

FORUM REVIEW ARTICLE

## Oxidative Stress and Autophagy in Cardiovascular Homeostasis

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### Abstract

**Significance:** Autophagy is an evolutionarily ancient process of intracellular protein and organelle recycling required to maintain cellular homeostasis in the face of a wide variety of stresses. Dysregulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to oxidative damage. Both autophagy and ROS/RNS serve pathological or adaptive roles within cardiomyocytes, depending on the context. **Recent Advances:** ROS/RNS and autophagy communicate with each other *via* both transcriptional and post-translational events. This cross talk, in turn, regulates the structural integrity of cardiomyocytes, promotes proteostasis, and reduces inflammation, events critical to disease pathogenesis. **Critical Issues:** Dysregulation of either autophagy or redox state has been implicated in many cardiovascular diseases. Cardiomyocytes are rich in mitochondria, which make them particularly sensitive to oxidative damage. Maintenance of mitochondrial homeostasis and elimination of defective mitochondria are each critical to the maintenance of redox homeostasis. **Future Directions:** The complex interplay between autophagy and oxidative stress underlies a wide range of physiological and pathological events and its elucidation holds promise of potential clinical applicability. *Antioxid. Redox Signal.* 20, 507–518.

### Introduction

OXYGEN IS A MOLECULE essential to life. It serves as the indispensable carrier of electrons in mitochondrial energy production. Further, unstable (reactive) derivatives of both oxygen and nitrogen act as intracellular second messengers, governing a wide range of cellular events. At the same time, accumulation of these reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be pathological. Maintenance of the optimal reductive/oxidative potential within the cell is critical to numerous cellular functions, and the nitroso–redox imbalance contributes to aging and disease development, including in muscle (10, 27, 36, 67).

Autophagy (“self-eating,” from the Greek *auto*=self and *phagein*=to eat) is a term that denotes multiple intracellular processes, which converge on a common degradation pathway mediated by lysosomes. This protein and organelle removal process maintains cellular homeostasis by eliminating damaged and/or toxic cellular components (7, 59, 84). As cardiomyocytes are postmitotic cells with high rates of energy

utilization and an unremitting requirement for ATP, they harbor an abundance of mitochondria and are uniquely vulnerable to damaged and dysfunctional mitochondria. Under basal conditions, cellular functions decline over time, accompanied by increases in ROS/RNS and perturbations in autophagy; aging ensues (62). Under stress conditions in which ROS and RNS accumulate, mitochondria and cellular components can become oxidized and damaged. Therefore, careful regulation of ROS/RNS and their interplay with autophagic degradation pathways is crucial for cellular homeostasis. Dysregulation of these mechanisms contributes to disease pathogenesis and may serve as a target for therapeutic manipulation (50).

### Fundamentals of Autophagy

Autophagic degradation of intracellular constituents is fundamental to cellular homeostasis owing to its critical role in removing toxic proteins and dysfunctional organelles and recycling these elements as nutrients and cellular building blocks. In particular, autophagy is a major mechanism for the

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elimination of bulk cellular components, such as protein aggregates, and the only mechanism for eliminating dysfunctional organelles (*e.g.*, mitochondria). Three types of autophagy have been identified: chaperone-mediated autophagy, microautophagy, and macroautophagy. All three types share the final common pathway of lysosomal fusion and consequent substrate degradation, but upstream mechanisms and governing circuitries differ (59, 84). Here we focus on macroautophagy, the most common and best characterized form of autophagy, and we use the term autophagy to denote this pathway. Importantly, macroautophagy also appears to operate in an organelle-selective manner, for example, selectively targeting the mitochondria (mitophagy), endoplasmic reticulum (reticulophagy), portions of the nucleus (nucleophagy), peroxisomes (pexophagy), microorganisms (xenophagy), ribosomes (ribophagy), lipid droplets (lipophagy), or protein aggregates (aggrephagy) (33).

The process of autophagy commences with the emergence of a double-membrane compartment, the phagophore, which derives from intracellular or *de novo* membrane sources. Beclin 1 is an essential element in autophagosome formation. Upon activation of autophagy, Beclin 1 dissociates from B-cell lymphoma-2 (Bcl-2), releasing Beclin 1 from the inhibitory effects of this protein-protein interaction (59, 84). It then localizes to the phagophore to promote recruitment of double-membrane elongation factors. Several autophagy-related proteins (ATGs) are recruited to the emerging double-membrane vacuole and are required for its progressive expansion. This elongation step is accomplished by activation of two parallel pathways at the phagophore. In one, the ubiquitin ligase E1-like enzyme, ATG7, mediates the conjugation of ATG12 to ATG5, which then couples with ATG16. In the other, microtubule-associated protein 1 light chain (LC3/ATG8) is cleaved by ATG4 to produce LC3-I. This new isoform is now available to be activated by ATG7 and ATG3 (an E2-like enzyme) through conjugation with phosphatidylethanolamine (PE), resulting in LC3-II. This lipidated isoform of LC3 is bound to both the inner and outer membranes of the autophagosome, making it a marker of autophagosome abundance. Then, as the two ubiquitin-like conjugation processes proceed, the nascent vacuole closes on itself, engulfing its cellular cargo, and is termed the autophagosome. The autophagic pathway

ultimately culminates in fusion of the autophagosome with a lysosome to form the autolysosome. As a consequence, autophagosomal cargo is degraded by lysosomal proteases and hydrolases, and the degraded materials are released into the cytoplasm *via* permeases (Fig. 1). The dynamic cascade of autophagosome formation, maturation, fusion with lysosomes, and degradation of cargo is termed autophagic flux. (59, 84).

## ROS Signaling

ROS are oxygen-based molecular species characterized by their high chemical reactivity; they include free radicals such as superoxide ( $O_2^-$ ) and hydroxyl ( $\cdot OH$ ) and nonradical species such as hydrogen peroxide ( $H_2O_2$ ). RNS include nitric oxide (NO) and peroxynitrite ( $ONOO^-$ ) (14).

