, studies of the mammalian lysosome were primarily pharmacological, biochemical and morphological in nature. Nonetheless, many of the questions raised, and conclusions drawn, from those investigations are still valid today. In more recent years, the discovery of autophagy in yeast, allowing the application of genetic and molecular genetic techniques, led to a greater understanding of the machinery required for this essential cellular process. In particular, the systematic isolation and characterization of autophagy-related (*ATG*) genes in the yeasts *Saccharomyces cerevisiae, Pichia pastoris* and *Hansenula polymorpha* has allowed identification of 27 gene products that appear to be specific to autophagy (Tab. 1.1) [9]. Accordingly, the proposed functions of lysosomes and the accepted cellular roles of autophagy have evolved as a more detailed characterization of this degradative pathway has been achieved. This chapter will serve to highlight the progression of our knowledge concerning autophagy, give a general introduction to the process and set the stage for a discussion of its role in cellular immunity.

Lysosomes were first identified in rat liver, recognizable in electron microscopy images by their intense acid phosphatase staining [12]. It was soon demonstrated that these "particles" harbored additional hydrolases. So-called "dense bodies" were identified during attempts to purify lysosomes; from the initial studies, it was unclear whether these structures were distinct from lysosomes, as they shared many of the same properties [13]. Subsequently, these intracellular structures were identified as compartments similar to, but distinct from, lysosomes and they were named autophagic vacuoles (AVs; also referred to as autophagosomes, particularly in yeast). One of the first clues as to the degradative capacity of the AVs was demonstrated in newborn mouse kidney cells, where it was shown that some vacuole-like structures contained dense, amorphous material and even whole organelles, including mitochondria [14]. Similar investigations continued, attempting to determine lysosomal function and the origin of the AVs through electron microscopy.

Two aspects of autophagy which have been intensely studied since the original studies mentioned above are the membrane source for the nascent AV and the induction of autophagy. Despite the focus of research on these facets of the process, they are still not completely known or understood. Early studies provided evidence supporting both the Golgi and the endoplasmic reticulum (ER), as well as an area of the cell termed GERL (Golgi endoplasmic reticulum lysosomes), as the source of the AV membrane (reviewed in Ref. [15]), although none of this evidence was conclusive. Later investigations similarly were unable to reach a consensus, although many of the conclusions implicated the ER [16–18]. The identity of the donor membrane is still not known with certainty, there being evidence implicating the Golgi, the plasma membrane, as well as the ER as the source for the AV (reviewed in Ref. [19]).

Table 1.1 Atg proteins, their orthologs and putative roles in autophagy-related processes

Atg protein	Orthologs in other species	Putative function or component of	Step involved in
1	*Sp, Nc, At, Dd, Ce, Dm, Mm, Hs*	protein kinase	induction, retrieval
2	*Sp, Pp, Ce, Dm, Hs*	Atg9 cycling	retrieval
3	*Sp, Nc, At, Ce, Dm, Mm, Hs*	Atg8 conjugation	expansion
4	*Sp, At, Ce, Dm, Mm, Hs*	Atg8 conjugation	expansion
5	*Sp, Dd, At, Ce, Dm, Mm, Hs*	Atg12 conjugation	expansion
6	*Sp, Nc, Dd, At, Ce, Dm, Mm, Hs*	PI3K complex	nucleation
7	*Sp, Pp, Nc, At, Dd, Ce, Dm, Mm, Hs*	Atg8 and Atg12 conjugation	expansion
8	*Sp, Nc, Dd, At, Ce, Dm, Mm, Hs*	Atg8 conjugation	expansion
9	*Sp, Nc, At, Ce, Dm, Hs*	membrane delivery	expansion
10	*At, Ce, Mm, Hs*	Atg12 conjugation	expansion
11	*Sp, Pp*	cargo specificity	cargo selection
12	*Sp, Nc, Dd, At, Ce, Dm, Mm, Hs*	Atg12 conjugation	expansion
13	*Sp, Nc*	regulates Atg1 activity	induction, retrieval
14		PI3K complex	nucleation
15	*Sp, Nc*	lipase	vesicle breakdown
16	*At, Ce, Dm, Mm, Hs*	Atg12 conjugation	expansion
17	*Sp, Nc*	Atg1–Atg13 complex	induction, formation
18	*Sp, Nc, At, Ce, Dm, Mm, Hs*	Atg9 cycling	retrieval
19		Cvt receptor	cargo selection
20		Atg1–Atg13 complex	induction
21		PI3P binding	
22	*Sp, Nc*	transmembrane protein	
23		cycling protein	formation, expansion
24		Atg1–Atg13 complex	induction
25[a]	*Hp*	coiled-coil protein	regulation
26	*Pp*	glucosyltransferase	
27		PI3P binding	

