Autophagy is a key catabolic process for cell survival against different types of stress. During macroautophagy, the cell carries out the sequestration of different substrates such as invading pathogens, proteins, lipids, or even damaged or superfluous organelles inside double-membrane vesicles, autophagosomes. To degrade their cargo, autophagosomes fuse with the lysosome/vacuole, which contains various hydrolases able to break down the sequestered substrates. After this event, the basic components obtained from cargo degradation are released into the cytoplasm for recycling.1

Autophagy is triggered by different stimuli such as hypoxia,2 oxidative stress,3 pathogen infection,4 endoplasmic reticulum (ER) stress,5 and most notably nutrient starvation.6

Autophagy can be divided into 3 main types: macroautophagy, microautophagy, and chaperone-mediated autophagy. Whereas microautophagy and macroautophagy can be selective or nonselective processes found in yeast and higher eukaryotes, chaperone-mediated autophagy is a selective process that has only been described in mammalian cells. During chaperone-mediated autophagy, specific protein substrates containing the amino acid sequence KFERQ are recognized by chaperones, unfolded and translocated into the lysosome through the lysosomal membrane protein LAMP2A (Figure 1A). In microautophagy uptake also occurs directly at the limiting membrane of the lysosome/vacuole. In this case, however, the process operates by directly sequestering...
the substrates through invagination of the vacuole/lysosome membrane. Of the 3 types mentioned, macroautophagy is clearly the most well-studied process (Figure 1B). Multiple autophagy-related (ATG) genes and proteins have been described as being involved in the different stages of autophagy, comprising what is now known as the core autophagy machinery that is required for autophagosome formation, and additional proteins that act in making the process selective, or in stages other than autophagosome biogenesis. Accordingly, macroautophagy (hereafter referred to as autophagy) can be dissected into different steps based on the proteins involved, including induction, nucleation of the autophagosome precursor (termed the phagophore), membrane expansion and maturation of the autophagosome, fusion with the lysosome/vacuole, and recycling of the degraded cargo.

**Autophagy Induction in Yeast**

During growth in nutrient-rich conditions autophagy activity is kept to a minimum by different nutrient sensing pathways including those regulated by the target of rapamycin (TOR) kinase and cAMP-dependent protein kinase A. The ability of TOR to sense nutrient levels, in particular amino acids, makes it a critical negative regulator of autophagy. The rapamycin-sensitive TOR complex 1 (TORC1) inhibits autophagy in part by preventing the activation of the Atg1 kinase complex. In yeast, the Atg1 kinase complex is formed by the Ser/Thr kinase Atg1, the regulatory subunit protein Atg13, and the Atg17–Atg31–Atg29 complex, which is thought to function as a scaffold. Although specific substrates of Atg1 may remain to be discovered, the Atg1 kinase complex plays an essential role in autophagy induction by recruiting other Atg proteins to what is known as the phagophore assembly site (PAS), a perivacuolar location found in yeast that is proposed to be the organizing center for phagophore formation. Upon nutrient starvation or rapamycin treatment TORC1 activity is inhibited and Atg13 is rapidly but partially dephosphorylated leading to the activation of Atg1. Autophosphorylation of Atg1 within its activation loop is also important for activating its kinase activity and inducing autophagy (Figure 2A). Atg13 dephosphorylation was linked to an increased interaction with Atg1 leading to a model in which starvation increased affinity between the 2 proteins; however, recent data as well as interaction studies in other model organisms support the idea that Atg1 and Atg13 interact independently of nutrient conditions. In addition to Atg13, TORC1 may also inhibit autophagy by directly phosphorylating Atg1.

Protein kinase A is also a negative regulator of autophagy. Protein kinase A suppresses autophagy by phosphorylating both Atg1 and Atg13. Although the 2 pathways target similar proteins, TOR and protein kinase A seem to work largely independently of one another by targeting different phosphorylation sites. Other nutrient-sensing kinases involved in autophagy induction are Snf1 and Gcn2. Although the former corresponds to the yeast homolog of 5′-AMP–activated protein kinase (AMPK), which will be discussed below, Gcn2 promotes autophagy during amino acid starvation by phosphorylating Sui2/Elf2α (suppressor of initiator codon 2). Phosphorylation of Sui2 blocks general protein synthesis and specifically activates

