The incidence of cardiovascular disease progressively increases with age in both men (from 10 per 1000 people at 45–54 years of age to 74 per 1000 people at 85–94 years of age) and women (from 4 to 65 per 1000 for the comparable age groups).1 Despite recent progress in cardiovascular treatment, the total prevalence of age-related diseases, including cardiovascular diseases, is still increasing.2 High blood pressure, obesity, and metabolic syndrome increase with age, and these conditions facilitate the development of heart disease, which is a major cause of chronic disability, morbidity, and mortality in the elderly.3,4

Examination of the cell biology of the aging process has revealed that it is often associated with dysfunctional organelles, which trigger oxidative stress, protein misfolding, and cell death and induce precipitous declines in the maintenance of cellular quality control mechanisms.5–7 In particular, an important pathological feature of aging is the development of mitochondrial abnormalities, where reactive oxygen species (ROS) progressively accumulate during aging as a result of damage to mitochondrial proteins, an imbalance between oxidative stress and antioxidant mechanisms and increases in electron leakage from dysfunctional electron transport.
Aging cardiomyocytes are less capable of undergoing compensatory hypertrophy and proliferation in response to increased workload.\textsuperscript{22,32–35} As a result, aging increases wall stress that is not normalized by ventricular remodeling.\textsuperscript{16} Furthermore, induction of cell protective mechanisms, such as expression of antioxidant and heat shock proteins, in response to pathological insults is attenuated in aging hearts.\textsuperscript{33,37,38} Optimal therapeutic interventions to alleviate the adverse effects of aging should prevent cell death and accumulation of senescent myocytes.\textsuperscript{22} For example, A2E, a major component of toxic lipofuscin, upregulates inflammatory cytokines and chemokines and contributes to the inflammatory response in aging tissues, but activation of autophagy seems to protect the tissue by eliminating both toxic aggregates and activation of inflammation.\textsuperscript{39} As we discuss later, however, autophagy and autophagic flux are generally downregulated in the aging heart.

**Molecular Mechanisms of Aging in the Heart**

Because aging increases the risk of diseases and reduces organ function, elucidation of the mechanisms that serve to counteract the adverse effects of aging has significant clinical implications.\textsuperscript{40} Evolutionarily conserved defined molecular mechanisms are involved in the regulation of lifespan in animals,\textsuperscript{40–42} and activation of these mechanisms may affect the aging of individual organs and the cells therein. Aging is a multifunctional process, with many mechanisms contributing to functional decline in organs and tissues. Accumulation of damaged proteins and mitochondria is commonly observed in aged cells. In addition, production of ROS in various organs is progressively enhanced over the years, and oxidative stress accumulates during the aging process.\textsuperscript{10,12} Likewise, increased ROS and accumulation of damaged proteins and organelles in the heart also occur in the presence of high blood pressure and metabolic abnormality, which facilitate senescence in the heart.\textsuperscript{44,45}

ROS progressively accumulate during aging, because of both electron leakage from mitochondria resulting from impaired mitochondrial oxidative phosphorylation and an imbalance between the expression of ROS-producing enzymes and antioxidant proteins.\textsuperscript{10,46} The accumulated ROS further promote the mtDNA mutations and deletions that accumulate over time, leading to a progressive reduction in mtDNA content.\textsuperscript{47} ROS can also impair the enzymes involved in oxidative phosphorylation, particularly those containing an iron–sulfur cluster.\textsuperscript{48} Finally, oxidative stress is associated with mitochondrial permeability transition pore opening, which promotes necrosis and cell death in mammalian cells.\textsuperscript{49} Mitochondrial dysfunction is a common feature of the aging process,\textsuperscript{50} and systemic mitochondrial overexpression of catalase extends lifespan and delays the aging process in mice.\textsuperscript{51} These observations suggest that the accumulation of ROS in mitochondria contributes to the aging process in mammals.\textsuperscript{52}

In addition to electron leakage from the electron transport chain, mitochondrial expression of Nox4, a major intracellular isoform of nicotinamide adenine dinucleotide phosphate oxidase in the heart, is upregulated in the aged heart, contributing to ROS production and the development of cardiac abnormalities in aging mice.\textsuperscript{53} Nox4 is upregulated by pressure overload through an nuclear factor-κB (NF-κB)–dependent

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### Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Atg</td>
<td>autophagy-related genes</td>
</tr>
<tr>
<td>CMA</td>
<td>chaperone-mediated autophagy</td>
</tr>
<tr>
<td>CR</td>
<td>caloric restriction</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FoxO</td>
<td>Forkhead box O</td>
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<tr>
<td>IGF-I</td>
<td>insulin-like growth factor I</td>
</tr>
<tr>
<td>mtDNA</td>
<td>mitochondrial DNA</td>
</tr>
<tr>
<td>mTOR</td>
<td>mammalian target of rapamycin</td>
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<tr>
<td>mTORC1</td>
<td>mTOR complex 1</td>
</tr>
<tr>
<td>NAD+</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>Nampt</td>
<td>nicotinamide phosphoribosyl transferase</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>UPR\textsuperscript{e}</td>
<td>mitochondrial unfolded protein response</td>
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**Manifestation of Aging in the Heart**