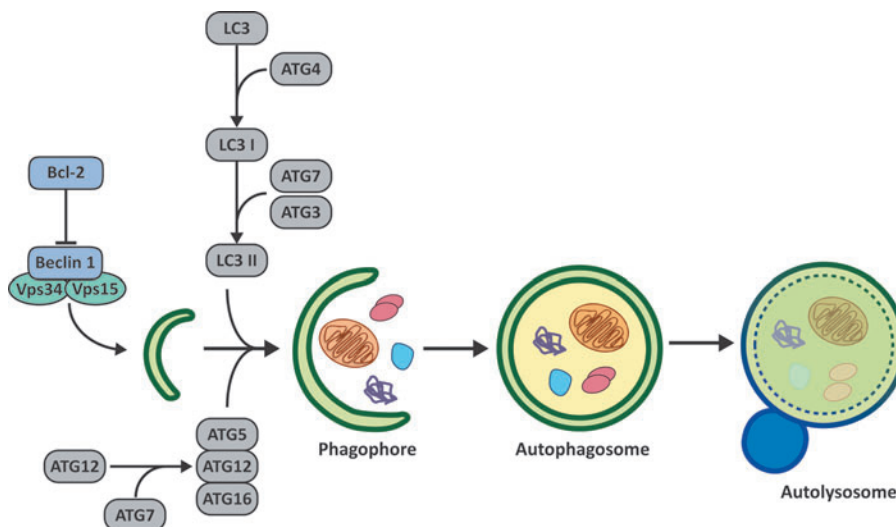
The balance between the production of ROS/RNS and the ability of chemical reducing systems to remove them is termed the redox (or nitroso-redox) state of the cell. A small, localized increase in ROS/RNS can act as an intracellular second messenger regulating a variety of signaling pathways (*e.g.*, NF $\kappa$ B). Robust increases in ROS/RNS, where the reducing systems are insufficient to process them, are termed redox or oxidative stress. In some instances, reductive stress has been inferred (1, 20, 58). During oxidative stress, cellular systems are damaged by oxidation, including membrane lipids, proteins, DNA, and other macromolecules. If the stress insult continues unabated, cell death by apoptosis, necrosis, or possibly autophagy can occur (18, 26).

## Sources of ROS/RNS in the Heart

A number of enzymatic sources of ROS and RNS have been identified in the heart: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), xanthine oxidase (XO), the mitochondrial electron transport chain (mETC), and nitric oxide synthase (NOS). Beyond this, nonenzymatic sources, such as the reduction of nitrite and the Fenton reaction, have been identified (36, 50, 57).

## Mitochondria

These organelles, particularly abundant in cardiomyocytes, are the main source of ROS in the heart. In physiological



**FIG. 1. The process of autophagy.** Beclin 1 localizes to the phagophore to promote autophagosome formation and recruit ATGs. Then, ATG7 mediates the conjugation of ATG12 to ATG5, which then bind ATG16 to promote autophagosome elongation. In parallel, LC3 is converted to LC3I by ATG4, and LC3I is subsequently lipidated to form LC3II by ATG7 and ATG3. The mature autophagosome fuses with a lysosome, an event that culminates in cargo degradation. ATG, autophagy-related protein. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/ars](http://www.liebertpub.com/ars)

conditions, 1%–2% of electrons passing through the electron transport chain lead to generation of  $O_2^-$  through mitochondrial complexes I and III (42). Under pathological conditions, however, the amount of  $O_2^-$  formed exceeds the clearance capacity of antioxidant systems. Then,  $O_2^-$  passes into the cytoplasm in its protonated form or through the voltage-dependent anion-selective channel, where it is converted to  $H_2O_2$ . A recently described process termed ROS-induced ROS release has also been characterized in mitochondria (89). In the setting of oxidative stress, localized elevations of ROS in mitochondria trigger opening of the mitochondrial permeability transition pore, destabilizing mitochondrial membrane potential. This event, in turns, triggers further increases in ROS production, leading to diffusion of these molecules to neighboring mitochondria to elicit a similar response (57, 89).

#### *NADPH oxidase*

This family of enzymes comprises seven members; NOX 1–5, DUOX1 and DUOX2. These enzymes are localized at the plasma membrane and consume nicotinamide adenine dinucleotide (NADH) or NADPH as a substrate for single-electron reduction of  $O_2$  to generate  $O_2^-$  (2).

#### *Xanthine oxidase*

Under certain conditions, xanthine dehydrogenase can be modified by oxidation or proteolytic cleavage and converted to XO. However, XO is still capable of catalyzing the conversion of hypoxanthine to uric acid, just as xanthine dehydrogenase, but the reaction uses molecular oxygen as the electron acceptor, generating  $O_2^-$  (21).

#### *Nitric oxide synthase*

Three NOS isoforms have been described in cardiac tissue: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible isoform NOS (iNOS). These enzymes catalyze the oxidation of L-arginine to L-citrulline with release of NO. The myocardium constitutively expresses nNOS and eNOS and can be induced to express iNOS. NO can react with  $O_2^-$  to produce the highly reactive ONOO<sup>-</sup>. Moreover, under uncoupling conditions, NOS is capable of generating  $O_2^-$  (87).

#### *Alternative sources of $H_2O_2$ and $^{\bullet}OH$*

Superoxide dismutase (SOD) dismutates  $O_2^-$  to generate  $H_2O_2$ . Subsequently,  $H_2O_2$  can be reduced to  $^{\bullet}OH$  via the Fenton reaction involving  $Fe^{2+}$  ion (54).