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**Nonstandard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AMPK</td>
<td>5′-AMP–activated protein kinase</td>
</tr>
<tr>
<td>Atg</td>
<td>autophagy related</td>
</tr>
<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
</tr>
<tr>
<td>HF</td>
<td>heart failure</td>
</tr>
<tr>
<td>HFD</td>
<td>high-fat diet</td>
</tr>
<tr>
<td>I/R</td>
<td>ischemia/reperfusion</td>
</tr>
<tr>
<td>PAS</td>
<td>phagophore assembly site</td>
</tr>
<tr>
<td>PtdIns3K</td>
<td>phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>PtdIns3P</td>
<td>phosphatidylinositol 3-phosphate</td>
</tr>
<tr>
<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
</tr>
<tr>
<td>TOR</td>
<td>target of rapamycin</td>
</tr>
<tr>
<td>TORC1</td>
<td>TOR complex 1</td>
</tr>
<tr>
<td>ULK</td>
<td>unc-51 like autophagy activating kinase</td>
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**Figure 1. The 3 main types of autophagy.** A. Yeast cells carry out both macroautophagy and microautophagy. Although macroautophagy consists of the bulk degradation of cytoplasmic material that is sequestered inside double-membrane autophagosomes that then fuse with the vacuole, microautophagy works by directly taking up the substrates through invagination of the vacuole. B. In mammals along with microautophagy and macroautophagy, chaperone-mediated autophagy enables the degradation of specific protein substrates that contain a KFERQ motif that is recognized by chaperones that mediate the translocation of the unfolded protein into the lysosome through LAMP2A.
the translation of the transcription factor Gcn4, which in turn induces the transcription of various ATG genes.20,21

**Autophagy Induction in Mammals**

In contrast to yeast, mammalian cells have multiple Atg1 homologs, and the ones most relevant to autophagy are unc-51 like autophagy activating kinase 1 (ULK1) and ULK2.22,23 Thus, in mammals the Atg1 kinase complex is known as the ULK kinase complex and is formed by ULK1/2, the mammalian homolog of Atg13 (ATG13), the functional homolog of Atg17 (RB1CC1), and the ATG13-stabilizing protein ATG101, which has no yeast counterpart. All members of the ULK kinase complex are required for autophagy induction in mammalian cells.24–26 As mentioned above, in mammalian cells the interaction between the members of the ULK kinase complex does not depend on nutrient conditions.27 Similar to the yeast Atg1 complex, regulation of the ULK kinase complex depends on the mechanistic target of rapamycin complex 1 (MTORC1). During nutrient-rich conditions, MTORC1 interacts directly with ULK1 through the scaffold protein regulatory associated protein of MTOR, complex 1 (RPTOR) and inhibits its kinase activity by phosphorylating both ATG13 and ULK1/2.25,26 Upon nutrient starvation or rapamycin treatment, MTORC1 is released from the ULK kinase complex leading to the dephosphorylation of both proteins and the activation of ULK kinase activity.25,27,28 Once activated, ULK1 phosphorylates ATG13, RB1CC1, and itself, stabilizing its enzymatic activity and inducing the autophagic process (Figure 2B).25,28,29

Another protein capable of sensing energy levels that is involved in autophagy regulation is AMPK. Through the upstream kinase serine/threonine kinase 11 (STK11/LKB1), AMPK is able to sense decreases in the cellular ATP/AMP ratio leading to its activation and autophagy induction.30 During glucose deprivation AMPK phosphorylates and activates the tuberous sclerosis complex, tuberous sclerosis (TSC) 1-TSC2, which in turn inactivates the GTPase-activating protein Ras homolog enriched in brain (RHEB), leading to MTORC1 inhibition and the release of the ULK kinase complex (Figure 3).31,32 Once MTORC1 leaves the ULK complex, AMPK directly phosphorylates ULK1, stimulating its catalytic activity and inducing autophagy.30 Interestingly, the ULK kinase complex also phosphorylates and inactivates AMPK,
through a mechanism that has been described as an inhibitory feedback loop.33