Aging hearts exhibit unique histological and biochemical features.\textsuperscript{22–25} Increases in apoptosis and necrosis, proliferation of myocyte nuclei, increased myocyte volume, and connective tissue accumulation are frequently observed in the myocardium of old animals.\textsuperscript{26–28} Senescence-associated ectopic β-galactosidase activity and dense bodies with autofluorescence, consisting of damaged proteins and lipid accumulates, called lipofuscin,\textsuperscript{29} are also increased. In addition, aging affects the abundance of some molecules, including the tumor suppressors p16\textsuperscript{INK4a} and p19\textsuperscript{ARF}.\textsuperscript{30,31} How do these changes affect cardiac function and susceptibility to heart failure in the elderly?
mechanism, but the mechanism by which Nox4 is upregulated in the aging heart is currently unknown. It would be interesting to test whether the increased susceptibility of aging hearts to stress is alleviated in cardiac-specific Nox4 knockout mice. However, it should be noted that endogenous Nox4 also possesses physiological functions, such as induction of angiogenesis and autophagy in response to glucose deprivation in cardiomyocytes. Thus, further investigation is required to fully elucidate the role of ROS in the overall responses of the heart during aging.

Mitochondrial stress triggers a series of events, including damage to proteins and mtDNA, activation of mitochondrial mechanisms of degradation of both individual proteins and partial or whole organelles, and mitochondrial biogenesis, thereby causing a condition in which misfolded proteins accumulate or there is a mismatch between mtDNA-encoded proteins and nuclear-encoded proteins. This condition activates a set of adaptive responses collectively termed the mitochondrial unfolded protein response (UPRmt), including the activation of chaperones and other mitochondrial quality control mechanisms such as mitophagy. The UPRmt is essential for long-term maintenance of mitochondrial quality and affects many mechanisms known to influence either lifespan or aging. Although the UPRmt is evolutionarily conserved and has been studied extensively in Caenorhabditis elegans, the detailed signaling mechanisms found in mammalian cells are poorly understood. Elucidating the molecular mechanisms by which the UPRmt maintains mitochondrial homeostasis and whether the UPRmt acts through autophagy and mitophagy in mammalian cardiomyocytes would be interesting and should provide clues for the development of novel interventions to achieve antisenescence therapy in the heart. It should be noted that endoplasmic reticulum (ER) stress also induces autophagy and a UPR to eliminate misfolded proteins and defective organelles. However, the connection between the ER stress and aging is, to date, less well defined than that between the UPRmt and aging.

In oxidative stress induce DNA damage, which, in turn, either induces a series of DNA repair mechanisms or activates either cell death or cellular senescence mechanisms. Increases in DNA damage and defective DNA repair have been shown to inhibit autophagy. Somatic mtDNA mutation inhibits autophagy, thereby allowing mutated mtDNA to escape degradation and promote mitochondrial dysfunction. When DNA damage induces senescence, it is accompanied by a unique phenotype, called the senescence-associated secretory phenotype, that mediates chronic inflammation, thereby further promoting DNA damage and senescence. DNA damage-induced cellular senescence stabilizes GATA4, thereby inducing senescence-associated secretory phenotype through the activation of NF-κB. Interestingly, p62-mediated selective autophagy degrades GATA4, whereas GATA4 is stabilized in human fetal lung fibroblasts undergoing senescence because of suppression of autophagy. These observations suggest that autophagy may inhibit the adverse effects of aging by negatively regulating senescence-associated secretory phenotype and aging-associated inflammation.

Telomeres are regions of repetitive nucleotide sequences located at the ends of chromosomes that protect the chromosomal end structures from damage. Each time cells divide, the telomere ends become shorter. Because telomere shortening causes senescence through the activation of the p53 and Rb pathways, the molecular mechanism controlling telomere length is intimately connected to aging. Although cardiomyocytes in the adult heart rarely proliferate, telomere damage is also caused by oxidative stress, which, in turn, activates signaling mechanisms leading to senescence. Importantly, the health of the heart is dependent on basal turnover of cardiomyocytes generated from cardiac progenitor cells. Telomere attrition and DNA damage induce senescence in cardiac progenitors, resulting in a loss of regenerative capacity and promotion of cardiac senescence. It has recently been shown that quiescent satellite cells in skeletal muscles are capable of maintaining their stemness by activating autophagy. However, autophagy is attenuated in aged satellite cells, leading to stimulation of senescence through accumulation of oxidative stress and damaged proteins and organelles.