### **Antioxidant Systems**

To counteract increases in ROS/RNS levels, cells possess a variety of antioxidant systems that scavenge and degrade these toxic molecules to stable, nontoxic species. These systems include enzymes, such as SOD, which converts  $O_2^-$  to  $H_2O_2$ , which is subsequently converted by catalase to  $H_2O$  and  $O_2$ . In addition, glutathione peroxidase (GSHPx) breaks down lipids and hydroperoxides, thioredoxin (TRX) catalyzes the reduction of protein disulfide bonds, and glutaredoxin deglutinates proteins. Among the nonenzymatic systems, glutathione (GSH) is fundamental to the maintenance of redox homeostasis. GSHPx catalyzes the oxidation of GSH to

glutathione disulfide (GSSG), while GSH NADPH-dependent reductase catalyzes reduction of GSSG to GSH. Nonenzymatic antioxidants in the cell include vitamin E, vitamin C, beta-carotene, lipoic acid, and urate (10).

### **Autophagy in Cardiovascular Physiology and Disease**

As with any cell, governance of protein quality control—proteostasis—is fundamental to cardiomyocyte health. In addition, as a postmitotic cell incapable of replication, the cardiac myocyte relies to a particularly great extent on the elimination of toxic protein elements and dysfunctional organelles (7, 53, 72, 73). On top of all this, the heart is rich in mitochondria, and these organelles must be eliminated rapidly once defective, lest they generate yet higher levels of ROS and trigger a catastrophic progression to programmed apoptotic cell death. Evidence in support of these contentions is seen in experimental models where ATG proteins are silenced, leading to accumulation of ubiquitinated protein aggregates, dysfunctional mitochondria, and pathological cardiac remodeling (40, 74). On the other hand, the antiaging effects of starvation derive, at least in part, from activation of autophagic degradation pathways (62).

In animal models of cardiovascular disease, excessive or insufficient autophagy are each maladaptive, and a wide range of cardiovascular disorders are accompanied by robust increases in the autophagic activity (11, 45, 51, 52, 82). For example, several studies have shown that robust activation of autophagy can be maladaptive in the load-stressed heart (8, 49, 88). Consistent with this, several reports have revealed evidence of increased autophagy in human samples from diseased and failing hearts (34). Moreover, left ventricular assist device-based support, a prominent therapy for advanced heart failure that reduces ventricular load stress, tempers upregulated autophagic activity (23, 32). Ischemia/reperfusion (I/R) stress triggers oxidative damage and declines in cellular ATP and a seemingly paradoxical suppression of autophagy, a response that appears to be maladaptive, possibly due to the lack of clearance of toxic intracellular substances. During reperfusion, when oxygen and nutrients are restored to the tissue, autophagy reactivates to levels that may exceed the baseline (16, 25, 47, 83). Whether this response is maladaptive or beneficial is the subject of ongoing investigation (Table 1).

While this review focuses on the role of autophagy in the heart, we will briefly note the protective role of autophagy in atherosclerosis. Recent evidence suggests that autophagy combats atherosclerotic lesions by hydrolyzing cholesterol deposits in macrophages, preventing plaque formation, and repressing the inflammatory response (39, 60). Overall, the functional consequences of autophagy in cardiovascular disease can be protective or maladaptive, depending on the context (61).

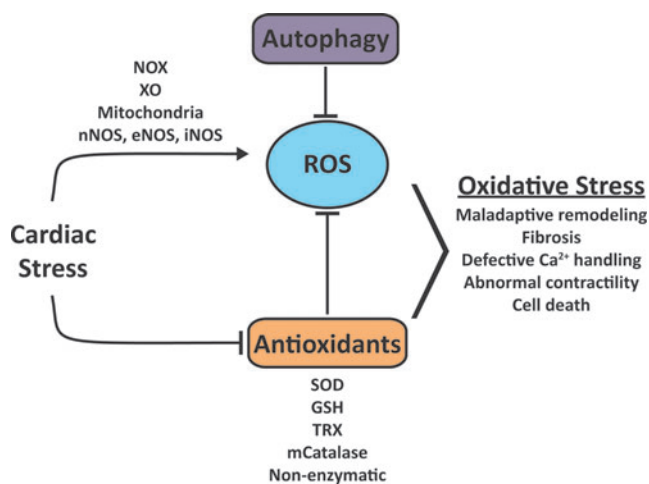
### **Consequences of Oxidative Stress in the Heart**

Oxidative stress, which is triggered by accumulation of toxic ROS and RNS, can lead to both functional and structural changes that culminate in pathological remodeling of the myocardium, fibrosis, and contractile dysfunction (Fig. 2). At the cellular level, oxidative stress provokes lipid peroxidation, DNA damage, and oxidation of proteins and other macromolecules (18). Lipid peroxidation is a process that occurs

TABLE 1. AUTOPHAGY IN CARDIAC DISEASE

Disease	Model	Sample	Cardiac phenotype	Autophagy	Refs.
Desmin-related cardiomyopathy	CryAB mutant	Ms, NRVMs	Hypertrophic cardiomyopathy	Adaptive induction	(53, 72, 73)
	CryAB mutant	Ms	Hypertrophic cardiomyopathy	Beclin 1 + / - shows accelerated cardiomyopathy	(72)
Danon disease	LAMP-2 mutant	Hu, Ms	Hypertrophic cardiomyopathy	Defective autophagosome-lysosome fusion	(45, 52)
Heart failure	Left ventricular myocardium	Hu	Hypertrophic cardiomyopathy	Increased autophagy	(34)
	TAC	Ms	Hypertrophy	Beclin 1 Tg shows exacerbated hypertrophy	(8)
	TAC	Ms	Hypertrophy	ATG5-/- shows rapid progression to failure	(8, 88)
	TAC	Ms	Hypertrophy	Maladaptive induction of autophagy	(8)
	sTAC	Ms	Pressure-overload HF	Maladaptive induction of autophagy	(88)
Cardiac unloading	LVAD support	Hu	Regression of hypertrophy	Decreased markers of autophagy	(32)
	DeTAC	Ms	Regression of hypertrophy	Increased markers of autophagy	(23)
Ischemic heart disease	Ischemia	Pig	Ischemia only	Increased autophagic adaptive response	(83)
	I/R	Ms, Rb	Reperfusion	Increased autophagic response (maladaptive?)	(16, 47)
	I/R	Ms	I/R	Beclin 1-/- shows reduced infarction	(47)

CryAB, alpha-crystallin B chain; HF, heart failure; Hu, human; I/R, ischemia/reperfusion; LAMP2, lysosomal-associated membrane protein-2; LVAD, left ventricular assist device; Ms, mouse; NRVMs, neonatal rat ventricular myocytes; Rb, rabbit; sTAC, severe thoracic aortic constriction; TAC, thoracic aortic constriction.

