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Oxidative Stress, Redox Signaling, and Autophagy: Cell Death *Versus* Survival

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Abstract

Significance: The molecular machinery regulating autophagy has started becoming elucidated, and a number of studies have undertaken the task to determine the role of autophagy in cell fate determination within the context of human disease progression. Oxidative stress and redox signaling are also largely involved in the etiology of human diseases, where both survival and cell death signaling cascades have been reported to be modulated by reactive oxygen species (ROS) and reactive nitrogen species (RNS). ***Recent Advances:*** To date, there is a good understanding of the signaling events regulating autophagy, as well as the signaling processes by which alterations in redox homeostasis are transduced to the activation/regulation of signaling cascades. However, very little is known about the molecular events linking them to the regulation of autophagy. This lack of information has hampered the understanding of the role of oxidative stress and autophagy in human disease progression. ***Critical Issues:*** In this review, we will focus on (i) the molecular mechanism by which ROS/RNS generation, redox signaling, and/or oxidative stress/damage alter autophagic flux rates; (ii) the role of autophagy as a cell death process or survival mechanism in response to oxidative stress; and (iii) alternative mechanisms by which autophagy-related signaling regulate mitochondrial function and antioxidant response. ***Future Directions:*** Our research efforts should now focus on understanding the molecular basis of events by which autophagy is fine tuned by oxidation/reduction events. This knowledge will enable us to understand the mechanisms by which oxidative stress and autophagy regulate human diseases such as cancer and neurodegenerative disorders. *Antioxid. Redox Signal*. 21, 66–85.

Introduction

AUTOPHAGY IS A transcendental homeostatic process in which certain components of a cell are engulfed by double-membraned autophagosomes and, subsequently, degraded in order to produce energy, or preserve cellular homeostasis and viability. It is most commonly seen in cells which are deprived of nutrients as a means for survival, but it has also been reported as an important phenomenon that regulates overall cellular homeostasis and disease progression. Autophagy breaks down compromised cellular components, such as damaged organelles and aggregated proteins, whose prevalence or accumulation within cells can lead to deleterious effects and, as a result, damage to tissues, organisms, and biological systems (22). Autophagy is a persistent homeostatic mechanism; almost all types of cells have basal levels of autophagy. Alterations in the autophagic cycle rate (flux), which begins with the formation of the phagophore and ends with the degradation of autophagosome cargo after its fusion with lysosome (Fig. 1), are commonly observed in