Another important mechanism regulating senescence is protein acetylation, which is regulated by a balance between protein acetylases and deacetylases. Spermidine, an acetyltransferase inhibitor, prolongs the lifespan of yeast, flies, and nematodes in an autophagy-dependent fashion, by inhibiting protein acetylation of cytoplasmic and nuclear proteins. In addition, the sirtuin family of protein deacetylases is particularly relevant in the regulation of lifespan and aging. Sirtuins are a family of NAD+-dependent protein deacetylases known to be involved throughout the evolutionary tree in lifespan extension in response to caloric restriction (CR) and resveratrol, a naturally occurring polyphenol that stimulates Sirt1. The Sirt1 isoform acts in both the nucleus and the cytoplasm, and its expression increases in the aging heart, most likely as a compensatory mechanism. Sirt1 can retard senescence and confer resistance to oxidative stress in the heart and promotes autophagy through deacetylation and stimulation of Forkhead box O (FoxO) 1 in cardiomyocytes. Sirt1 negatively regulates p66shc expression in human umbilical vein endothelial cells. p66shc is a multifunctional adapter protein and its deletion prolongs lifespan in mice. p66shc positively regulates oxidative stress by stimulating mitochondrial oxidative phosphorylation and downregulating FoxO-mediated protection against oxidative stress. Sirt3, a mitochondrial sirtuin isoform, can stimulate oxidative phosphorylation by directly deacetylating the electron transport chain complexes. Sirt3 also inhibits pathological cardiac hypertrophy by deacetylating cyclophilin D, a protein that regulates mitochondrial permeability transition pore opening and prevents the adverse effects of aging in the heart.

Mammalian target of rapamycin (mTOR), a serine threonine kinase that regulates protein synthesis, transcription of mitochondrial proteins and autophagy, has also been implicated in the regulation of lifespan and aging. mTOR suppresses autophagy through phosphorylation of Unc-51-like autophagy activating kinase 1 (Ulk1), primarily through the activation of mTOR complex 1 (mTORC1). mTOR has drawn particular attention in the area of aging because the mTOR inhibitor rapamycin has been shown to increase lifespan in mammals. A recent report showed that the activity of mTOR does not increase with age in C57BL/6J mice. Although these findings suggest that endogenous mTOR may not be directly involved
Role of Autophagy During Aging of the Heart

Damage to proteins, DNA, and cellular organelles plays an important role in aging. Accumulation of damaged proteins and organelles leads to the age-associated malfunction of many biological processes. Thus, reduced degradation of proteins and organelles may contribute to the aging process.

In cardiomyocytes, the accumulation of dysfunctional organelles and toxic proteins results in global cardiac dysfunction. Because autophagy plays a crucial role in the degradation of long-lived proteins and organelles, it is an essential mechanism for maintenance of tissue homeostasis in the heart during the aging process. Three types of autophagy have been identified: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy.

In microautophagy, cytoplasmic cargo is directly trapped and engulfed through membrane invagination by lysosomes. Although it is thought to be a random process, recent evidence suggests that parts of mitochondria are also degraded through this mechanism. CMA, on the contrary, specifically degrades cytosolic proteins with a KFERQ motif, by directly transferring them to lysosomes. In macroautophagy, hereafter referred to as autophagy, small vesicular sacs, called isolation membranes or phagophores, are initially formed. The phagophores enclose cytosolic long-lived proteins and organelles, resulting in the formation of double-membrane structures called autophagosomes. The autophagosomes then fuse with lysosomes, which leads to the degradation of the sequestered cellular contents through digestion of the cargo by lysosomal hydrolases. Essentially nothing is known about the role of microautophagy and CMA during aging in the heart. Thus, we focus on macroautophagy in the discussion below.

Autophagy Is Downregulated During the Course of Aging

Autophagy is downregulated in the heart during the course of aging. The general mechanisms of aging described above may also contribute to this downregulation of autophagy (Figure 1). Seemingly paradoxically, although ROS and misfolded proteins can stimulate autophagy, suppression of autophagy is commonly observed in aging hearts with increased oxidative stress, misfolded proteins, and dysfunctional mitochondria. One possible explanation for this apparent paradox is that continuous activation of autophagy caused by higher oxidative stress and protein misfolding may lead to exhaustion of the autophagic machinery, eventually causing suppression of autophagy. A similar phenomenon, termed autophagy exhaustion, was reported to have been observed after HIV-1 infection. Aging is also characterized by attenuation of stress-induced adaptations, which may result, in part, from the suppression of autophagy. In particular, activation of autophagy is required to potentiate the protective effect of ischemic preconditioning, raising the possibility that autophagy exhaustion may contribute to the increased susceptibility of elderly patients to myocardial ischemia. Whether autophagy exhaustion actually takes place in aging hearts remains to be tested.