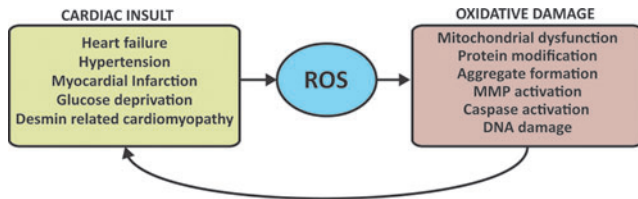


**FIG. 2. Balance of ROS/RNS and antioxidants in cardiac stress.** A critical balance between oxidants and antioxidants is essential to maintain normal cardiac signaling and function. Similarly, adequate basal autophagic flux is required for homeostasis. A variety of cardiac insults can lead to ROS accumulation and the decline of antioxidant systems. This loss of redox balance is related to the development of maladaptive remodeling. In this context, autophagy may be induced as a bulk anti-oxidative response. GSH, glutathione; NOX, NADPH oxidase; XO, xanthine oxidase; eNOS, endothelial NOS; iNOS, inducible isoform NOS; nNOS, neuronal NOS; SOD, superoxide dismutase; TRX, thioredoxin; ROS, reactive oxygen species; RNS, reactive nitrogen species. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/ars](http://www.liebertpub.com/ars)

when free radicals react with membrane lipids, capturing electrons from the latter to produce nonradical species. In this process, oxidized lipids and their products are fragmented to produce malondialdehyde (MDA) and 4-hydroxynonenal, accompanied by loss of membrane integrity. Depending on the proximity of ROS sources, lipid membranes of some organelles are more susceptible than others to lipid peroxidation reactions. For example, unsaturated fatty acids within the mitochondrial membrane are susceptible to peroxidation owing to their close proximity to mitochondrial ROS (3).

As previously described, the cellular oxidative state is important for ROS signaling, and a modest increase in ROS can induce cardioprotection. An example is the oxidative modification of the ryanodine receptor by glutathionylation (17). On the other hand, excessive increases in ROS induce irreversible oxidation and amino acid nitration to trigger protein denaturation, protein aggregation, and protein degradation. Moreover, the oxidation of such proteins can also be generated by conjugation with lipid peroxidation products (Fig. 3) (18).

In addition, some products of lipid peroxidation, such as MDA, are capable of forming adducts with DNA bases. The consequences of this include alterations in gene expression and even induction of apoptosis and cell death (75). Finally, increased ROS levels within mitochondria can lead to apoptosis by inducing apoptosome formation. Mitochondrial damage can occur in other ways, as in the oxidation of mitochondrial DNA or by oxidation of cardiolipin, a lipid within the inner mitochondrial membrane. Such peroxidation facilitates release of cytochrome c and inhibits cytochrome oxidase and electron flow across mitochondrial complexes I and III (31).



**FIG. 3. Effects of elevated ROS in heart disease.** A variety of cardiac insults can lead to ROS accumulation, provoking oxidative damage in cardiomyocytes. In a feed-forward manner, accumulation of oxidative damage can result in exacerbation of disease-related stress. MMP, metalloproteinase. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/ars](http://www.liebertpub.com/ars)

**Oxidative Stress and Autophagy in Cardiac Pathology**

*ROS regulation of autophagy*

Increasing evidence points to ROS as important activators of autophagy (Fig. 4). Nutrient deprivation is a canonical activator of autophagy, and under starvation conditions, ROS are produced and accumulate (44). Glucose starvation also decreases GSH levels. Treating cardiomyocytes with the antioxidant N-acetylcysteine blocks the increased autophagic response and recovers GSH levels (86).

Autophagy plays a major role in cardiac pathologies caused by oxidative stress, such as ischemia and I/R. In a mouse model, it has been reported that oxidative stress leads to increased autophagy during ischemic conditions, which is considered a beneficial response to eliminate oxidized and

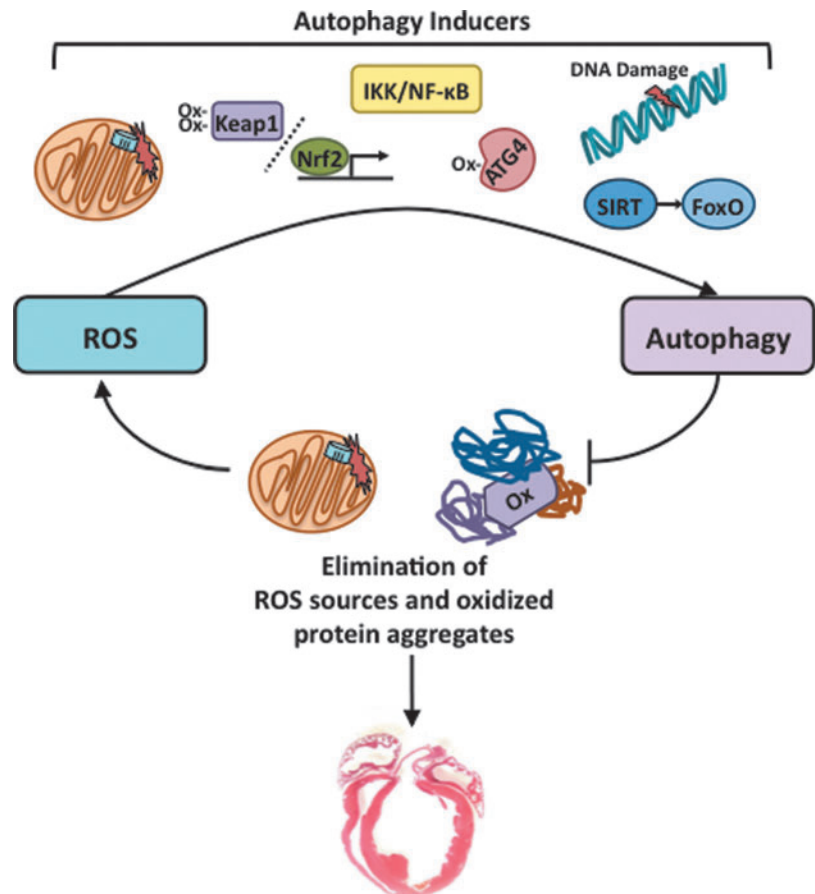
damaged cellular components and prevent cell death (69). In I/R injury, autophagy may be even further upregulated, exacerbating cell death due to aberrant levels of oxidative damage. Cardiomyocytes exposed to H<sub>2</sub>O<sub>2</sub> manifest elevated autophagic processing, which can be prevented by treatment with the antioxidant N-2-mercaptopyrionyl (MPG) (25). Mice treated with MPG manifest decreased oxidative stress, decreased LC3-II degradation, and less ischemia-induced injury (25, 69).