Although functioning in part in a hormone-sensing pathway, AKT/PKB can also regulate autophagy by controlling mTORC1 activation. On ligand binding, dimerization, autophosphorylation, and activation of insulin receptor or insulin-like growth factor 1 receptor, the class I phosphoinositide 3-kinase is recruited to the plasma membrane and activated.34 Phosphoinositide 3-kinase catalyzes the phosphorylation of phosphatidylinositol(4,5)bisphosphate generating the lipid second messenger phosphatidylinositol(3,4,5)trisphosphate (PIP3), which in turn recruits AKT to the plasma membrane where it is activated via phosphorylation by PDK1 and mTORC2.34,35 AKT-dependent phosphorylation of TSC2 prevents RHEB inhibition, leading to mTORC1 activation and autophagy inhibition.36,37 As a consequence, the tumor suppressor and lipid phosphatase and tensin homolog (PTEN) can induce autophagy by dephosphorylating PIP3 and downregulating the AKT-phosphoinositide 3-kinase pathway (Figure 3).38

Membrane Nucleation and Source

Once autophagy is induced, assembly of the phagophore is initiated by membrane nucleation. As mentioned above, in yeast the PAS corresponds to the location at which several Atg proteins are recruited to assemble the phagophore. In contrast, mammalian cells lack a single defined PAS, and autophagosome formation seems to be initiated at different

Mammals

![Diagram of mammalian autophagy](http://circres.ahajournals.org/)

**Figure 4. Class III phosphatidylinositol 3-kinase (PtdIns3K) complexes.** Three class III PtdIns3K complexes can be observed in mammals. All of them require phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3/VPS34), phosphoinositide-3-kinase, regulatory subunit 4 (PIK3R4/VPS15) and beclin 1, autophagy related (BECN1). Specific subunits regulate the function of the different complexes. Binding of ATG14 and autophagy/beclin-1 regulator 1 (AMBRA1) leads to autophagy induction. UV radiation resistance associated (UVRAG) and SH3-domain GRB2-like endophilin B1 (SH3GLB1/Bif-1) promotes autophagosome formation.46,47 The complex formed by UVRAG and KIAA0226/RUBICON downregulates autophagy by impairing autophagosome maturation (Figure 4).44,45 Other BECN1-interacting proteins include the antiapoptotic protein B-cell CLL/lymphoma 2 (BCL2), which inhibits the PtdIns3K complex by sequestering BECN1 under

**Figure 5. Autophagosomes have a diverse range of potential membrane sources.** The trans-Golgi network, mitochondria, mitochondrial-associated membrane, and endoplasmic reticulum (ER) have been postulated as membrane donors. Omegasomes have been described as the ER structures that work as a platform for autophagosome formation. The phagophore (shown in red) elongates and engulfs part of a cisternae before it buds off the ER and becomes an autophagosome. MAM indicates mitochondria-associated ER membrane.
nutrient-rich conditions.\textsuperscript{58} Besides alterations in protein inter-
actions, BECN1 post-translational modifications also regulate
PtdIns3K activity. For example, BECN1 phosphorylation by
DAPK promotes dissociation of BCL2 and autophagy induc-
tion.\textsuperscript{53} ULK1-dependent phosphorylation of BECN1 activates
the ATG14- and UVRAG-containing PtdIns3K complexes in-
ducing autophagy during amino acid starvation.\textsuperscript{52} Activation
of both these PtdIns3K complexes by ULK1-mediated
BECN1 phosphorylation would argue for the importance of
this post-translational modification for autophagosome induc-
tion and later maturation and provides a link between the ULK
kinase initiation complex and the membrane nucleation com-
xplex. Most recently, AMPK was described as regulating the
activity of different PtdIns3K complexes by phosphorylating
BECN1 and PIK3C3.\textsuperscript{53}