Activation of major suppressors of autophagy, such as Mst1 and mTOR, or suppression of major activators of autophagy, such as Sirt1, may also cause downregulation of autophagy in the heart. Further experimentation is required to test these hypotheses.

In addition, a variety of other mechanisms have been shown to contribute to the age-dependent downregulation of autophagy. The genes involved in general and mitochondria-specific autophagy are regulated by transcription factors, including FoxO, HIF-1 (hypoxia inducible factor-1), p53, E2F1 (E2F transcription factor 1), NF-κB, KLF4 (Kruppel-like factor 4), TFEB (transcription factor EB), and ZKSCAN3 (zinc finger with KRAB and SCAN domains). The transcriptional activity of FoxO is negatively regulated by Akt-mediated phosphorylation and by lysine acetylation resulting from decreases in NAD+ and consequent inactivation of Sirt1 in the heart. This, in turn, may contribute to decreased expression of autophagy-related genes in the aged heart. Aging has also been shown to upregulate miR-216a, which, in turn, downregulates Beclin1, thereby inhibiting oxidized low-density lipoprotein–induced stimulation of autophagy in endothelial cells. In contrast, atrogin-1, an E3 ubiquitin ligase known to be activated in response to muscle atrophy, induces autophagy through degradation of charged multivesicular body protein 2B, a component of the endosomal sorting protein complex that is essential for autophagy. However, although the activity of the ubiquitin proteasome system declines with aging, whether atrogin-1 is downregulated in aging hearts is unknown. Finally, saturated and unsaturated fatty acids induce metabolic profiles that differentially affect aging through the activation of distinct forms of autophagy in the liver: the former activates PI3K- and Beclin1-dependent autophagy by depleting autophagy-inhibitory amino acids, whereas the
latter activates PIK3C3- and Beclin1-independent autophagy by increasing the level of NAD+. Whereas the effects of saturated fatty acids on health are detrimental, those of unsaturated fatty acids are beneficial.105 However, how the metabolic perturbation observed in age-associated heart disease affects metabolomic profiles and autophagy remains to be elucidated.

**Autophagy Is Essential for Preventing Aging in the Heart**

Increasing lines of evidence suggest that autophagy is intimately involved in the regulation of lifespan and aging.21 Loss-of-function mutations in autophagy-related genes, Atg1, Atg7, Atg18, and Beclin1, key autophagy genes, decrease the lifespan of the nematode *C. elegans*.106 Similarly, in a genetic screen of budding yeast, several short-lived mutants had autophagy defects, including 10 ATG mutations found among a total of 117 short-lived mutants.107 Deficient expression of Atg1 facilitates accumulation of ROS and muscle degeneration, mimicking the aging phenotype, in the fruit fly *Drosophila melanogaster*.108 Mutations in Atg8 also induce accumulation of insoluble proteins, increase sensitivity to ROS, and shorten lifespan in *Drosophila*, whereas increased expression of Atg8 in the nervous system of *Drosophila* extends its lifespan.109 Autophagy-related genes have also been shown to promote survival in worms and flies exposed to prolonged starvation,109 by alleviating age-associated pathologies, including mitochondrial and cardiac dysfunction.108

In mammals, tissue-specific knockout of ATG genes induces multiple age-associated symptoms, including accumulation of intracellular inclusion bodies containing ubiquitinylated proteins, accumulation of lysosomes containing lipofuscin, disorganized mitochondria, and protein oxidation.111–115 In contrast, adenovirus-directed overexpression of Atg7 that corrected a hepatic autophagic defect was shown to diminish ER stress and counteract insulin resistance.115 Stimulation of autophagy reduces oncogenesis, maintains neuronal function,116 improves immune responses,117 reduces inflammation,118 and improves lipid mobilization119 in the organism as a whole, thereby improving overall fitness during aging.16,21 However, more studies may be needed to clarify whether the lifespan of mice can be prolonged by selectively correcting aging-associated defects in autophagy.