These proteins were first identified in the yeast *S. cerevisiae*. The species with known orthologs are abbreviated as follows: *At, Arabidopsis thaliana*; *Ce, Caenorhabditis elegans*; *Dd, Dictyostelium discoideum*; *Dm, Drosophila melanogaster*; *Hp, Hansenula polymorpha*; *Hs, Homo sapiens*; *Mm, Mus muscularis*; *Nc, Neurospora crassa*; *Pp, Pichia pastoris*; *Sp, Schizosaccharomyces pombe*. The orthologs were compiled from the references listed below and from Homologene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=homologene). The information presented in this table is a compilation of information from Refs. [1, 9–11], as well as those cited throughout the text.

a) This protein has not been identified in *S. cerevisiae*, only in the species of yeast indicated.

Despite their inherent limitations, the morphological studies provided important information on the basic membrane dynamics involved in autophagy. For example, these studies suggested that AVs must acquire their resident enzymes through fusion with mature lysosomes [20]. These and more recent analyses have led to a model in which AVs develop into mature degradative autophagolysosomes in a series of discrete steps: (1) following induction, an initial isolation membrane or phagophore forms in a nucleation step; (2) this membrane expands into an AV; (3) the AV fuses with an endosome to form an intermediate structure known as an amphisome; (4) the amphisome acidifies; (5) fusion with a lysosome allows the AV/amphisome to acquire hydrolases [18, 21, 22].

Another mechanistic aspect of autophagy that has been the focus of much research concerns regulation and, in particular, induction. Initially it was not known whether autophagy was a random process, indiscriminately enwrapping and degrading cytoplasmic components, or whether it was a more directed action, selecting substrates according to the cellular needs at that particular moment. At present, we know that autophagy occurs at a basal level, but that in many cell types it is also an inducible process. In addition, although autophagy is generally considered to be nonspecific, there are various examples of specific types of autophagy. As the control of autophagic induction is important for defense against extracellular pathogens [22, 23], it is addressed in more detail later in this chapter.

Many of the questions that were raised shortly after the discovery of autophagy are still relevant today. The source of the sequestering membrane is still not known and it is possible that the forming AV may derive its membrane from multiple sources within the cell. Some of the molecular components that induce autophagy are now known, but the precise manner in which they act to bring about autophagic degradation is still unclear. Finally, the steps involved in the maturation of the newly formed AV are partially understood, but there are still aspects of this process that need to be clarified. As mentioned previously, over 20 genes have been identified that are involved in some form of autophagy, but the functions of the gene products are still largely unknown; however, based on recent genetic, molecular genetic and biochemical studies, along with new morphological analyses, a model describing a series of discrete steps and the components involved in these steps has been postulated.

1.3
Mechanistic Aspects of Autophagy

Autophagy is an evolutionarily conserved process in which the basic components are similar from unicellular (i.e. yeast) to multicellular eukaryotes. Very few of the autophagy proteins have motifs that provide insight into their function. Accordingly, the role of most of the Atg proteins is unknown; however, their interacting partners and order of action have been determined through various studies. Although autophagy is a dynamic process, the pathway has been delineated into