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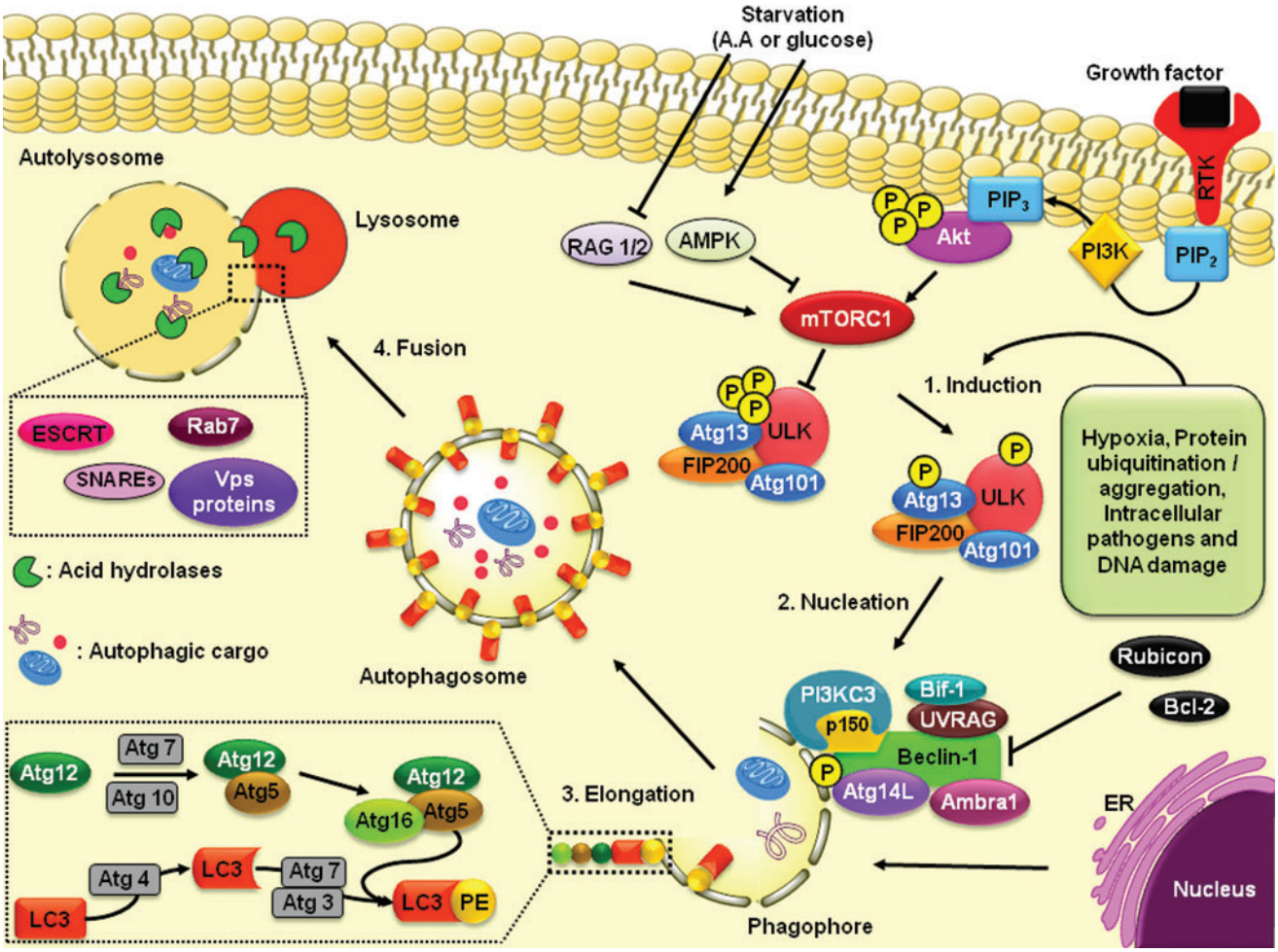

FIG. 1. Signaling machinery involved in macroautophagy. Macroautophagy requires the formation of distinct complexes during four sequential stages: (1) induction and (2) nucleation of the phagophore; (3) elongation and closure of the autophagosomes; and (4) fusion between autophagosomes and lysosomes. *See text for details*. The mTORC1 complex is regulated by stimulation of the class I PI3K–AKT pathway by growth factors, and *via* the regulation of RAG and AMPK proteins by amino acid (A.A.) or glucose starvation, respectively. mTORC1 negatively regulates the ULK1 complex, and starvation or growth factor withdrawal inhibits mTORC1, leading to its dissociation and dephosphorylation/activation of ULK1. Other mTOR-dependent or -independent stimuli such as hypoxia, ER stress, proteasome inhibition (proteasome aggregation and ubiquitination), apoptosis, and pathogen infection could lead to the induction autophagy by the activation of diverse signaling pathways. Autophagic cargo include any unwelcomed intracellular content such as long-lived or aggregated proteins/lipids, damaged organelles, and invading microbes. ER, endoplasmic reticulum. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

response to stress (78). In most cases, the induction of autophagy in response to stress acts as a pro-survival mechanism, while very few examples exist where autophagy has been clearly demonstrated to mediate cell death (31, 130).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive molecules that are generated as by-products from cellular metabolism under both normal and pathological conditions or upon exposure to xenobiotic agents. Similar to autophagy, basal or physiological levels of ROS/RNS formation play an important homeostatic role in regulating signal transduction involved in proliferation and survival (37). However, when ROS/RNS formation is dysregulated and surpasses antioxidant defenses, oxidative stress takes place, and when oxidative stress exceeds the capacity of the cell to repair biomolecule oxidation (nucleic acids, lipids, and proteins), oxidative damage occurs. Oxidative stress and ROS/RNS formation have been largely shown to regulate cell signaling involved in programmed cell death by apoptosis and/or necrosis (42).