Although membrane nucleation has been established as a
key step in the autophagic process, the origin of the mem-
brane that gives rise to the phagophore, and subsequently
the autophagosome, remains an open question. Different
studies have described the ER, mitochondria, plasma mem-
brane, and trans-Golgi network as possible membrane donors
(Figure 5). Evidence supporting the ER as a possible mem-
brane source include 3-dimensional tomography studies show-
ing a connection between the phagophore and ER, as well as
ATG14-containing PtdIns3K complex localization to the ER to
initiate autophagosome formation.\textsuperscript{48,57} Generation of PtdIns3P
at the ER triggers the recruitment of the PtdIns3P-binding pro-
tein ZFYVE1/DFCP1 (zinc finger, FYVE domain containing
1) and one of the mammalian homologs of Atg18, WD re-
peat domain, phosphoinositide interacting 2 (WIP2). Both of
these proteins have been linked to autophagosome formation
from a PtdIns3P-enriched ER-associated structure termed the
omegasome for its \(\Omega\)-like shape.\textsuperscript{58,59} Omegasomes have been
described as platforms for autophagosome formation, which
seem to depend on PtdIns3P, because ATG14 depletion leads
to omegasome disappearance.\textsuperscript{48} Although the role of ZFYVE1
in autophagy is not well defined, WIP2 silencing results in
accumulation of omegasome structures and failure to mature
into autophagosomes, suggesting that WIP2 is involved in the
transition between omegasomes and autophagosomes.\textsuperscript{59} Other
ATG proteins that have been associated with omegasomes in-
clude the ULK kinase complex, which localizes transiently to
omegasomes in a PtdIns3P-dependent manner.\textsuperscript{58,60} The precise
mechanism by which the ER gives rise to autophagosomes via
an omegasome intermediate is unknown, and several questions
remain to be answered about the conditions, specific proteins
involved, and the selectivity of the process (Figure 5).\textsuperscript{61}

As mentioned above, mitochondria are another organ-
elle that have been proposed as a membrane source for the
phagophore. During starvation conditions an outer mito-
ochondria membrane fluorescent marker colocalizes with au-
tophagosomes; mitochondrial lipids also seem to transit to
autophagosomes.\textsuperscript{54} The same study showed that autophago-
some formation during nutrient starvation is impaired in cells
lacking the ER-mitochondria tethering protein mitofusin 2.
Mitochondria-associated ER membrane, which are sites where
the mitochondria and ER are in close proximity to each other,
have been implicated in autophagosome formation. ATG14
and other autophagy markers localize to the mitochondria-as-
associated ER membrane during starvation conditions. In mito-
fusin 2-depleted cells, which are unable to tether the ER to the
mitochondria, ATG14 localization to the mitochondria-associated
ER membrane is impaired. In addition, the omegasome marker ZFYVE1 localizes to the mitochondria-associated
ER membrane upon starvation.\textsuperscript{53} This remarkable finding
opens the possibility that the functions of omegasomes and
mitochondria in autophagosome formation are essentially one
and the same, unified by the association between the 2.

Other studies on the transmembrane protein Atg9 have
advanced our understanding of the membrane source from
which phagophores are assembled. Atg9 has been character-
ized as a self-interacting protein containing 6 putative trans-
membrane domains, with both its carboxyl and amino termini
facing the cytosol.\textsuperscript{63,64} In yeast, Atg9 cycles from the PAS to
peripheral membranes; Atg9-containing vesicles are thought
to be part of the initial membranes that will generate the pha-
gophore.\textsuperscript{65,66} The Atg9-containing membrane reservoir seems
to be composed of tubules and vesicle clusters formed through
the ER-Golgi trafficking pathway;\textsuperscript{64} however, Atg9 also cycles
between perimitochondrial sites and the PAS.\textsuperscript{67} Although the
precise mechanisms by which Atg9 cycling is controlled
remains unknown, several Atg proteins are involved in the
regulation of Atg9 movement. Atg9 anterograde transport,
which is defined as movement from the peripheral sites to
the PAS, depends on Atg11, Atg23, and Atg27,\textsuperscript{11,68,69} whereas
retrograde transport, that is from the PAS to the peripheral
sites, is directed by the Atg1–Atg13 complex, Atg2, Atg18,
and the PtdIns3K complex.\textsuperscript{11} In mammalian cells, nutrient
starvation induces ATG9 redistribution from the trans-Golgi
network to phagophores. Both ULK1 silencing and PtdIns3K
inhibition block ATG9 trafficking to phagophores, suggesting
both complexes are involved in mammalian ATG9 cycling.\textsuperscript{60,70}
The MAPK pathway is also implicated in mammalian ATG9
traffic, SUPT20H/FAM48A/p38IP (suppressor of Ty 20 homolog
[Saccharomyces cerevisiae]) interacts with ATG9 and
induces its redistribution leading to autophagy activation.
Conversely, binding between SUPT20H and MAPK14/p38\(\alpha\)
inhibits ATG9 interaction with SUPT20H and autophagy.\textsuperscript{71}