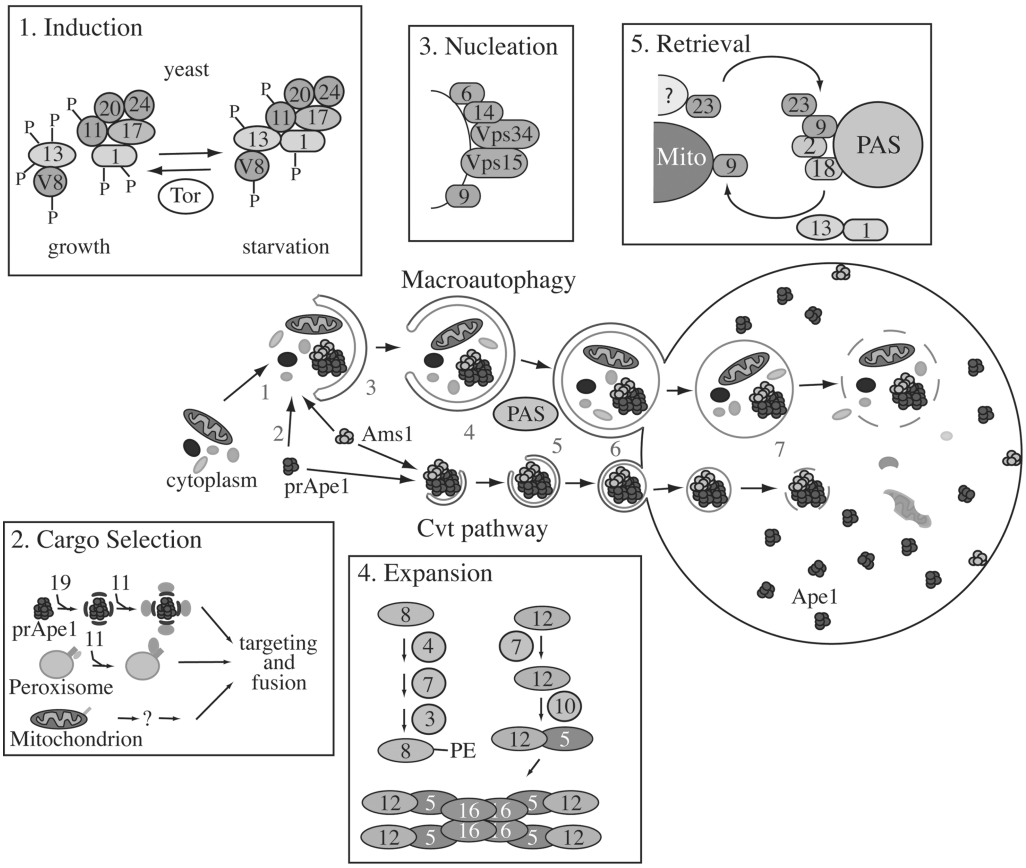

Fig. 1.2 Autophagy and the Cvt pathway. Autophagy and the Cvt pathway can be depicted as a series of separate steps. The roles of Atg and other proteins, shown to participate in different parts of the pathway, are depicted. The proteins classified by only a number are the corresponding Atg gene product. Otherwise, the protein name is specified, except for Vac8, which is indicated as "V8". "P" denotes phosphorylation of the indicated protein. (1) *Induction.* TOR kinase becomes inactivated upon nutrient limitation, eliciting a series of events, which result in the induction of autophagy. These include partial dephosphorylation of Atg13, which alters its association with Atg1. Atg1 is thought to play a key role in the switch between growth and starvation. Autophagy-specific proteins are shown in light gray, whereas Cvt-specific proteins are depicted in dark gray. (2) *Cargo selection and packaging.* Examples of specific autophagy include the Cvt pathway, pexophagy and possibly mitophagy. During growth, the Cvt pathway is active. The cargo, prApe1, is synthesized as an inactive precursor and rapidly oligomerizes. Atg19, the cargo receptor, binds to the oligomer, followed by Atg11 binding to the complex. Upon induction of pexophagy, the peroxin, Pex3, is degraded, thus exposing the docking protein, Pex14. Although it is not proven, Atg11 is proposed to bind to the newly exposed Pex14. The mechanism of mitophagy is unknown. Once these binding events occur, the cargo are enwrapped by a double-membrane vesicle and delivered to the lysosome/vacuole. (3) *Vesicle nucleation.* Membrane is acquired from an unknown location and the cargo associates with the forming vesicle. Membrane formation re-

several static steps for the convenience of description: (1) induction, (2) cargo selection and packaging, (3) vesicle nucleation, (4) vesicle expansion and completion, (5) retrieval, (6) targeting, docking and fusion of the vesicle with the lysosome/vacuole, and (7) breakdown of the vesicle and its contents (Fig. 1.2) [1, 25].

