Primer - Autophagy

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Summary

Maintaining cellular functions and homeostasis depends on efficient recycling mechanisms to degrade unwanted, superfluous or damaged material. Nonfunctional or superfluous proteins are degraded by the ubiquitin-proteasome system. However, more complex cellular components including protein aggregates and composite structures such as ribosomes or even entire organelles also need to be degraded. This material is in terms of size and shape extremely heterogeneous and its degradation thus requires a specialized and highly versatile recycling process, termed autophagy. The hallmark of autophagy is the delivery of cytoplasmic materiel to lysosomes for degradation. As its substrates, autophagy is diverse and includes specialized pathways such as macroautophgy, microautophagy and chaperone mediated autophagy. The mostly studied and best characterized process is macroautophagy, which will be the major focus of this Primer.

Main Text

In 1955, the biologist and Nobel Prize laureate Christian de Duve discovered that cells possess specialized organelles filled with hydrolytic enzymes, that he called lysosomes. At the same time, electron microscopy studies by Novikoff and colleagues showed that dense bodies, which later turned out to be lysosomes, contain cytoplasmic components. Together, these groundbreaking observations revealed that cells can deliver cytoplasmic components to lysosomes for degradation. The hallmark of the related pathway, which de Duve called autophagy, is the formation of double-membrane vesicles. Further morphological characterization of these autophagosomes revealed that they mainly contain bulk cytoplasm.

Although this suggested that autophagy leads to a nonselective degradation of cytoplasmic materiel, de Duve anticipated that also a regulated and selective type of this pathway must exist. Today we know that under normal conditions, macroautophagy is a highly selective pathway that sequesters damaged or superfluous material from the cytoplasm through the formation of double-membrane limited autophagosomes. Upon fusion with lysosomes, the content of autophagosomes is degraded and the resulting building blocks are released in the cytoplasm. However, in response to cytotoxic stress or starvation cells start to produce autophagosomes that capture bulk cytoplasm nonselectively. This stress response is essential for cells to survive adverse environmental conditions, whereas the selective sequestration of cargo is important to maintain cellular homeostasis.

In general, the term autophagy describes an ensemble of pathways that deliver cytoplasmic components to lysosomes. Morphologically, theses pathways are remarkably diverse including not only the already introduced generation of autophagosomes in a process referred to as macroautophagy, but also direct uptake of cytoplasm or cytoplasmic components by lysosomal membrane invaginations, termed microautophagy. Moreover, lysosomal channels import a dedicated set of proteins into the lumen of lysosomes in a process that requires assistance by chaperones and has thus been termed chaperone-mediated autophagy. Here, we will focus on macroautophagy (autophagy in the following), owing to its versatile nature and its essential contribution to cellular survival and homeostasis.

From membranes to molecules – identification of the autophagy machinery in yeast

The morphological characterization of autophagy by electron microscopy established general properties of the pathway but until the nineteen-nineties mechanistical or even molecular insights into the biogenesis of autophagosomes as well as its regulation remained uncharacterized. Maybe inspired by genetic screens that Randy Schekman and colleagues developed to identify genes that regulate protein secretion in yeast, Yoshinori Ohsumi and coworkers established a genetic screen to identify autophagy-defective mutants in Saccharomyces cerevisiae. The ground-breaking discoveries of both researchers were honored with the Nobel Prize in Physiology or Medicine, first for Schekman in 2013 followed by Ohsumi in 2016, for their characterization of fundamental cellular transport processes. The set of autophagy-deficient mutants that Ohusmi's group identified in their screen comprised 15 apg (for AutoPhaGy) genes. Similar screens were subsequently employed to expand the

repertoire of autophagy genes. However, these studies were based on defects in selective autophagy, leading to the discovery of the cytoplasm to vacuole targeting (CVT) pathway that delivers vacuolar hydrolases to the vacuole and pathways that direct peroxisomes (pexophagy) or mitochondria (mitophagy) to vacuoles for degradation. Selective and nonselective autophagy pathways were initially thought to be distinct and the corresponding genes received pathway specific names including apg and aut (non-selective and selective autophagy) as well as cvt (CVT-pathway). However, many of the corresponding proteins were later found to be involved in all autophagy pathways, leading to the unification of the initially diverse nomenclature by defining AuTophaGy (Atg) proteins and genes.