Oxidative stress has been shown to lead to the accumulation of autophagosomes in different types of somatic cells (138). However, the redox events involved at the molecular level remain unclear. More importantly, the inter-relationship between alterations in the redox balance, oxidative stress or oxidative damage and autophagy, and their further pathological implications are unclear. A number of excellent reviews have been recently published regarding the role of autophagy and/or oxidative stress in human diseases such as brain ischemia (16, 50), neurodegeneration (55, 90, 146), cancer (90, 95, 144), diabetes (49, 118), immune (50) and cardiovascular diseases (8, 50, 102). The role of mitochondria and ROS formation in autophagy regulation has also been

recently reviewed (60, 88, 127). In this article, we will approach this issue from a different perspective and discuss the molecular mechanisms by which oxidative stress/damage and redox signaling regulate autophagy in the context of cell survival or cell death. We will focus on three specific topics: (i) the recent advances in our understanding of the molecular mechanism by which ROS/RNS generation, redox signaling, and/or oxidative stress/damage alter autophagic flux rates; (ii) the role of autophagy as a cell death process or survival mechanism in response to oxidative stress; and (iii) alternative mechanisms by which autophagy-related signaling regulates mitochondrial function and antioxidant response.

Overview of Autophagy and Signaling Processes Involved

There are three major recognized types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy is the most understood form of autophagy. It is responsible for the breakdown of proteins and organelles in the cell, and it is considered necessary for cell survival.

Macroautophagy

Macroautophagy, referred to here as autophagy, starts with the formation of a phagophore, which matures into a double-membrane autophagosome (Fig. 1). The phagophore has been hypothesized to be generated *de novo* from pre-existing intracellular precursor molecules or multiple sources that include the endoplasmic reticulum (ER), the outer mitochondrial membrane, and the plasma membrane. The autophagosome then fuses with a lysosome, forming an autolysosome in which cellular cargo is degraded by lysosomal hydrolyses in order to eliminate damaged or harmful components *via* catabolism and recycling to maintain nutrient and energy homeostasis. This process is regulated by a variety of signaling proteins and complexes. One class of these proteins is designated as ''Atg,'' or autophagy-related genes originally identified in yeast (78) (Fig. 1).

Macroautophagy molecular mechanism

Mammalian autophagy requires five molecular components, including (1) the Atg1/unc-51-like kinase (ULK) complex for initiation; (2) the Bcl-2 interacting myosin/moesin-like coiled-coil protein 1 (Beclin-1)/class III phosphatidylinositol 3-kinase (PI3KC3) complex for nucleation; (3) the ubiquitin-like protein conjugation systems Atg12 and LC3 for elongation and closure; (4) the transmembrane proteins Atg9 and vacuole membrane protein 1 (VMP1); and (5) proteins that mediate fusion between autophagosomes and lysosomes (78).

Induction. Growth factors stimulate the class I phosphatidylinositol 3-kinase–AKT pathway and other nutrient-related signals, which *via* the mammalian (also known as mechanistic) target of rapamycin (mTOR) macromolecular complex 1 (mTORC1), negatively regulate the ULK1, Atg13, Atg101, and FIP200 complex. Starvation is a major inducer of autophagy by the inhibition of mTORC1, leading to its dissociation from the ULK complex and dephosphorylation/activation of ULK1 (or ULK2) (101) (Fig. 1). Recently, it was demonstrated that ULK1 and/or ULK2 are not required for the autophagy response to the enhanced amino-acid catabolism which is induced by the deprivation of glucose or direct exposure to ammonia (20).