1.3.1 Induction

During vegetative growth, autophagy operates at a basal level both in yeast and mammalian cells. In addition, during growth in yeast, a second, more specific pathway operates, the Cvt pathway, which mediates delivery of the resident vacuolar hydrolase aminopeptidase I (Ape1) [26]. Upon a change in nutrient status, or other stress conditions, autophagy is induced. Therefore there must be some intracellular stimulus signaling the need to degrade intracellular components. As stated earlier, the mechanism of induction is not precisely understood, but some of the molecules involved are known. The best characterized regulatory component in yeast is the protein kinase target of rapamycin (Tor) [27]. Tor either directly or indirectly controls a putative protein complex that is sometimes called "the switching complex". This complex includes Atg1, a serine/threonine protein kinase, Atg13, a protein that modulates Atg1 kinase activity, and pathway-specific proteins including the autophagy-specific protein Atg17, and Cvt-specific factors Atg11, Atg20, Atg24 and Vac8 [28, 29]. Although the associations among these proteins have been demonstrated as bimolecular interactions, it is not known whether all of these proteins are ever present in a single complex.

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quires the PI3K complex I; the components of this complex are shown in Step 3. The PI3-phosphate (PI3P) generated by this complex recruits a number of Atg proteins to the PAS, including Atg18, Atg20, and Atg21, Atg24 and Atg27 [24]. (4) *Vesicle expansion and completion*. There are two sets of Atg proteins, which participate in a series of ubiquitin-like (Ubl) conjugation reactions. These generate Atg12–Atg5–Atg16 and Atg8–PE (see text for details). The functions of these proteins are not known but they are needed for expansion and completion of the sequestering vesicle. (5) *Retrieval*. As most of the Atg proteins are not included in the completed vesicle, there must be a mechanism to release and return these components back to their original site. Atg9 and Atg23 have been shown to be cycling proteins, moving between the PAS and other punctate structures. Atg9 has been shown to cycle betweent he mitochondria and the PAS. The non-PAS localizations of Atg23 are as yet unidentified. These two proteins may aid in the recovery of Atg components, allowing them to be reused for another round of delivery. (6) *Targeting, docking and fusion of the vesicle with the lysosome/vacuole*. The docking and fusion of the completed vesicle requires a number of components (see text for details). The fusion event results in a single-membrane vesicle within the lumen of the lysosome/vacuole. (7) *Breakdown of the vesicle and its contents*. Once inside the lysosome/vacuole, the autophagic or Cvt body must be degraded in order for the cargo to be released. The lipase responsible for vesicle lysis is thought to be Atg15. Upon release into the lumen, the cargoes of pexophagy and bulk autophagy are broken down for re-use in the cell, while the cargoes of the Cvt pathway carry out their function as hydrolases. (This figure also appears with the color plates).

Nutrient limitation results in inactivation of Tor and induction of autophagy, whereas during vegetative growth conditions, Tor is active. Tor may mediate the activity of autophagy directly or indirectly: Tor activity alters the phosphorylation state of Atg13, thereby changing its binding affinity for Atg1; Tor also controls global transcription and translation through various downstream effectors [29–31]. Regulation through additional factors in yeast is not well understood. For example, protein kinase A is another negative regulatory component, but it is not known whether this protein acts downstream of TOR or in parallel [32]. In higher eukaryotes, mammalian (m) TOR is controlled through a phosphatidylinositol-3-kinase (PI3K) pathway that includes phosphoinositide-dependent kinase 1 (PDK1) and Akt/protein kinase B [33].

1.3.2 Cargo Selection and Packaging

Just as a signal must exist to dictate which of the various types of autophagy are functioning at a given moment, there also must be components that confer specificity on the cargo to be sequestered. The biosynthetic Cvt pathway involves the specific sequestration of the resident vacuolar hydrolases Ape1 and α-mannosidase (Ams1), and their subsequent delivery to the vacuole [26]. This is a receptor-mediated route of transport, although the primary cargo, precursor Ape1 (prApe1), is not concentrated via binding to Atg19, the Cvt receptor [34]; the ability of prApe1 to assemble into a large oligomeric complex appears to be an inherent property of the precursor protein. None of the other Atg proteins are needed for the interaction between Atg19 and the prApe1 propeptide [26]; however, in the absence of Atg11 the prApe1–Atg19 complex (termed the Cvt complex) is not localized at the pre-autophagosomal structure (PAS), the presumed site of Cvt vesicle and autophagosome formation. Therefore, Atg11 is thought to act in part as a tethering factor. Following delivery to the PAS, Atg19 binds Atg8 conjugated to phosphatidylethanolamine (Atg8–PE) that is present on the PAS membrane. Atg11 is not found in the completed Cvt vesicle, so it is thought to leave the Cvt complex at this time [35]. The interaction between Atg19 and Atg8–PE may trigger completion of the sequestering vesicle.