Currently, more than 40 Atg proteins have been discovered in yeast and a subset of them constitute the autophagy core machinery that is essential for selective and non-selective autophagy as well as the CVT-pathway. This core machinery comprises five major complexes that are recruited to a specific location in yeast, termed phagophore assembly site (PAS) at which autophagosomes are being generated. The recruitment of the Atg1 kinase complex to the PAS is the earliest event upon starvation-induced (nonselective) autophagy. This complex comprises the kinase Atg1, the regulatory subunit Atg13, as well as a scaffolding and tethering subcomplex that consists of Atg17, Atg31 and Atg29. The activity of the Atg1 kinase complex is regulated by the Target of Rapamycin (TOR) complex. TOR constitutively phosphorylates Atg13 under growing conditions. The inactivation of TOR upon starvation leads to a rapid dephosphorylation of Atg13 which in turn allows the Atg1 kinase complex to assemble. The activated and assembled complex recruits downstream components of the core machinery to the PAS including Atg9-vesicles, which donate lipids in order to generate the autophagic precursor membrane, termed phagophore (Figure 1). At a molecular level, the Atg1 kinase complex subunit Atg17 tethers Atg9 vesicles to bring them into close proximity which initiates their subsequent fusion. The fusion process itself leads to the formation of the early phagophore and depends on the activity of SNARE proteins, which are a family of proteins that catalyze virtually all intracellular membrane fusion reactions. Furthermore, the Atg1 kinase complex recruits an autophagy specific class III phosphatidylinositol (PtdIns) 3-kinase complex to the phagophore, which catalyzes the conversion of PtdIns to PtdIns(3)phosphate. This phospholipid is recognized by two members of the PROPPIN (β-propellers that bind polyphosphoinositides) protein family Atg18 and Atg21. Both proteins orchestrate the transition from phagophore initiation to phagophore expansion (Figure 1). A recent study

reported that Atg18 cooperates with Atg2 and Atg9 to form phagophore-ER-contact sites, which might be involved in transferring lipids form the ER to the phagophore during expansion. Atg21 recruits Atg16, a component of an autophagy specific E3-ligase complex, to the PAS. This ligase contains in addition to Atg16 the Atg12-Atg5 conjugate. The conjugation of ubiquitin (Ub)-like Atg12 to Atg5 is catalyzed by the sequential action of the E1-like enzyme Atg7 and the E2-like enzyme Atg10. The Atg12–Atg5 ligase promotes the conjugation of the Ub-like protein Atg8 to the lipid phosphatidylethanolamine (PE) within phagophores. This conjugation is also preceded by a cascade of enzymatic reactions. First, Atg4 primes Atg8 by proteolytic cleavage of its inhibitory C-terminal arginine residue. The newly exposed C-terminal glycine is conjugated to the E1-like enzyme Atg7. Atg8 is then transferred to E2-like Atg3, which catalyzes in collaboration with the E3-ligase complex the lipidation of Atg8.

Atg8 coordinates many steps during the biogenesis of autophagosomes. The pool that is conjugated to the inner membrane of autophagosomes functions as membrane anchor for autophagy substrates and binds cargo either directly or through its interaction with autophagy receptors (Figure 1). A large number of substrate specific receptors have already been characterized and their coordinated interaction with cargo and Atg8 represents a fundamental principle in selective autophagy. The substrate of the Cvt-pathway ApeI is, for example, bound by its receptor Atg19. The tight interaction of Atg8 and Atg19 tethers ApeI complexes to the phagophore to ensure that they are selectively engulfed by autophagosomes by excluding other cytoplasmic components.

In non-selective autophagy, Atg8 appears to provide in cooperation with its E3-ligase complex Atg12–Atg5-Atg16 structural support for the expansion of phagophores to allow them to capture bulk cytoplasm in the absence of templating cargo. This function of Atg8 is restricted to the outer membrane of autophagosomes, at which Atg8 assembles together with Atg12–Atg5-Atg16 into a stiff membrane coat. Atg8 also binds and thus recruits regulators of phagophore expansion, maturation and fusion including Atg1, Rab GTPases such as Ypt1 and Ypt7, as well as the vacuolar SNAREs Vti1 and Vam3. Many of these interactions depend on a common peptide motif in Atg8 binding partners, termed Atg8-interacting motif (AIM) that binds into a conserved pocket in Atg8. The consensus sequence of this motif is WxxL, but tryptophan (W) can be replaced by other bulky aromatic amino acids such as tyrosine, and lysine (L) by isoleucine or valine.