Autophagy may be especially important in nonproliferating cells because there is no dilution of toxic materials accumulated during aging through cell division. In terms of cardiac aging, mice in which the Atg5 protein is cardiac-specifically deleted through constitutive αMHC-Cre expression develop dilated cardiomyopathy with severe systolic dysfunction during aging, accompanied by sarcomeric disarray and accumulation of dysfunctional and abnormal mitochondria.96 Cardiac-specific deletion of glycogen synthase kinase (GSK)-3α also promoted the development of cardiac aging, and this was accompanied by the suppression of autophagy.120 One cautionary note is that the use of αMHC-Cre, originally generated by Dr Michael Schneider’s laboratory for the use in aging studies, may potentially be problematic because cardiac-specific Cre alone induces significant time-dependent cardiac dysfunction.121 Nevertheless, these results strongly

![Figure 1. Regulation of cardiac aging by autophagy](http://circres.ahajournals.org/). Aging inhibits autophagy in cardiomyocytes through multiple mechanisms. Aging-induced suppression of autophagy induces accumulation of misfolded proteins and dysfunctional organelles, sterile infection caused by undigested mitochondrial DNA, inflammation, and lipotoxicity, thereby leading to a metabolically unhealthy environment, precipitous mitochondrial dysfunction and eventual cell death. FoxO indicates forkhead box O; mTOR, mammalian target of rapamycin; ROS, reactive oxygen species; and UPR, unfolded protein response.)
suggest that autophagy is required for cardiac homeostasis during aging and that downregulation of autophagy contributes to cardiac pathology during aging. Indeed, a genome-wide association study of aging identified a single nucleotide polymorphism near the Atg4c gene as being associated with a higher risk of death, suggesting that autophagy may be intimately involved in the risk of heart disease in elderly patients. Furthermore, autophagy also alleviates accumulation of advanced glycation end products and preamyloid oligomers. However, determining whether downregulation of autophagy mechanistically contributes to the development of cardiac aging awaits further experimentation to test whether restoring the level of autophagy rescues the aging phenotype.

The lysosome-associated membrane protein 2a, a protein required for CMA, is also downregulated during the aging process in the mouse liver. Restoration of lysosome-associated membrane protein 2a prevents aging-associated defects in CMA and decreases the abundance of oxidized proteins, polyubiquitinated protein aggregates, and apoptotic cells in the liver. The role of CMA in cardiac aging remains to be elucidated.

Role of Mitophagy

Damaged mitochondria can be eliminated through a specialized form of autophagy termed mitophagy or mitochondrial autophagy. Autophagosomes containing only mitochondria have been observed in electron microscopic analyses of the hearts of adult mice, providing evidence for the existence of mitophagy in the heart. The molecular mechanisms mediating mitochondrial autophagy and its functional significance in the heart have been reviewed recently. In perhaps the most well-characterized mechanism of mitochondrial autophagy, depolarized mitochondria are marked by a PTEN-induced putative kinase 1 (PINK1)-Parkin-mitofusin 2 (Mfn2)–dependent mechanism and engulfed by autophagosomes through an LC3–receptor–dependent mechanism. A recent report showed that PINK1-induced phosphorylation of ubiquitin recruits LC3 receptor proteins, including NDP52 and optineurin, which in turn recruit the autophagy factors, including Ulk1, DFCP1 (double FYVE domain–containing protein 1), and WIP1 (WD repeat domain, phosphoinositide interacting 1), to mitochondria. However, more investigation is needed to fully elucidate how depolarized mitochondria are marked and recognized by LC3 and the involvement of Parkin-Mfn2 in this process. On the contrary, increasing lines of evidence suggest that mitochondrial autophagy can take place in a Parkin-independent manner or even in the absence of Atg5/7, suggesting that there are multiple mechanisms by which mitochondria may be degraded, perhaps in a stimulus-dependent fashion. In addition, mitochondria can be degraded by microautophagy and mitochondria-derived vesicles. Given that mitochondrial dysfunction can develop with aging and that mitochondria are the major source of ROS in aging hearts, it is critically important to understand how mitochondrial autophagy is regulated in aging hearts (Figure 2).

It should be noted that there have been no studies quantitatively evaluating the changes in mitophagy during cardiac aging to date. Because mitochondrial dysfunction is a common feature of cardiac aging, in theory, more mitophagy and mitochondrial autophagy should be observed, at least in the early phase of aging. In C. elegans, age-associated stress such as oxidative stress stimulates both mitochondrial biogenesis and mitophagy through SKN-1 (skinhead-1), a homolog of Nrf2 (nuclear factor [erythroid-derived 2]-like 2), thereby coordinating mitochondrial turnover. As noted above, to date, most studies of mitophagy in the

Figure 2. Mechanisms of mitochondrial autophagy in the heart. Molecular mechanisms mediating mitochondrial degradation are summarized. The contribution of each mechanism to the regulation of cardiac senescence at baseline and under stress remains to be elucidated. Mfn2 indicates mitofusin 2; Pink1, PTEN-induced putative kinase 1; and UPS, ubiquitin proteasome system.
heart have focused on PINK1-Parkin–mediated mitophagy. One study showed that although young Parkin knockout mice exhibit a normal cardiac phenotype, abnormal mitochondria accumulate in cardiomyocytes with age in these mice. This suggests that endogenous Parkin may mediate mitophagy in aging hearts. It is unclear, however, how Parkin expression is regulated during aging. Furthermore, considering the fact that Parkin has multiple functions besides mitophagy, whether downregulation of Parkin directly promotes accumulation of abnormal mitochondria through a defect in mitophagy remains to be tested. Damaged mitochondria can be eliminated by multiple mechanisms, including mitochondrial proteases, ubiquitin proteasome-dependent mechanisms, Parkin-independent macroautophagy, a nonconventional form of autophagy, microautophagy, and mitochondria-derived vesicles.