Upon fusion with the vacuole, the inner vesicle is released into the lumen and is now termed a Cvt body. Precursor Ape1 is activated by removal of the propeptide [36]. Although this pathway has only been reported in the yeast *S. cerevisiae*, it provides an ideal model system to analyze specific autophagy-related pathways, variations of which are likely to operate in all cell types. For example, the degradation of peroxisomes (pexophagy) is another form of specific autophagy, which has been detected in yeast as well as in plants and mammalian cells [37]. When yeasts are grown on carbon sources that require peroxisome function, this organelle proliferates. Shifting to a preferred carbon source results in rapid elimination of the now superfluous organelle. This is a highly specific process – the sequestering vesicles contain solely peroxisomes and it is presumed that this is also receptor driven, although a receptor similar to Atg19 has

not been identified [38]. The peroxisomal tag for pexophagy in yeast appears to be Pex14 [39]. Other examples of selective autophagy involve exclusive packaging and delivery of ER and mitochondria, as well as certain cytosolic proteins and even the nucleus [40–43]. As investigations continue to reveal these different forms of selective autophagy, they serve to highlight the point that autophagy is not only a random process, but can also be highly discriminatory, capable of degrading only the specific components necessary at a given time.

1.3.3
Vesicle Nucleation

The putative site for vesicle formation is the PAS [44, 45]. This is the structure believed to be the organizing center for the assembly and organization of the autophagic machinery. Very little is understood about this process, but it seems that autophagic vesicles may form *de novo*, meaning that they are not generated in one step by segregation of membrane from a pre-existing organelle. Rather, the sequestering vesicles appear to begin at some nucleation site and then appropriate additional intracellular membrane to form a cup-shaped structure around the cargo. The intermediate structure is termed a phagophore, or isolation membrane. Formation of the double-membrane cup requires PI3K activity, which is mediated by a complex containing Atg6/Vps30, Atg14 (in yeast), Vps15 and the PI3K Vps34 [46, 47]. Atg5 is one of the first Atg proteins that can be visualized on this structure, but whether it is the first protein to arrive at the PAS is unknown [47].

1.3.4
Vesicle Expansion and Completion

In order to completely enclose the cargo, the membrane must undergo an expansion step. Involved in this process are two groups of Atg proteins that include some ubiquitin-like (Ubl) proteins, which participate in conjugation reactions [48]. One set of proteins is involved in the covalent attachment of the Ubl Atg12 to Atg5. Atg5 binds Atg16 noncovalently and the subsequent tetramerization of Atg16 forms a large multimeric complex. Atg8 is another Ubl important for membrane expansion. This protein is proteolytically cleaved at its C-terminus to expose a glycine residue and is then used as a modifier of PE [49]. In mutants lacking Atg8, autophagosomes can still be generated but they are of reduced size [50]. These and the other proteins depicted in Fig. 1.2 are thought to be delivered to the forming autophagosome, and possibly control the size and curvature of the nascent vesicle; however, the exact function of these proteins is not known.

1.3.5 Retrieval

Protein targeting pathways generally utilize components that are reused, enabling them to be used for multiple rounds of substrate delivery. Atg8 and Atg19 are the only components that are known to be included in the completed autophagosome, suggesting that all of the proteins involved in the previously described steps must dissociate from the forming vesicle before completion. This is particularly problematic for integral membrane proteins such as Atg9 [51], which cannot simply dissociate from the vesicle. Retrieval of Atg proteins has been recently demonstrated for two factors, Atg9 and Atg23, which have been shown to cycle between punctate cytosolic structures and the PAS [52, 53]. Interestingly, the Atg9-containing structures have been identified as corresponding at least in part to mitochondria [54]; it remains to be determined whether this is also the case for Atg23. Atg1 and Atg13 are required for cycling of both of these proteins (although higher Atg1 kinase activity is needed for Atg23), whereas Atg2 and Atg18 are required only for cycling of Atg9 [52]. The function of Atg9 and Atg23 cycling is unknown – Atg9- and Atg23-containing structures may contribute membrane to the expanding autophagosome or these proteins may serve to mediate delivery of other necessary components to the PAS.