Before or shortly after autophagosomes and lysosomes fuse, the outer membrane pool of Atg8 is recycled by Atg4 through proteolytic cleavage of the Atg8-PE bond. The fusion reaction itself is coordinated by the membrane tethering HOPS complex through its interaction with Ypt7 on autophagic and vacuolar membranes, as wells as vacuolar SNARE proteins Vam3, Vam7 and Vti1 and the autophagic SNARE Ykt6.

During selective autophagy, the composition of the initiating Atg1 kinase complex differs by substitution of the inactive Atg17 tethering subunit by Atg11. The latter is also a coiled coil protein which binds not only Atg9 and the Atg1-Atg13 subcomplex, but also cargo through conserved interactions of its C-terminal domain with cargo receptors. Although the precise function of Atg11 is not entirely clear, Atg11 appears to activate the kinases activity of Atg1 to promote phagophore expansion. Furthermore, Atg11 contributes to recruit Atg17 to the PAS upon induction of non-selective autophagy by starvation. How the recruitment of Atg17 by Atg11 and the exclusive functions of both Atg1 kinase scaffolds in nonselective and selective autophagy are coordinated remains, however, to be investigated.

From molecules to diseases – autophagy in human cells

Autophagy is a highly conserved mechanism and the fundamental principles of autophagy in yeast and humans are very similar. Many yeast Atg proteins have human homologs and their interactions with other proteins and membranes are often similar or identical. However, the human machinery is more complex, comprising additional ATG proteins that are not present in yeast as well as different ATG isoforms. This increased molecular complexity allows human ATG proteins to fulfill diverse functions in autophagy-related and unrelated processes, such as development and immunity.

The higher complexity of human autophagy also pertains to its regulation. The human homolog of the Atg1 kinase complex is the ULK1 (unc-51-like kinase 1) kinase complex and its activity is not only regulated through phosphorylation by the mammalian TOR complex 1 and the AMP-activated protein kinase (AMPK1), but also by other posttranslational modifications including ubiquitination and acetylation. Similar to autophagy induction in yeast, amino acid starvation leads to an inactivation of mTORC1 in human cells. However, mTORC1 phosphorylates not only ATG13, but also ULK1 and both modifications lead to an inactivation of the ULK1 kinase complex. Decreasing energy levels of cells by glucose starvation induces nonselective autophagy by activating AMPK1. This kinase inhibits mTORC1 by phosphorylating

its subunit RAPTOR. Furthermore, AMPK1 phosphorylates ULK1 at multiple sites leading to its activation as well as ATG13 which, counterintuitively, inhibits autophagy. Together, the diversity of these regulatory mechanisms suggests that the activity of the ULK1 complex is fine-tuned and integrates various metabolic and non-metabolic signals.

The ULK1 kinase complex is composed of ATG13, FIP200 and ATG101 but is in contrast to the yeast complex constitutively assembled. Although FIP200 and ATG101 do not have direct homologs in yeast, FIP200 has been suggested to be homologous to yeast Atg17. Activation of the ULK1 kinase complex leads to its recruitment to the ER, where it coalesces with ATG9 vesicles. Previous ultrastructural characterization revealed that a cradle-like ER domain, termed omegasome, is the major platform for autophagosome biogenesis in humans (Figure 2A). In contrast to yeast cells which possess a unique PAS, human autophagosomes are generated at many places simultaneously. Furthermore, most omegasomes are not only in intimate contact with ER membranes but also in close vicinity to ER-mitochondria contact sites, implying that mitochondria participate in phagophore formation. The formation of omegasomes depends not only on the recruitment of the activated ULK1 kinase complex to the ER, but also on PtdIns(3)P. This lipid is generated through phosphorylation of PtdIns by the class III PtdIns(3)-kinase complex, which is recruited through an interaction of its subunit ATG14 (Barkor) with the ULK1 kinase complex subunit ATG13. In addition to ATG14, the PtdIns(3)-kinase complex further comprises VPS34 (harboring the kinase activity), p150 and Beclin1. The activity of this complex is extensively regulated and tightly controlled. Beclin1, for example, interacts with Bcl2 and $BclX_L$ and both interactions decrease the activity of VPS34, thereby inhibiting autophagy. By contrast, other Beclin1 interaction partners including Ambra1 stimulate formation of the PtdIns(3)P kinase complex and promote autophagy. Moreover, Beclin1 forms together with p150 and VPS34 two distinct complexes, containing either ATG14 or UVRAG. The latter complex is not involved in autophagy but essential for endocytosis by generating the endosomal pool of PtdIns(3)P.