On the other hand, I/R reduces autophagosome formation in HL-1 cardiomyocytes *in vitro* (22). In this condition, activation of autophagy protects against apoptosis (22, 29). Furthermore, in a murine model of I/R, autophagosome accumulation was found to result from impaired flux stemming from defective lysosomal processing (43). Consistent with this, we have evidence that cardiomyocyte autophagosome formation is blunted in response to surgical I/R (unpublished observations).

Ischemia and I/R are distinct stressors, and further studies are required to characterize the role of autophagy in each condition. Furthermore, the degree and extent of the autophagic response are each critical to the cellular outcome to stress; too much or too little autophagy can each be detrimental (61). For example, while inducing autophagy may be protective in I/R, overly robust activation of autophagy can be maladaptive (8, 88).

*ATG4 cysteine oxidation*

Oxidation of the amino acid cysteine modifies the structure and function of some proteins. As a consequence,



**FIG. 4. Reciprocal ROS-induced activation of autophagy and autophagic elimination of ROS sources.** ROS activate autophagic flux *via* a variety of inducers. Meanwhile, autophagy can eliminate mitochondrial sources of ROS, as well as ROS-oxidized protein aggregates, which can contribute to cardiomyopathy. FoxO, Forkhead box-O; IKK, IκB kinase; Keap1, Kelch-like ECH-associated protein-1; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; SIRT, sirtuin (silent mating type information regulation 2 homolog) 1. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/ars](http://www.liebertpub.com/ars)

cysteine-harboring proteins function as sensors of the oxidative state of the cell. ATG4 is a cysteine-rich protease that plays an important role in autophagosome formation. ATG4 cleaves an arginine at the C-terminus of LC3. This cleavage allows for the lipidation of LC3 and subsequent conjugation with PE. As noted above, this event is necessary for the attachment of lipidated LC3 to the autophagosomal membrane and development of mature autophagosomes. In addition, ATG4 can cleave lipidated LC3 and remove it from the autophagosomal membrane.

It has been reported recently that during starvation, production of  $H_2O_2$  can oxidize cysteine 81 at the C-terminus of ATG4. This modification does not affect the cleavage of LC3 at the C-terminal arginine, but inhibits the delipidation action of ATG4, resulting in accumulation of lipidated LC3 and induction of autophagy (6). While this mechanism has been shown to regulate starvation-induced autophagy, additional definition of the role of ATG4 in the regulation of autophagy in cardiac oxidative stress is warranted.

#### IKK/NF $\kappa$ B pathway

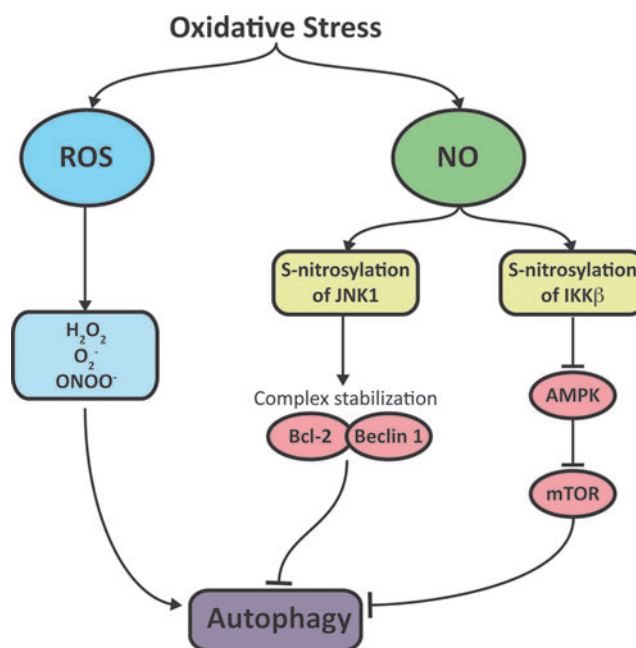
The transcription factor NF $\kappa$ B regulates the expression of genes involved in both cell survival and anti-inflammatory responses (27). Under basal conditions, NF $\kappa$ B forms a complex with I $\kappa$ B proteins, which negatively regulates its transcriptional activity by sequestering the protein within the cytosol. Increased ROS levels activate I $\kappa$ B kinase (IKK), which phosphorylates I $\kappa$ B leading to degradation by the proteasome. The free NF $\kappa$ B translocates to the nucleus, where it is transcriptionally active (27). NF $\kappa$ B has a dual role in the regulation of autophagy, as NF $\kappa$ B promotes the expression of Beclin 1, LC3, and ATG5 in myotubes (12). In addition, as a negative feedback mechanism, NF $\kappa$ B promotes the expression of autophagy inhibitors, such as Bcl-2 and B-cell lymphoma-extra large (Bcl-xL), as well as activation of mammalian target of rapamycin (mTOR) (63). However, activation of IKK by ROS can stimulate autophagy independent of NF $\kappa$ B (13). While both NF $\kappa$ B and autophagy are protective in the context of cardiac ischemia, the precise interplay between these processes remains unclear.