Currently, how these mechanisms are affected by cardiac aging is unknown. In erythroid cells in which mtDNA mutation is stimulated, mitochondrial autophagy is downregulated by aging. Accumulation of damaged mtDNA activates mTOR, which inhibits autophagy, thereby preventing elimination of mitochondria containing damaged mtDNA and initiating a feed-forward mechanism that causes rapid accumulation of dysfunctional mitochondria and cell death. It will be interesting to test whether similar mechanisms exist in aging hearts.

Mito-Timer is a time-sensitive fluorescent protein targeted to the mitochondrial matrix that can be used to evaluate mitochondrial age by quantifying the proportionate integration of young (green) and old (red) Mito-Timer protein. Transgenic mice with cardiac-specific expression of Mito-Timer have recently been developed. These mice should be useful for evaluating age-dependent changes in mitochondrial turnover. Age-associated deterioration in the mitochondrial quality control mechanisms, including decreases in mitophagy, may be indicated by a slower mitochondrial turnover rate in this animal model.

**Relevant Intracellular Signaling Mechanisms Controlling Autophagy During Aging**

**Sirtuins**

Yeast silent information regulator 2 (Sir2) is an evolutionarily conserved molecule that mediates lifespan extension in yeast and *Drosophila* in response to CR. Sir2 is an NAD+-dependent protein deacetylase and functions in a wide array of cellular processes, including gene silencing, rDNA recombination, lifespan extension, and DNA damage repair. Overexpression of Sir2 increases the lifespan of many organisms, including yeast, *C. elegans* and *Drosophila*. Recently, the concept that lifespan extension in terminally differentiated cells might depend on mechanisms similar to those that regulate chronological lifespan in yeast has been challenged. Nonetheless, we have shown that Sir1, a mammalian ortholog of Sir2, is able to retard aging of the heart, an organ whose major component is terminally differentiated cardiomyocytes. Sir1 deacetylates p53 and the FoxO family transcription factors, thereby inhibiting apoptotic cell death in mice and humans. Mice deficient in Sir1 exhibit developmental abnormality in the heart and only infrequently survive postnatally. Sir1 is upregulated by CR and regulates fat metabolism by inhibiting fat cell differentiation and fat accumulation through regulation of peroxisome proliferator-activated receptor-γ. Three- to 6-fold overexpression of Sir1 attenuates aging-induced cardiac pathology, including hypertrophy, fibrosis, apoptosis, and upregulation of senescence markers, and protects the heart against oxidative stress. Furthermore, endogenous Sir1 protects the heart against ischemia/reperfusion injury, in part, through deacetylation of FoxO1 and consequent upregulation of antioxidants. Sir1 also mediates fasting-induced deacetylation of FoxO1 in the heart, thereby stimulating autophagosome formation and autophagosome–lysosome fusion through transcriptional upregulation of Rab7 in cardiomyocytes. Sir3 mediates the effect of CR on age-related hearing loss and oxidative stress in mice. Given that their functions favor longevity and cell survival, and that they are coupled with the metabolic state of cells and with autophagy, both Sir1 and Sir3 are attractive candidates for critically regulating cell survival in cardiomyocytes in response to stresses such as energy starvation.

**NAD⁺ and Protein Acetylation**

NAD⁺ is an electron acceptor in the mitochondrial electron transport chain and also acts as an essential substrate for NAD⁺-dependent enzymes, including sirtuins and poly ADP ribose polymerase, a DNA repair enzyme. Cellular levels of NAD⁺ are regulated by the balance between synthesis through the de novo and salvage pathways and consumption through sirtuins and poly ADP ribose polymerase. NAD⁺ in turn regulates the level of protein acetylation through regulation of sirtuins. We have shown previously that nicotinamide phosphoribosyl transferase (Nampt), a key enzyme in the salvage pathway of NAD⁺ synthesis in cardiomyocytes, is downregulated in the heart in response to prolonged ischemia, which leads to decreases in the level of NAD⁺ in the heart, inhibition of autophagic flux, and consequent increases in cell death. However, restoration of the NAD⁺ level by overexpressing Nampt restores the level of autophagy during prolonged ischemia and reduces the extent of myocardial infarction. Autophagic flux is negatively affected by decreases in NAD⁺ during ischemia due to resultant decreases in lysosomal acidification. We hypothesize that maintaining the NAD⁺ level is essential for maintaining the activity of the H⁺ pump on the lysosomal membrane. Decreases in NAD⁺ also increase protein acetylation through suppression of sirtuins, which in turn suppresses autophagy.