The model that omegasomes are ER-derived membranes has very recently been challenged by a study that reported that recycling endosomes act as platforms for autophagosome assembly. This endosome population is in close proximity to the ER and to ER-mitochondria contact sites, suggesting that recycling endosomes and omegasome are in fact identical membranes. As consensus, human phagophores are formed on membrane platforms at which the ULK1- and the PtdIns(3)P-kinase complexes assemble together with

ATG9 vesicles. Although these membranes are in close proximity to various other organelles including ER and mitochondria, the exact contribution of each organelle to phagophore formation remains to be investigated. It is also not clear how the phagophore itself is generated although common thinking suggests that the membrane is formed de novo, i.e. without budding from an organellar membrane. Consequently, its nucleation would depend on membrane input from other sources and ATG9 vesicles, ER-derived COPII vesicles as well as vesicular-tubular structures from the ER-Golgi-intermediate compartment have been implicated in this process.

The generation of the phospholipid PtdIns(3)P is an essential step during the nucleation of phagophores by recruiting a number of PtdIns(3)P binding proteins to phagophore assembly sites, including WIPI2, which is a homolog of the yeast PROPPIN Atg18. Due to the presence of two distinct PtdIns(3)P-kinase complexes which act on autophagic and endosomal membranes, PtdIns(3)P is highly enriched in autophagosomes and early endosomes. Thus, additional interaction partners are required to target PtdIns(3)P binding proteins to their correct compartment. This so called coincidence detection has recently been reported for WIPI2, which binds PtdIns(3)P and the small GTPase RAB11 on recycling endosomes, providing another piece of evidence that recycling endosomes could function as platforms for phagophore assembly.

The recruitment of WIPI2 is essential for the progression of autophagy after the phagophore has been nucleated. WIPI2 recruits ATG16L1 to the phagophore, which is similar to its yeast homolog Atg16 a component of the Ub-like E3-ligase complex. This ligase forms part of the human Ub-like conjugation system that comprises as its yeast counterpart the E1-like enzyme ATG7 as well as E2-like ATG3 and ATG10 enzymes. However, in contrast to the yeast system, six ATG8 family proteins (hATG8s) are expressed in human cells. Based on their sequence homology, hATG8s can be grouped into LC3 and GABARAP subfamilies. The LC3 family comprises members LC3A, LC3B and LC3C which are thought to be involved in phagophore expansion while GABARAP, GABARAP-L1 and GABARAP-L2 promote sealing of autophagosomes and/or their fusion with lysosomes. Consistent with this division of labor, hATG8s are ubiquitously expressed with some variations in the expression levels among human tissues. Similar to their yeast homolog, hATG8s are conjugated to both, inner and outer autophagic membranes. The pool of hATG8 at the inner membrane tethers cargo to the phagophore and thus promotes selective uptake of cytoplasmic material (Figure 2A).

Many autophagy receptors have been identified in humans. Some of them are specific for certain cargo, including the mitochondrial receptor Nix and FUNDC1 or the endoplasmic reticulum receptor FAM134B. Others, such as p62, NDP52, NBR1 or Optineurin have broad substrate specificity and promote degradation of different cellular components. Whether these receptors execute distinct and nonredundant functions depends on the nature of cargo. The receptors p62, NDP52 and Optineurin are, for example, essential for xenophagy, the selective degradation of intracellular pathogens. This implies that they cooperate to fulfill distinct functions. By contrast, the two receptors NBR1 and p62 take on similar and partially redundant functions in recruiting and tethering protein aggregates to phagophores during aggrephagy.