#### Nitric oxide

While the prevailing notion is that ROS activate autophagy, recent evidence suggests that RNS can exert an inhibitory effect on autophagic pathways. NO inhibits the activity of c-Jun N-terminal kinase-1 (JNK1) by S-nitrosylation, leading to the hypophosphorylation of Bcl-2. This, in turn, induces dimerization of Bcl-2 with Beclin 1, which suppresses autophagy initiation. Consistent with this, overexpression of iNOS inhibits autophagosome formation. NO can also activate the mTOR complex 1, a well-established inhibitor of autophagy (Fig. 5) (64).

#### Sirtuins/FoxO

Sirtuins are protein deacetylases that participate in cardiac remodeling, regulating energy production, oxidative stress, autophagy, and cell survival. Oxidative stress activates sirtuins, which leads to the promotion of autophagy, a process governed, at least in part, by Forkhead box-O (FoxO) transcription factors (65). Activation of both sirtuins and FoxO1



**FIG. 5. Differential regulation of autophagy by ROS and RNS.** ROS can induce autophagic degradation in cardiomyocytes. However, the role of specific RNS in autophagy in cardiomyocytes remains relatively less well characterized. Recently, it was shown in neurons that NO leads to S-nitrosylation and inhibition of JNK1 and IKK $\beta$ . In consequence, downstream signaling events inhibit autophagy. JNK1, c-Jun N-terminal kinase-1; mTOR, mammalian target of rapamycin. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/ars](http://www.liebertpub.com/ars)

inhibits oxidative damage in cardiomyocytes and is correlated with the induction of autophagy (28). In H9c2 cells, oxidative damage by  $H_2O_2$  was rescued by resveratrol, an activator of sirtuins (41). In this model, resveratrol triggers autophagic activation and promotes cell survival in the setting of oxidative stress (41). Resveratrol is known to have several intracellular targets beyond functioning as a sirtuin activator and a potent antioxidant. In addition, it has been reported that resveratrol can also induce expression of eNOS and iNOS (79).

FoxO1 can ameliorate oxidative stress and increase autophagic degradation by inducing expression of antioxidants and ATG genes. In addition, deacetylation of FoxO1 by Sirt1 in cardiomyocytes is necessary for the induction of the autophagic response triggered by starvation (24). In the case of murine cardiac I/R, deficiency in FoxO1 in cardiomyocytes results in significant increases in cell death, a response that correlates with decreased expression of both SOD2 and LC3-II (66).

#### DNA damage

A model has been proposed, where oxidative stress-induced DNA damage promotes pathological cardiac remodeling leading to end-stage cardiomyopathy (68). These investigators showed that nutrient deprivation leads to impaired base excision repair as well as loss of the base excision repair enzyme 8-oxoguanine glycosylase 1 (OGG1). Reconstitution of OGG1 improved DNA repair and cardiac function. The proposed role of autophagy in this oxidative response is based on observations that OGG1 degradation is blunted when autophagy is suppressed. This response

requires the presence of a nutrient starvation stressor, as inducing autophagy alone is insufficient to degrade OGG1. Therefore, elevated levels of ROS in the heart can lead to DNA damage, which is exacerbated by the activation of autophagy and degradation of OGG1 (68).

#### Metalloproteinases

Accumulation of extracellular matrix proteins and consequent tissue fibrosis are hallmark features of heart failure. Recent reports demonstrate that ROS can lead to the activation of matrix metalloproteinases (MMPs) in the heart, resulting in increased collagen deposition (78). Two mechanisms have been described (56, 77). One involves homocysteine-induced increases in mitochondrial calcium, which are activated by the homocysteine receptor N-methyl-d-aspartate receptor-1 (NMDA-R1). Cardiomyocyte-specific knockout of *NR1* (gene coding for NMDA-R1) results in decreased NO production, inactivation of mitochondrial MMP-9, and improved cardiac function (77). A similar result was observed in a mouse model of pulmonary hypertension, where increased production of ROS, increased activity of MMPs, and increased autophagy were observed in the right ventricle (56). Treatment with the antioxidant folic acid led to decline in ROS levels, and a consequent reduction in autophagy and MMP activity. Importantly, deposition of collagen within the right ventricle was decreased, and cardiac function improved (56).

#### Keap1/Nrf2

Recently, a link between the antioxidant transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and autophagy has been described (80). Nrf2 is a key regulator of the expression of glutathione-S-transferase and NADPH quinone oxidoreductase. Nrf2 can also induce expression of p62, a protein involved in autophagy activation and responsible for targeting ubiquitinated proteins for degradation (4). When the oxidative balance within the cell is optimal, Nrf2 is sequestered within the cytosol by Keap1, resulting in Nrf2 degradation by the proteasome. Increased ROS oxidizes a cysteine in Kelch-like ECH-associated protein-1 (Keap1) and disrupts its interaction with Nrf2, leading to the translocation of Nrf2 to the nucleus. In addition, Keap1 can interact with p62 and LC3, and preventing these interactions leads to accumulation of ubiquitinated proteins. Together, these findings suggest that Keap1, in addition to its regulation of Nrf2, may promote autophagic clearance in response to oxidative stress by interacting with specific ATG proteins (19).