The level of NAD⁺ decreases with age in many organs, because of downregulation of Nampt, defective circadian rhythm, oxidative stress, and accumulation of DNA damage. Although the level of Nampt in the mouse heart is upregulated at 1 year of age, whether it is downregulated thereafter has not been shown. We speculate that, as in the case of prolonged ischemia, aging-induced decreases in NAD⁺ may induce lysosomal dysfunction, which in turn contributes to age-dependent increases in the susceptibility of the heart to ischemic injury. In the rate-limiting step of the salvage pathway of NAD⁺ synthesis, Nampt produces nicotinamide mononucleotide, which is then converted to NAD⁺. Exogenous supplementation of...
nicotinamide mononucleotide increases NAD⁺ content in cardiomyocytes, stimulating Sirt1 and inducing deacetylation of cellular proteins, including FoxO, in the heart, which, in turn, protects the heart from ischemia/reperfusion injury. This suggests that supplementation of NAD⁺ via its precursors, including nicotinamide mononucleotide and nicotinamide riboside, may allow suppression of cardiac aging through the activation of sirtuins, including Sirt1 and Sirt3, increases in protein deacetylation in the nucleus and mitochondria, and activation of autophagy.

**Insulin-Like Growth Factor I Signaling**

In lower organisms, inhibition of insulin-like growth factor (IGF-I) signaling, such as by Daf2 mutation in *C. elegans*, causes lifespan extension. This is mediated by a loss of suppression of Daf16 or FoxO transcription factors, which regulate expression of antioxidants (MnSOD) and DNA damage repair enzymes (GADD45). In mammals, Ames and Snell dwarf mice lacking growth hormone/IGF-I signaling and IGF-I receptor heterozygous knockout mice have longer lifespans. Systemic overexpression of klotho, a hormone known to inhibit insulin/IGF-I signaling, also extends lifespan in mice. It should be noted, however, that whether inhibiting IGF-I signaling (and thus stimulating FoxOs) positively affects senescence and aging-related diseases in mammals without affecting normal function is not fully understood. IGF-I may only produce a trade-off between current benefits to reproduction and later costs in senescence, like the relationship between inotropic agents and exacerbation of heart failure. Interestingly, activation of Akt weakens cardiac adaptation to stress and exacerbates the aging phenotype in the heart, effects that are accompanied by suppression of autophagy. However, the specific role of Akt in cardiac aging and how autophagy affects this process are not yet known. Considering the apparent cell survival–promoting effects of the IGF-I-Akt axis, this issue seems to represent a paradox in longevity research and will require clarification in each organ.

**GSK-3, AMP-Activated Protein Kinase, and mTOR**

mTOR inhibits autophagy through phosphorylation of Ulk1, a mammalian ortholog of Atg1, at Ser 757. Conversely, suppression of mTORC1 by rapamycin stimulates autophagy in cardiomyocytes. Although inhibition of mTORC1 by rapamycin has been shown to attenuate the adverse effects of aging in the heart, the specific role of Akt in cardiomyocytes, stimulating Sirt1 and inducing deacetylation of cellular proteins, including FoxO, in the heart, which, in turn, protects the heart from ischemia/reperfusion injury. This suggests that supplementation of NAD⁺ via its precursors, including nicotinamide mononucleotide and nicotinamide riboside, may allow suppression of cardiac aging through the activation of sirtuins, including Sirt1 and Sirt3, increases in protein deacetylation in the nucleus and mitochondria, and activation of autophagy.

**Oxidative Stress**

Oxidative stress can either activate or inactivate autophagy in cardiomyocytes in a context-dependent manner. We have shown previously that oxidative stress during ischemia/reperfusion in the heart stimulates autophagy by upregulating Beclin1. In addition, glucose deprivation and hypoxia activate autophagy through the upregulation of Nox4 in the ER and consequent activation of PERK-dependent mechanisms. Although increases in oxidative stress induce tissue damage, mitochondrial dysfunction and cell death, whether age-dependent increases in oxidative stress suppress autophagy in the heart has not yet been clearly demonstrated.

**How to Stimulate Autophagy**

Because autophagy is downregulated with age and downregulation of autophagy promotes senescence of the heart, interventions to increase the level of autophagy may prevent or slow the progression of aging in the heart. Furthermore, considering...
the fact that cardiac aging is accompanied by the accumulation of insoluble polymeric materials, such as lipofuscin, and damaged organelles, it would seem to be advantageous to have a degradation mechanism with a large capacity (Figure 3).