Most cargo receptors possess an LC3 interacting region (LIR) which has as its yeast counterpart the consensus sequence WxxL. Furthermore, the two hydrophobic residues in the LIR bind into conserved hydrophobic pockets of hATG8s. The structural conservation of the LIR binding region in all six hATG8s results in similar binding affinities of interaction partners. Consequently, interactions of binding partners that are based on canonical LIRs do not distinguish between different hATG8s. NDP52 was the first receptor for which a selective interaction with one hATG8 member, in this case LC3C, was observed. The corresponding LC3C specific CLIR motif in NDP52 has the consensus sequence I-L-V-V and binds to an extended, LC3C specific hydrophobic patch that partially overlaps with the binding site for canonical LIRs. Other autophagy regulators including PLEKHM1, a regulator that promotes fusion of autophagosomes and lysosomes, specifically interact with GABARAPs and possess the GABARAP interacting motif (GIM) with the consensus sequence W/F-V/I-x-V. The selectivity of PLEKHM1 for GABARAPs is consistent with the observation that GABARAPs promote later steps in autophagy, while LC3s do not. This might also explain why deletion of LC3s has little effect on autophagy while that of GABARAPs leads to the production of smaller autophagosomes whose fusion with lysosomes is impaired. A recently published comprehensive structural characterization of GABARAP-LIR complexes revealed that canonical LIR motifs can also become GABARAP specific. Their specificity for GABARAP is encoded in C-terminal residues adjacent to the canonical LIR motif.

For more than a decade, LIR motifs served as a paradigm for the conserved interaction of hATG8 with autophagy receptors. However, recently a new class of binding motif, which is homologous to canonical ubiquitin interacting motifs (UIMs), was identified. These peptide

motifs bind a C-terminal hydrophobic region in ATG8 that is distinct from the canonical LIR binding region and target a distinct set of autophagy substrates such as CDC48 to phagophore membranes.

The evolution of highly conserved LIRs and UIMs in hATG8 binding proteins suggests that hATG8s play essential functions in autophagy. This is contrasted by studies showing that autophagosomes can form even in the absence of all hATG8s. The number of autophagosomes, their size and the maturation of phagophores into autophagosomes were, however, strongly impaired. A consensus of these apparently contradicting observations might be that hATG8s are important regulators of phagophore initiation, maturation and fusion with lysosomes but are not essential for the formation of autophagosomes. Interestingly, deletions of the Ub-like conjugation enzymes ATG7 or ATG5 lead to a complete block in autophagy, which was for a long time considered to be a proof for the importance of ATG8 conjugation to phagophores. However, accumulating evidence suggest that this strong effect is at least partially related to non-autophagic functions of ATG7 and ATG5.

Many other functions of hATG8s in autophagosome biogenesis have been identified, including membrane tethering and fusion during phagophore closure and recruitment of autophagy regulators such as PLEKHM1 or ULK1. Although some functions of hATG8s can be clearly correlated with their spatial distributions to the inner or outer autophagic membranes, all hATG8s are conjugated to both membranes. Our understanding of the spatially correlated timing of hATG8 conjugation to the phagophore and the persistence of ATG8s at the phagophore or autophagosome remains, however, limited.

Interestingly, the number of Atg8-like proteins has increased during evolution from one (yeast), to two (insects) and four (nematodes) to reach six variants in mammals. Thus, the most complex eukaryotes evolved the largest number of ATG8 variants. This correlation suggests that ATG8s possess other, non-autophagic functions that are related to cellular processes in high eukaryotes. It is, for instance, known that hATG8s are involved in the rapid degradation of pathogen-containing phagophores, a process that has been termed LC3-associated phagocytosis. The intersection of autophagy with other cellular pathways and non-autophagic activities of ATG proteins is a rapidly growing field and became a focus of current research.

The cleavage of hATG8s from the outer autophagic membrane by ATG4 coincides with closure of phagophores and their fusion with lysosomes. The activity of ATG4 thus needs to

be tightly regulated. Surprisingly, ULK1 which is functioning during autophagy induction is also a key regulator of ATG4 activity. Phosphorylation of ATG4 by ULK1 inhibits the protease activity whereas dephosphorylation by the PP2A-PP2R3B phosphatase activates ATG4 which leads to the cleavage of hATG8s from the outer membrane of autophagosomes. The complexity of this ATG8 recycling reaction is further increased by the presence of four ATG4 variants (ATG4A to D) in human cells. Recent studies demonstrated that the different ATG4 variants have different substrate preferences. ATG4B, for example, has been found to prime hATG8 by orders of magnitudes faster than it cleaves them from membrane. In general, ATG8-recycling appears to be a slow process involving all ATG4 proteases with some substrate preferences, indicating that the combination of ATG4 and ATG8 during recycling might be a well-controlled and tightly regulated process which correlates with the progression of autophagosome biogenesis by promoting different stages during autophagosome maturation and fusion with lysosomes.