1.3.6 Targeting, Docking and Fusion of the Vesicle with the Lysosome/Vacuole

There must be some mechanism for preventing fusion of the incomplete Cvt vesicle or autophagosome with the lysosome or vacuole. This may be achieved by the presence of coat proteins that sterically interfere with the interaction of soluble *N*-ethylmalemide-sensitive fusion protein (NSF) attachment receptor (SNARE) proteins; however, the presence of coat proteins has not been clearly demonstrated. Once the vesicle is complete, it fuses with the degradative organelle. As noted previously, in mammalian cells the initial fusion step may involve an endosome. The proteins required for fusion appear to be common to those involved in other lysosomal/vacuolar fusion events including SNARE proteins, NSF, soluble NSF attachment protein (SNAP) and GDP dissociation inhibitor (GDI) homologs, a Rab protein, and the class C Vps/HOPS complex [55]. After fusion of the double-membrane vesicle with the lysosome/vacuole, the inner vesicle is released into the organelle's lumen.

1.3.7 Breakdown of the Vesicle and its Cargo

The outer membrane of the sequestering vesicle becomes continuous with the limiting membrane of the lysosome/vacuole. This membrane may be removed through a microautophagic process. The membrane of the Cvt or autophagic body must be broken down within the lumen to release the contents. The lipase

thought to be responsible for breakdown of the membrane is Atg15 [56]. In the Cvt pathway, lysis of the Cvt body allows release and subsequent activation of prApe1. For pexophagy and nonspecific autophagy, release of the vesicle cargo results in its subsequent degradation by resident vacuolar hydrolases. These macromolecular components are then made available for reuse in the cell. These processes are only depicted in general in Fig. 1.2; a more detailed summary can be found in other reviews [1, 25, 55].

The details of the steps outlined above have been best characterized in yeast, but many of the components also have homologs in higher eukaryotes cells (Tab. 1.1). In addition, the development of novel techniques is allowing investigators to determine if the mammalian components function similar to their yeast counterparts.

1.4 Autophagy and Immunity

One of the most important functions of autophagy appears to be its role in the host defense against cellular pathogens. In general, bacterial pathogens enter the cell via an endocytosis-like pathway, enclosed within a vesicle called a phagosome that ultimately fuses with and is degraded by the lysosome [57]. This was demonstrated to be the method cells use to avoid infection by *Streptococcus pyogenes* [58]. In contrast, it has recently been shown that certain bacteria undermine the autophagic machinery to promote their replication and survival [22, 59, 60]. This evasion is accomplished in different ways by different bacteria. In the case of *Listeria monocytogenes*, *Shigella* and certain other bacteria, the microbes induce lysis of the phagosome, causing their release into the cytoplasm and enabling them to replicate in that environment [61, 62]. Other invasive bacteria including *Mycobacterium tuberculosis* modify the phagosome in which they are contained, to prevent fusion with the lysosome [63]. Still other pathogens such as *Legionella pneumophila* induce the autophagic pathway and replicate within autophagosome-like compartments [59, 64]. In organisms such as *L. pneumophila* and *Coxiella burnetii*, induction of autophagy enhances the replication and survival of the invading bacteria [65].

The role of autophagy in defense against viral infection is also dual in nature, both protecting against infection and being exploited to promote viral invasion. For example, induction of autophagy increases MHC class II antigen presentation and contributes to the immune response by aiding in antigen processing of certain viruses [66, 67]. Like bacteria, some viruses can also use the autophagic machinery to their advantage. For example, coronavirus replication and viability is increased by autophagy [68], and stimulation of autophagy increased the yield of poliovirus [69].

With these recent discoveries, it is now clear that autophagy can aid in defense against pathogens, but the microbes can also employ this pathway to promote their viability. This is similar to the role of autophagy in cancer and other

diseases – depending on the progression of the disease, autophagy may be a protective mechanism, eliminating damaged organelles or even damaged cells, or it may have harmful effects by causing cell death or in the case of cancer by promoting the survival of tumor cells under limiting nutrient conditions [70]. These varying effects of autophagy only serve to emphasize the importance of being able to control the activity of this degradative pathway if it is ever to be used therapeutically. In addition, as will become evident in the following chapters, the various analyses of autophagy and its role in immunity have led to a series of intriguing new questions. For example, is the mechanism of induction of autophagy the same during pathogen infection as it is during nutrient deprivation? What signals allow bacterial pathogens to induce autophagy and how do pathogens prevent maturation or fusion of autophagosomes? Is the sequestration of these pathogens specific, enclosing only the bacteria or virus, or is it a bulk degradation process, which includes other cytoplasmic components? Continued investigations of autophagy will aid in our understanding of this important process, and hopefully lead to treatments for the human conditions and diseases in which autophagy is implicated.

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