**Phagophore Expansion**

Elongation and expansion of the phagophore membrane are
key steps in the autophagic process. The Atg12–Atg5–Atg16
and Atg8 conjugation systems, 2 inter-related ubiquitin-like
conjugation pathways, regulate this stage in both yeast and
mammals. Before being covalently linked to their final sub-
strates, both Atg12 and Atg8 go through an activation and
conjugation reaction, triggered by an E1-like and an E2-like
enzyme, respectively. In the Atg12–Atg5–Atg16 system, Atg12
is first activated in an ATP-dependent manner by the E1-like
activating enzyme Atg7, forming a thioester bond between the
2 proteins.\textsuperscript{72,73} After this event, Atg12 is transferred to the E2-
like conjugating enzyme Atg10, generating the Atg12–Atg10
intermediate through the formation of another thioester bond.\textsuperscript{74}
Finally, Atg12 is covalently attached to a specific lysine resi-
due on Atg5 in a process that, unlike ubiquitination, seems to
be constitutive, irreversible and does not require an E3-like

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**Figure 5.** Evidence supporting the ER as a possible membrane source include 3-dimensional tomography studies showing a connection between the phagophore and ER, as well as ATG14-containing PtdIns3K complex localization to the ER to initiate autophagosome formation. Generation of PtdIns3P at the ER triggers the recruitment of the PtdIns3P-binding protein ZFYVE1/DFCP1 (zinc finger, FYVE domain containing 1) and one of the mammalian homologs of Atg18, WD repeat domain, phosphoinositide interacting 2 (WIP2). Both of these proteins have been linked to autophagosome formation from a PtdIns3P-enriched ER-associated structure termed the omegasome for its \(\Omega\)-like shape. Omegasomes have been described as platforms for autophagosome formation, which seem to depend on PtdIns3P, because ATG14 depletion leads to omegasome disappearance. Although the role of ZFYVE1 in autophagy is not well defined, WIP2 silencing results in accumulation of omegasome structures and failure to mature into autophagosomes, suggesting that WIP2 is involved in the transition between omegasomes and autophagosomes. Other ATG proteins that have been associated with omegasomes include the ULK kinase complex, which localizes transiently to omegasomes in a PtdIns3P-dependent manner. The precise mechanism by which the ER gives rise to autophagosomes via an omegasome intermediate is unknown, and several questions remain to be answered about the conditions, specific proteins involved, and the selectivity of the process. As mentioned above, mitochondria are another organelle that have been proposed as a membrane source for the phagophore. During starvation conditions an outer mitochondria membrane fluorescent marker colocalizes with autophagosomes; mitochondrial lipids also seem to transit to autophagosomes. The same study showed that autophagosome formation during nutrient starvation is impaired in cells lacking the ER-mitochondria tethering protein mitofusin 2. Mitochondria-associated ER membrane, which are sites where the mitochondria and ER are in close proximity to each other, have been implicated in autophagosome formation. ATG14 and other autophagy markers localize to the mitochondria-associated ER membrane during starvation conditions.
Further interaction between Atg12–Atg5 and Atg16 leads to the formation of the Atg12–Atg5-Atg16 complex. Unlike Atg12, Atg16 is not covalently bound to Atg5 and is able to self-interact when bound to Atg12–Atg5 forming a large multimeric protein complex. The Atg12–Atg5-Atg16 complex is essential for autophagy and localizes to the phagophore. In yeast the second ubiquitin-like conjugation system catalyzes the lipidation of Atg8 by covalently linking it to phosphatidylethanolamine. The first event in this process corresponds to the cleavage of the carboxyl terminus of Atg8 by the cysteine protease Atg4, exposing a glycine residue. In the next step, Atg7, again working as an E1-like enzyme, activates Atg8. The activated protein is then conjugated to the E2-like enzyme Atg3, before finally being linked to phosphatidylethanolamine through an amide bond. Different studies have proposed that the E3-like enzyme that facilitates the Atg8–phosphatidylethanolamine linkage is the Atg12–Atg5-Atg16 complex. Atg8 lipidation is a reversible process because Atg8–phosphatidylethanolamine bound to the external autophagosome membrane can be cleaved by Atg4, releasing it from the autophagosome (Figure 6). Both ubiquitin-like conjugation pathways are conserved and work similarly between mammals and yeast with the specific difference being that mammalian cells have several Atg8 homologs further divided into the microtubule-associated protein 1 light chain 3 (MAP1LC3/LC3) and GABA(A) receptor-associated protein (GABARAP) subfamilies. Although all of the homologs go through a similar conjugation process, each subfamily works at different stages of autophagy. The LC3 subfamily is involved in expansion of the phagophore and the GABARAP subfamily participates at a later stage in autophagosome maturation.