Sealing of phagophores is one of the last steps during the biogenesis of autophagosomes (Figure 2A). Although, the underlying molecular mechanisms are not well understood, early evidence suggested that hATG8s are involved in this process. This hypothesis was based on the observation that hATG8s promote membrane fusion in vitro. However, a recent study reported that ESCRTs, which promote the formation of intraluminal vesicles in late endosomes, are involved in this process. The ESCRT-III complex is a membrane scission machine that promotes the detachment of nascent intraluminal membrane buds from limiting membranes of endosomes. This process is topologically similar to sealing of phagophores. Thus, a function of ESCRT-III in autophagy was anticipated for a long time. The recently obtained experimental evidence that the ESCRT-III subunit CHMP2A promotes fusion has improved our understanding of phagophore closure. It remains, however, possible and plausible that both, hATG8s and ESCRT-III collaborate during this process. ESCRT-III promotes only the scission of narrow membrane necks and hATG8s might be involved in closing the phagophore to reach neck dimensions that can become substrates for ESCRT-III.

After sealing, autophagosomes fuse with lysosomes, a process coordinated by various factors. Again, hATG8 proteins appear to play a central role in orchestrating the fusion reaction. GABARAP, for example, recruits PLEKHM1 to autophagosomes, which in turn tethers autophagosomes and lysosomes through its interaction with RAB7. The small GTPase RAB7 recruits EPG5 and, in collaboration with PLEKHM1, the HOPS complex. Both EPG5 and HOPS

are additional tethering factors that promote fusion by bringing the outer autophagic membrane and the lysosomal membrane in close proximity. Furthermore, they facilitate priming of the lysosomal SNARE VAMP8 and autophagic SNAREs SNAP29 and STX17. This is followed by merging of both membranes through the formation of a four helical bundle between these SNAREs. As a result, the autophagic body, which is delimited by the inner autophagic membrane, is released into the lumen of lysosomes and degraded (Figure 2A).

From Disease to Treatments

Autophagy takes a central position in the metabolism of human cells and is intimately interconnected with metabolic, homeostatic, and trafficking pathways. Furthermore, autophagy allows cells to cope with environmental and endogenous stresses including hypoxia, starvation, reactive oxygen species and cellular damage. It is therefore not surprising that impaired autophagy has severe consequences for cells. Notably, accumulation of protein aggregates as well as of non-functional and damaged organelles contribute significantly to the development of many human diseases including cancer, neurodegenerative diseases, metabolic diseases and auto-immune diseases.

The role of autophagy in cancer is ambiguous. On the one hand, autophagy protects against malignant transformation of cells by promoting genome stability. Beclin 1, component of the PtdIns(3)kinase complex and major autophagy regulator, has been shown to be a bona fide tumor suppressor and mutations in Beclin1 are often found in human cancers. Moreover, mutations in other autophagy regulators and ATG proteins including Ambra1, ATG4C, Parkin or ULK1 are associated with the development of various human cancers. The most obvious mechanism by which autophagy protects against malignant transformation involves the degradation of damaged mitochondria through mitophagy, which limits the release of reactive oxygen species. Furthermore, autophagy negatively regulates inflammatory processes which are known to significantly contribute to the development of cancers. On the other hand, autophagy promotes tumor survival during metabolic stress and accumulating evidence suggests that autophagy also promotes metastasis by regulating focal adhesion and integrin function. The ambivalent contribution of autophagy in the onset and progression of cancer makes it difficult to use autophagy modulators in anti-cancer therapy. However, several clinical trials are currently ongoing in which autophagy inhibitors are used to boost the efficacy of chemotherapeutic treatments.