#### Lipopolysaccharide and inflammation

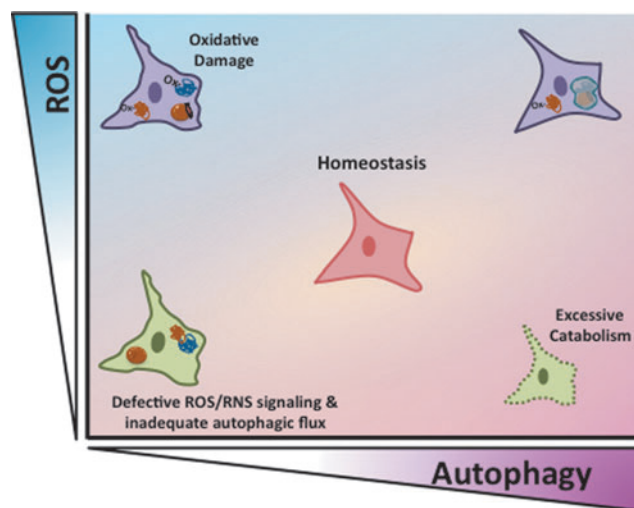
A multitude of insults to the heart can provoke myocardial inflammation. As part of this, the role of oxidative stress and autophagy in lipopolysaccharide (LPS)-induced inflammation has been the focus of considerable attention. It is well established that LPS triggers cardiomyocyte autophagy. Recently, it has been reported that this activation of autophagy is mediated by oxidative stress, where LPS treatment prevents the expression of GSH and leads to ROS accumulation. In this study, autophagy was induced by exposing HL-1 cells to NO or H<sub>2</sub>O<sub>2</sub>, while treatment with N-acetylcysteine (antioxidant) or L-NMMA (NOS inhibitor) reduced the autophagic response to LPS (86). Induction of autophagy before LPS treat-

ment resulted in reduced ROS and inflammation (86). Similarly, cardiomyocyte-specific overexpression of catalase rescued the contractile dysfunction caused by LPS treatment, reduced levels of ROS, and blunted autophagy (76). In addition, isolated adult cardiomyocytes treated with N-acetylcysteine or 3-methyladenine (3MA) manifested improved contractility in the background of LPS (76). In contrast, whereas overexpression of metallothionein can rescue LPS-induced oxidative stress in cardiomyocytes, it did not prevent induction of autophagy (9).

#### Mitochondria

Damage to mitochondria is a hallmark consequence of oxidative stress in several contexts, including aging, hypertrophy, heart failure, ischemia, and reperfusion. Selective autophagic elimination of mitochondria by autophagy (mitophagy) is a key quality control process (37, 38). In mammals, Nix is a specific regulator required for degradation of erythrocyte mitochondria (85). Elimination of altered mitochondria is mediated by PTEN-induced putative protein kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin. Both PINK1 and Parkin accumulate on dysfunctional mitochondria, stimulating their segregation from the mitochondrial network, and targeting them for autophagic degradation in a process that requires Parkin-dependent ubiquitination of mitochondrial proteins (5). In addition, accumulating evidence suggests that during stress, damaged mitochondria undergo fission, generating smaller organelle compartments that are then easily engulfed and degraded (30).

A recent study proposed that the mETC complex III has a role in mediating autophagy. In this study, antimycin A, an inhibitor of the mETC complex III, specifically inhibited autophagy (42). A second, structurally distinct inhibitor, myxothiazol, also inhibited autophagy. Two alternative mechanisms were proposed for how mitochondrial complex III regulates autophagosome initiation. In one, complex III regulates HIF-BNIP3-dependent control of autophagy; in the



**FIG. 6. Balance between redox state and autophagic activation.** ROS, RNS, and autophagy must each be maintained within a narrow range; too little, or too much, of each can be maladaptive. To see this illustration in color, the reader is referred to the web version of this article at [www.liebertpub.com/ars](http://www.liebertpub.com/ars)

other, mitochondrial sources of autophagic membrane are implicated (42).

The importance of oxidative mechanisms in mitochondria and their regulation of autophagy were highlighted in a recent study in which, induction of ROS by angiotensin II provoked mitochondrial oxidative damage and autophagy (15). Dai

*et al.* overexpressed catalases specifically targeted to mitochondria and peroxisomes, finding that only the catalase expressed in mitochondria rescued cardiac hypertrophy and decreased fibrosis and mitochondrial damage (15). Importantly, angiotensin II-induced autophagy was inhibited by the expression of this antioxidant enzyme (15).

TABLE 2. RECENT PATENTS RELEVANT TO THE REGULATION OF AUTOPHAGY AND OXIDATIVE STRESS

Patent type	Invention	Suggested mechanism of action	Patent number	Year
Autophagy	High-throughput screen for small molecule modulators of autophagy	Discover novel modulators of autophagy	WO2008122038 A1	2008
Autophagy	Modulate the expression or activity of ATG14L and Rubicon in several diseases, including HF and I/R	ATG14L increases autophagic activity, while Rubicon inhibits it, both through the PI3K/Vps34 pathway	WO2010030936 A2	2010
Autophagy	Use of Tat-ATG5 and Tat-Becn1 in the context of HF and I/R	Tat-ATG5 inhibits, while Tat-Becn1 induces autophagy	WO2011106684 A2	2011
RNS	Therapeutic use of NO precursors	Administration of NO to treat several oxidative stress conditions, including cardiac surgery and hypertension	EP2335693A2	2011
RNS	Pyrrole inhibitors of SNO	Inhibition of SNO blocks the metabolism of NO and enhances its biological action	EP2315590	2011
ROS	Use of collismycin A as oxidative stress inhibitor	Potential oxidative stress inhibitor during I/R, MI	EP1909912 (A2)	2008
ROS	Methods to diagnose acute cardiac ischemia	Detection of metabolic by-products of XO to diagnose cardiac ischemia	WO2009020860 A1	2009
ROS/ Autophagy	Composition and methods for the treatment and/or prevention of disorders relating to oxidative stress	Increase Nrf2 biological activity or expression to promote an antioxidant response during ischemia	WO2007005879 A2	2007
ROS/ Autophagy	Food product with resveratrol derivatives (0.1–1%)	Control blood pressure by inducing an antioxidant response	WO2009020860 A1	2009
ROS/ Autophagy	Resveratrol compounds and methods of use	Prevention and treatment of cardiovascular diseases	WO2012006065 A1	2012
ROS/ Autophagy	Resveratrol-containing composition as sirtuin agonists for treating HF	Treat impaired cardiac contraction in HF and I/R	WO 2010020959 A1	2010
ROS/ Autophagy	Inhibitory peptide of Rubicon	Rubicon induces ROS production by activating NADPH Oxidase and inhibiting autophagy.	WO2011156637 A1	2011
ROS/RNS	Method for detecting biomarkers of oxidative stress in biological samples	Use of blood samples to detect markers of oxidative damage to determine the extent of oxidative injury	WO2007041868 A1	2007
ROS/RNS	Thiazolium compounds treat and prevent cardiac disease associated by high-fat diet	Decrease NADPH Oxidase expression and ameliorate mitochondrial superoxide production associated with high-fat diet	WO2009042213 A1	2009
ROS/RNS/ Autophagy	Administration of resveratrol following coronary intervention	Resveratrol isomers decrease ROS production and promote vascular relaxation by generating NO	EP1338278A1	2003
ROS/RNS/ Autophagy	Oxidative Stress Induced Peptides (OSIPs) to elicit an antioxidant response.	Peptides purified from the secretions of various oxidatively stressed human cells.	WO2012075549 A2	2012