Interestingly, both CR and suppression of mTOR interventions that alleviate the adverse effects of aging and increase lifespan, promote autophagy in many cell types and organs, even when autophagy is suppressed by aging. Importantly, CR has been shown to reduce age-related pathologies and diseases in animals. However, whether the beneficial effects of these interventions are mediated primarily through activation of autophagy in the heart requires further study.

Voluntary exercise activates autophagy by stimulating phosphorylation of Bcl-2 and its dissociation from Beclin1. Exercise leads to a reduction in protein aggregates in the heart, which is accompanied by increases in autophagic flux, but not in the ubiquitin proteasome system or UPR. We speculate that the antisenescence effect of exercise on the heart may be, in part, mediated through the activation of autophagy and consequent amelioration of protein aggregate formation.

Toll-interacting proteins interact with LC3 and ubiquitin, thereby binding to ubiquitin conjugates more tightly than p62 and effectively clearing highly aggregation-prone proteins such as Huntington poly-glutamine protein. Thus, an intervention that upregulates them in vivo might be effective in clearing protein aggregates associated with aging, such as preamyloid bodies and lipofuscin. A recent report shows, however, that Toll-interacting protein promotes inflammation and apoptosis in the heart after myocardial infarction. Thus, further investigation is needed to clarify the function of this protein in the heart.

Trehalose is a naturally occurring disaccharide that is induced in response to dehydration in plants and promotes their survival by inducing autophagy, thus fulfilling the definition of a hormetic response. We have shown recently that trehalose treatment increases autophagy in the mouse heart and the cardiomyocytes therein. It will be interesting to test whether trehalose also inhibits the adverse effects of aging in the heart by stimulating autophagy.

It should be noted that the interventions discussed above have multiple effects in the heart besides autophagy. Furthermore, their effects may be mediated through noncardiac cells. For example, growth factors, miRNA, and exosomes excreted from distal organs may mediate autophagy and inhibit senescence in the heart after reaching the heart through the systemic circulation. Thus, where such is likely to be the case for a particular antisenescence intervention, further investigation is needed to identify the responsible cell type and the mechanism of transmission.

Concluding Remarks
Accumulating lines of evidence suggest that the ability of cardiomyocytes to maintain appropriate levels of autophagy may decline during aging of the heart. However, many questions remain unanswered. First, it is unclear when the level of autophagy becomes significantly altered during the course of aging in the human heart. Currently, evaluating the level of autophagy and autophagic flux is challenging in the human heart in vivo. Developing convenient and reliable methods to accurately evaluate cardiac autophagy is essential. Second, more investigation is needed to elucidate the molecular mechanism by which autophagy or mitochondrial autophagy is regulated during the course of aging in the heart. Autophagy is regulated not only at the level of autophagosome formation but also at the levels of autophagosome–lysosome fusion and lysosomal degradation. In particular, how the function of lysosomes is affected by aging requires further investigation. Third, more investigation is needed to clarify the functional role of autophagy or mitophagy during cardiac aging. Currently, none of the available molecular interventions allow purely specific modification of either autophagy or mitophagy in mammalian cells. Whether protein aggregates, such as accumulated lipofuscin, are causatively involved in cardiac aging has not been formally addressed. Development of a more selective intervention or improvement of the selectivity by combining multiple interventions seems essential. Fourth, it is important to clarify the molecular mechanisms by which autophagy or mitophagy regulates cardiac aging. Although autophagy is generally thought to be a nonspecific mechanism of protein degradation, there is increasing evidence that some proteins may be specifically degraded through their association with LC3 receptor proteins. Identification of specific targets of autophagy during aging may allow a better understanding of the molecular mechanisms of cardiac aging. Fifth, most of the investigations reported to date focused only on autophagy in cardiomyocytes during aging. It is important to determine whether autophagy in other cell types, such as inflammatory cells, also affects cardiac aging and, if so, how these cells communicate with cardiomyocytes, such as through secretion of paracrine factors, circulating miRNA, or exosomes. Likewise, a better understanding of the function of autophagy in cardiac progenitor cells is also highly relevant for aging research in the heart. Finally, it is essential to develop more convenient and specific interventions to normalize the level of autophagy in the heart during aging.

Autophagy mediates many lifespan-extending and antisenescence mechanisms. In particular, considering the emerging importance of the UPR as a fundamental mechanism of longevity enhancement, elucidating the connection between the UPR and autophagy/mitophagy is of great interest. Together with the recent advancement in understanding the molecular mechanisms of autophagy, investigating the role of autophagy/mitophagy during cardiac aging should eventually lead to the development of more efficient and specific interventions to slow senescence and increase stress resistance in the heart.

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Disclosures

None.

References


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