Impaired autophagy is also a major risk factor for the development of neurodegenerative disorders. Mutations in PINK1 and Parkin that impair mitophagy are considered as paradigm for the contribution of autophagy in Parkinson's disease. Moreover, mutations in autophagy receptors such as optineurin and p62 are frequently associated with early onset frontotemporal dementia or amyotrophic lateral sclerosis. The neuroprotective function of autophagy is mainly related to its capacity to degrade protein aggregates, amyloids and mitochondria. Thus, a reduction of the autophagic activity leads ultimately to the accumulation of protein aggregates and damaged organelles (Figure 2B). Combined with the formation of pathological protein aggregates in many neurodegenerative diseases, impaired autophagy is an important risk factor and mutations in ATG proteins are consequently associated with such diseases. The relationship between autophagy and neurodegeneration has been extensively studied but many open questions remain to be answered before potential treatment options can be considered. This is even more true for the relationship between autophagy and metabolic diseases. Although autophagic dysfunction correlates with an increased risk for obesity, impaired lipid metabolism and diabetes, the underlying mechanisms are very complex and not well understood. Common for these pathologies is an increased pro-inflammatory response which provides a possible link to the involvement of autophagy in such diseases.

Taken together, our understanding of autophagy, its underlying molecular mechanisms and its regulation has strongly improved during the past decades. A major future challenge remains to identify autophagy modulators that target specific autophagic functions in well-defined pathologies in a tissue specific manner. A better characterization of autophagy is thus indispensable before autophagy activators or inhibitors can be safely used to treat human diseases by avoiding adverse reactions.

Further Reading:

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Figure Legends:

Figure 1: Autophagy in yeast. Autophagosomes are formed at the phagophore assembly site to which Atg9 vesicles are recruited by the Atg1-kinase complex. These vesicles fuse, giving rise to an early phagophore. The PtdIns(3)-kinase complex enriches the phagophore in the PtdIns(3)P which leads to the recruitment of the PROPPINs Atg18 and Atg21. The expansion of autophagosomes involves lipid supply from various sources such as COPII vesicles. Furthermore, the ubiquitin-like proteins Atg8 is conjugated to the phagophore by an ubiquitin-like conjugation system. Atg8 fulfills many functions in autophagy including cargo selection, phagophore expansion, maturation, sealing and its fusion with the vacuole. For details see text.

Figure 2: Autophagy in humans. (**A**) Autophagy in humans is initiated at the endoplasmic reticulum (ER) from which a domain with distinct morphology (the omega-(Ω)-some) serves as a platform for the nucleation of the isolation membrane (IM). The coordinated activity of the ULK1-kinase complex, the PtdIns(3)-kinase complex and the ATG9 compartment leads to the nucleation of the isolation membrane. Its expansion depends on the ubiquitin-like conjugation system that conjugates six human ATG8-like proteins to the membrane. ATG8 proteins regulate many steps in autophagy by tethering cargo to the inner membrane of autophagosomes, by recruiting autophagy-regulators at the outer membrane, and by promoting sealing of autophagosomes and their fusion with lysosomes. (**B**) Autophagy in health and disease. Neural Stem Cells expressing GFP-tagged lysosomal marker (green), RFP-tagged autophagosome marker (red) are immunostained for protein aggregates (grey). In healthy cells, autophagosomes deliver cargo to lysosomes for degradation (left). In cells, in which the fusion of autophagosomes and lysosomes is impaired (right, disease), autophagosomes and cargo cluster at lysosomes but cannot be degraded.

Figure 1: Autophagy in yeast.

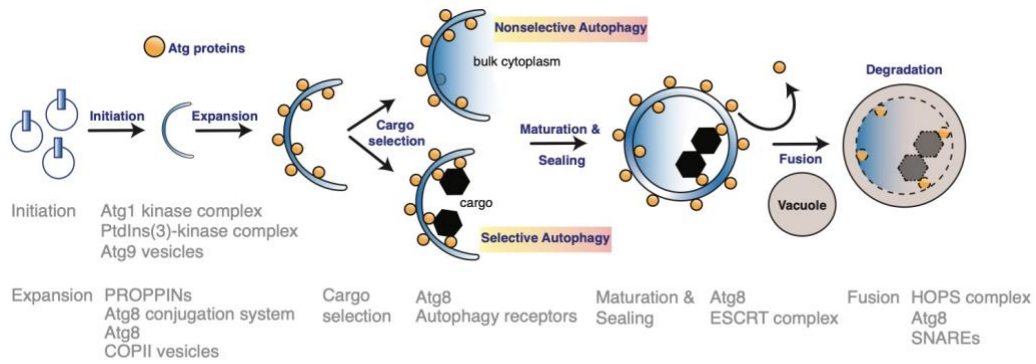


Figure 2: Autophagy in humans.