Lysosome/Vacuole Fusion and Recycling of the Degraded Cargo

The fusion of autophagosomes with lysosomes/vacuoles results in the generation of autolysosomes in higher eukaryotes and autophagic bodies in yeast. In either case, the fusion process seems to involve similar machinery that plays a role in other transport processes that terminate at these degradative organelles. In yeast, this machinery includes the class C Vps/HOPS complex, the SNARE family proteins Ykt6, Vti1, Vam3, and Vam7, the small GTPase Ypt7, and the proteins Mon1 and Ccz1. Fewer details are known in mammalian cells; however, the Ypt7 homolog RAB7 is required. One difference between yeast and mammals is that there is a clear convergence between autophagy and the endocytic pathway in the latter; autophagosomes can fuse with endosomes to form amphisomes that subsequently fuse with the lysosome. Once fusion occurs, the inner autophagosomal membrane and its cargo are degraded inside the lysosome/vacuole by various hydrolases. The resulting macromolecules such as amino acids that are obtained after cargo degradation are transported back into the cytoplasm for recycling. In yeast this process is regulated by protein permeases such as Atg22.

Autophagy in the Cardiovascular System

According to the World Health Organization, cardiovascular disease is the leading cause of mortality in the globalized world, accounting for 30% of all deaths. As expected, considerable resources have gone toward understanding the nature of cardiovascular disease and to search for possible therapeutic targets.

Figure 6. Two ubiquitin-like conjugation systems. Autophagy related (Atg) 8 and Atg12 go through subsequent activation, mediated by Atg7 and conjugation mediated by Atg3 and Atg10, respectively, before covalently binding to phosphatidylethanolamine (PE) in the case of Atg8, and Atg5 in the case of Atg12. Atg8–PE binds both the inner and outer membrane of the autophagosome, but can be deconjugated by Atg4, the same protein that removes the C-terminal arginine initially present at the Atg8 C terminus. Atg12–Atg5 bind Atg16 creating a large multimeric complex that localizes to the phagophore and enhances Atg8 lipidation and membrane expansion. GR indicates glycine-arginine.

Figure 7. Autophagy regulators in cardiomyocytes. A wide variety of stimuli can regulate autophagy in cardiomyocytes. Some of them are autophagy activators and are associated with cardiovascular diseases. However, acetylcholine, catecholamines, aging, insulin-like growth factor 1 (IGF1), and insulin (INS) are capable of inhibiting autophagy. INS and IGF1 are well-known cardioprotective agents. AchR indicates acetylcholine receptor; AGT II, angiotensin II receptor; AGTR, angiotensin II receptor, type 1; IGF1R, IGF1 receptor; INSR1, INS receptor 1; and PKA, protein kinase A.

Vps/HOPS complex, the SNARE family proteins Ykt6, Vti1, Vam3, and Vam7, the small GTPase Ypt7, and the proteins Mon1 and Ccz1. Fewer details are known in mammalian cells; however, the Ypt7 homolog RAB7 is required. One difference between yeast and mammals is that there is a clear convergence between autophagy and the endocytic pathway in the latter; autophagosomes can fuse with endosomes to form amphisomes that subsequently fuse with the lysosome. Once fusion occurs, the inner autophagosomal membrane and its cargo are degraded inside the lysosome/vacuole by various hydrolases. The resulting macromolecules such as amino acids that are obtained after cargo degradation are transported back into the cytoplasm for recycling. In yeast this process is regulated by protein permeases such as Atg22.
Autophagy has been described widely in the cardiovascular system, and our understanding of the molecular machinery as described above provides the possibility for specific therapeutic intervention in treating cardiovascular disease. Autophagic activity is linked to cardiovascular development, preserving heart and vascular homeostasis, as well as in the onset and progression of several cardiovascular diseases. Interestingly, whether autophagy plays a survival role or has a deleterious effect during heart disease it is still a matter of discussion.