MI, myocardial infarction; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; ROS, reactive oxygen species; RNS, reactive nitrogen species; SNO, S-nitrosoglutathione reductase; XO, xanthine oxidase.



### Autophagic Regulation of ROS

While the role of ROS as an upstream trigger of autophagy has been characterized, the role of autophagy to govern ROS and oxidative stress is also gaining attention (Fig. 4). As noted, oxidative stress triggers protein damage, formation of toxic protein oligomers, protein aggregation, and accumulation of oxidized cellular components. In this setting, autophagy serves as a major mechanism of clearance of toxic elements that cannot be degraded by the proteasome. In addition, mitophagy eliminates mitochondria damaged by oxidative stress. In doing so, autophagic mechanisms not only eliminate damaged mitochondria, but they remove a major source of potentially damaging ROS (37).

The beneficial role of autophagy in oxidative stress has been highlighted in genetic models of defective autophagy, which manifest increased sensitivity to oxidative stress. *ATG5*, *ATG6/Beclin1*, and *ATG7* knockout mice, as well as *ATG3*-deficient T-cells, harbor increased ROS and accumulate enlarged and defective mitochondria (46, 55, 70, 71). *ATG7* knockout mice manifest deficient energy production and oxygen consumption (81). Similarly, decreased mitochondrial degradation and accumulation of ubiquitinated proteins are seen in *unc-51-like kinase-1 (ULK1)* knockout mice (35). In addition, *LC3*-deficient macrophages generate more superoxide in response to LPS (48). Thus, the autophagic process itself can be considered a bulk antioxidant mechanism.

### Conclusions and Perspective

Oxidative stress and autophagy are key elements in the pathological progression of many cardiovascular diseases, and they interact in intricate and important ways (Fig. 6). As the complex roles of these two processes are context dependent, a challenge in translating this biology to the clinical realm is the reality that neither can be completely abolished nor robustly activated. Translational efforts are already underway, as evidenced by several patents filed in recent years (Table 2). Moving forward, careful titration of the oxidative state and autophagic flux within the cardiomyocyte must be maintained to ensure proper cellular function. A comprehensive approach that considers the intricate interplay between these vital processes is likely to be required for success.

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#### Abbreviations Used

3MA = 3-methyladenine  
 ATG = autophagy-related protein  
 Bcl-2 = B-cell lymphoma-2  
 Bcl-xL = B-cell lymphoma-extra large  
 CryAB = alpha-crystallin B chain  
 DUOX = dual oxidase  
 eNOS = endothelial NOS  
 FoxO = Forkhead box-O  
 GSH = glutathione

GSHPx = glutathione peroxidase  
 GSSG = glutathione disulfide  
 HF = heart failure  
 I $\kappa$ B = inhibitor of kappa B  
 IKK = I $\kappa$ B kinase  
 iNOS = inducible isoform NOS  
 I/R = ischemia/reperfusion  
 JNK1 = c-Jun N-terminal kinase-1  
 Keap1 = Kelch-like ECH-associated protein-1  
 LAMP2 = lysosomal-associated membrane protein-2  
 L-NMMA = L-NG-monomethyl arginine citrate  
 LPS = lipopolysaccharide  
 LVAD = left ventricular assist device  
 MDA = malondialdehyde  
 mETC = mitochondrial electron transport chain  
 MI = myocardial infarction  
 MMP = metalloproteinase  
 MPG = N-2-mercaptopyrionyl  
 mTOR = mammalian target of rapamycin  
 NADH = nicotinamide adenine dinucleotide  
 NADPH = nicotinamide adenine dinucleotide phosphate  
 NAMDA-R1 = N-methyl-d-aspartate receptor-1  
 NF $\kappa$ B = nuclear factor kappa-light-chain-enhancer of activated B cells  
 nNOS = neuronal NOS  
 NO = nitric oxide  
 NOS = nitric oxide synthase  
 NOX = NADPH oxidase  
 Nrf2 = nuclear factor (erythroid-derived 2)-like 2  
 NRVMs = neonatal rat ventricular myocytes  
 OGG1 = 8-oxoguanine glycosylase 1  
 PE = phosphatidylethanolamine  
 RNS = reactive nitrogen species  
 ROS = reactive oxygen species  
 SNO = S-nitrosoglutathione reductase  
 SOD = superoxide dismutase  
 sTAC = severe thoracic aortic constriction  
 TAC = thoracic aortic constriction  
 TRX = thioredoxin  
 ULK1 = unc-51-like kinase-1  
 VPS = vacuolar protein sorting  
 XO = xanthine oxidase