**Autophagy and Cardiovascular Development**

Autophagy plays a role in the regulation of mammalian cardiac development starting at an early stage. Morpholino knockdown of atg5, atg7, and becn1 result in abnormal heart structure, including defects in cardiac looping, abnormal chamber morphology, and aberrant valve development in zebrafish. Similarly, Atg5 knockout mice display defects in heart valve development and chamber septation indicating that autophagy regulates cardiac progenitor cell differentiation and is involved in heart development.

**Autophagy in the Genesis of Cardiovascular Diseases**

Several risk factors underlie the genesis and progression of cardiovascular diseases. These factors include age, tobacco, unhealthy diet, insufficient physical activity, excessive weight/obesity, hypertension, diabetes mellitus, and hyperlipidemia/hypercholesterolemia. Hypertension is one of the largest contributors to the worldwide burden of cardiovascular diseases, and its prevalence is close to 30% in the world population. The main mechanisms involved in the regulation of blood pressure are the sympathetic, parasympathetic, renin–angiotensin–aldosterone and anti-diuretic hormone systems. Dysregulation of these systems, as well as obesity and diabetes mellitus, is associated with the genesis and development of hypertension. Because most of the peptides and hormones belonging to these systems are capable of regulating autophagy, it is possible to speculate that dysregulation of autophagy could be associated with hypertension, obesity, diabetes mellitus, and end organ damage (Figure 7).

**Sympathetic and Renin–Angiotensin–Aldosterone Systems**

Although there is not a lot of information on the parasympathetic system and autophagy, studies involving the sympathetic system have shown that autophagy protects cells against excessive β-adrenergic stimulation. Regarding the renin–angiotensin–aldosterone system, autophagy is stimulated by angiotensin II via the angiotensin II receptor, type 1 (AGTR1) but diminished via AGTR2 in neonatal rat cardiomyocytes overexpressing AGTR1, AGTR2, or both receptors after adeno viral transduction. Cardiomyocytes derived from a genetic rat model of heart hypertrophy are more susceptible to AGTR1-induced autophagy, but also show a strong reduction of autophagy via AGTR2. Therefore, besides their well-known favorable hemodynamic and neurohumoral effects in the treatment of heart failure (HF), AGTR1 antagonists may also downregulate excessive autophagic cell death and consequently preserve cardiomyocytes.

**Obesity**

Autophagy is upregulated in adipose tissue of obese patients, correlating with the degree of obesity, visceral fat distribution, and adipocyte hypertrophy. Xu et al. showed that the autophagosome maturation process is involved in high-fat diet (HFD)–induced and AKT2 knockout–induced cardiac hypertrophy and contractile dysfunction. In the heart, HFD promotes the initiation and accumulation of autophagy, although it disrupts autophagosome maturation probably at the step of autophagosome–lysosome fusion. However, although AKT2 knockout does not affect the initiation of autophagy by HFD, it rescues HFD-induced disruption of the autophagosome maturation process and facilitates the transition from autophagosomes to autolysosomes, indicating a cardioprotective effect of cardiac autophagy in the presence of a HFD.

**Diabetes Mellitus**

On insulin resistance, pancreatic β cells enhance their insulin secretion to compensate for hyperglycemia. However, the progressive diminution of the number of pancreatic β cells, mainly because of apoptosis, and the decrease of their function lead to pathological myocardial remodeling. Along these lines, cells from human patients with T2DM may display elevated levels of autophagy and increased cell death; however, it is not possible to verify in these cells that autophagy is the cause of cell death.

**Autophagy in Cardiovascular Diseases**

In the heart, isolated cardiomyocytes, vascular epithelial cells, and vascular smooth muscle cells, autophagy is strongly induced by physiological conditions, such as nutrient starvation. In those conditions, autophagy is important for the turnover of organelles and protein aggregate degradation at low basal levels under normal conditions. During conditions of cardiovascular stress, including ischemia/reperfusion (I/R) and HF, among others, autophagy is also activated. However, whether autophagy in these contexts is beneficial or detrimental is not well defined.

**Ischemia/Reperfusion**

During ischemia, autophagy is triggered as an adaptive mechanism providing nutrients and eliminating damaged mitochondria. The selective autophagic removal of dysfunctional
organelles can be a critical aspect of maintaining cellular homeostasis. For example, damaged mitochondria can result in the production of excess reactive oxygen species that can further harm organelles or cause DNA damage, leading to apoptosis. During mild ischemic stress, autophagy activation depends on AMPK-mediated inhibition of MTOR.\textsuperscript{140,141} Pharmacological inhibition of autophagy increases cardiomyocyte death, indicating that autophagy functions as a pro-survival mechanism.\textsuperscript{142} During chronic ischemia, autophagy can inhibit apoptosis and diminish tissue damage.\textsuperscript{143} During reperfusion, cardiomyocyte autophagy is upregulated dramatically in rat,\textsuperscript{144} rabbit,\textsuperscript{145} and swine\textsuperscript{146} hearts, and primary neonatal cardiomyocytes.\textsuperscript{140,142} Cardiac autophagy triggered by reperfusion can be either adaptive or detrimental and involves BECN1 activation.\textsuperscript{148} In cultured neonatal cardiomyocytes exposed to simulated I/R, inhibition of autophagy with 3-methyladenine enhances cell viability.\textsuperscript{142} In contrast, other studies describe protective actions of autophagy in simulated I/R.\textsuperscript{146,147} One possible explanation to these discrepancies is the dual role of BECN1 during I/R. While BECN1 is critical in the initial stage of autophagy induction, excessive amounts of this protein during reperfusion may result in an autophagic imbalance that leads to cell death.\textsuperscript{148} providing another example of the dual relationship between autophagy and cardiovascular physiology.\textsuperscript{149}

**Heart Failure**

The initial response of the heart to several stresses is hypertrophy.\textsuperscript{110} If the stresses persist, a pathological hypertrophy is developed followed by heart failure,\textsuperscript{150} and there is some indication that autophagy plays a role in the progression of structural remodeling that leads to HF in the pressure-overloaded human heart.\textsuperscript{151} Cardiomyocyte-specific overexpression of BECN1 amplifies the pathological remodeling response.\textsuperscript{151,152} Conversely, \textit{Becn1} haploinsufficiency partially rescues HF.\textsuperscript{151} Collectively, these data provide yet another example of autophagy having a maladaptive role in cardiovascular disease. Analysis of human samples has supported additional evidence that autophagic cell death contributes to HF pathogenesis\textsuperscript{153,154}; however, it is important to keep in mind that autophagy is fundamentally a cytoprotective response, and a complete block in this process generally has severe negative consequences. For example, in adult mice acute cardiac-specific \textit{Atg5} deficiency leads to cardiomyopathy,\textsuperscript{115} consistent with the notion that basal levels of cardiomyocyte autophagy are required for cellular proteostasis. Thus, whether elevated autophagy observed in human failing heart promotes cell death and contributes to the progression of HF, or if it represents an adaptive response of the heart to promote survival remains to be elucidated. Accordingly, with regard to therapeutic intervention, we propose that there is an optimal zone of cardiomyocyte autophagy that may be beneficial, and that treatments resulting in either decreased or elevated levels of autophagy are likely to be deleterious (Figure 8).\textsuperscript{108}

**Conclusions and Perspectives**

As one of the major catabolic pathways in the cell, autophagy has become an important focus of research in a diverse range of biological fields. Autophagy is essential in normal cardiovascular homeostasis. However, alterations in autophagic flux are seen in all forms of cardiovascular diseases. The relationship of autophagy to cardiovascular physiology is complex and can be either beneficial or harmful, depending on the timing and magnitude. Despite all knowledge accumulated to date, a substantial amount of work must be done to clarify the real contribution of autophagy in cardiovascular systems. This knowledge will not only help develop our basic understanding on how and to what end autophagy is activated but may also give rise to potential drug treatment for several diseases. To this end, we must advance our current comprehension of the autophagic pathway and its various modulators. We are optimistic that in the near future pharmacological targeting of autophagy will emerge as a therapeutic alternative to treat cardiovascular diseases.

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**Disclosures**

None.
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For the *Circulation Research* article by Gatica et al (Molecular Mechanisms of Autophagy in the Cardiovascular System. *Circ Res.* 2015;116:456-467. doi: 10.1161/CIRCRESAHA.114.303788.), it was brought to our attention that a few sections of the text of the article were very similar to passages in a review article simultaneously published elsewhere by some of the same authors. Those passages have now been significantly revised, and this revised version of the article is available at http://circres.ahajournals.org/content/116/3/456.full. The authors apologize for this oversight.