Nucleation. The Beclin-1 complex includes Beclin-1, PI3KC3, p150, Atg14L, and Ambra1, which mediate the allosteric activation of the class III PI3K PI3KC3 to generate phosphatidylinositol-3-phosphate (PI3P) (Fig. 1). PI3P recruits effectors such as the double FYVE domain-containing protein 1 (DFCP1) and WD-repeat protein interacting with phosphoinositides (WIPI) family proteins to mediate the initial stages of vesicle nucleation/autophagosome formation. Atg14L (Barkor) is essential for PI3K activity. UVRAG (UV radiation resistance-associated gene), Ambra1, and Bif-1/endophilin B1 interact with Beclin-1, inducing autophagy. The binding of the anti-apoptotic proteins Bcl-2, Bcl-xl, or Mcl-1 as well as Rubicon (RUN domain protein as Beclin-1 interacting and cysteine-rich containing) to Beclin-1 inhibit autophagy. In contrast, Beclin-1 phosphorylation and BH3-only Bcl-2 proteins, which compete for anti-apoptotic Bcl-2 members, induce autophagy (78, 119) (Fig. 1). Noncanonical, Beclin-1-independent autophagy has also been reported during apoptosis, differentiation, and bacterial toxin uptake (26).

Elongation and fusion. Autophagosomal elongation requires the ubiquitin-like conjugation systems Atg5–Atg12 and the microtubule-associated protein light chain 3 (LC3) conjugation systems (Fig. 1). The covalent conjugation of Atg12 to Atg5 occurs *via* the E1-like enzyme Atg7 and the E2-like enzyme Atg10 and is organized into a complex by a noncovalent association with Atg16. This complex is essential for the elongation of the preautophagosomal membrane, but dissociates from fully formed autophagosomes. The Atg12-Atg5-Atg16 complex can function as the E3 ligase of LC3. In mammals, three human LC3 isoforms (LC3A, LC3B, and LC3C) have been identified, which exhibit distinct expression patterns in different human tissues. Although mammalian cells contain several variants of LC3, LC3B is the most widely used marker in autophagic assays, as LC3B is expressed in nearly all tissues (56). The conjugation of phosphatidylethanolamine (PE) to soluble LC3 (LC3-I) is mediated by the sequential action of the protease Atg4, the E1-like enzyme Atg7, and the E2-like enzyme Atg3. LC3-II (autophagic vesicle-associated form or lipidated form) is specifically targeted to the elongating autophagosome and remains on autophagosomes until their fusion with lysosomes (Fig. 1). Then, LC3-II on the cytoplasmic face of autolysosomes is delipidated by Atg4 and recycled, while LC3-II found on the internal surface of autophagosomes is degraded in the autolysosomes (78). Mouse cells lacking Atg5 or Atg7 can still form autophagosomes/autolysosomes, which do not correlate with LC3-II accumulation (108). Autophagosomes move along microtubules, which require the function of dynein motor proteins. Depolymerization of microtubules or inhibition of dynein-dependent transport results in the inhibition of autophagy. The fusion step of autophagosomes with lysosomes involves proteins such as ESCRT, SNAREs, Rab7, and the class C Vps proteins (140).

Selective autophagy forms

Autophagy is, in many cases, considered a nonselective bulk degradation pathway. However, several forms of

selective autophagy have been recently identified as participating in organelle clearance such as ER-phagy, pexophagy (peroxisomes), mitophagy (mitochondria), and ribophagy (ribosomes). Selective autophagy is not limited to the degradation of organelles. Lipid (macrolipophagy) glycogen and pathogen (xenophagy) degradation have also been described. Autophagic adaptors p62/SQSTM1 (sequestrome 1) and NBR1 (neighbor of Brca1 gene) are selectively degraded by autophagy, and they act as cargo receptors for the degradation of ubiquitinated substrates. A direct interaction between these autophagic adapters and the autophagosomal marker protein LC3 is mediated by the LIR (LC3-interacting region) motif, while their Ub-associated (UBA) domain binds to mono- and poly-ubiquitin (65, 74).

Mitochondria are dynamic organelles that undergo continuous events of fission and fusion. These opposing processes work in concert to maintain the shape, size, number of mitochondria, and their physiological function. Fusion enables mitochondrial content to be mixed between neighboring organelles. Fission enables the distribution of mitochondria to daughter cells after mitosis and also represents a quality control mechanism where damaged mitochondria can be turned over by autophagy (15, 79, 124). Two types of mitophagy pathways have been described to date. Mitophagy has been shown to be regulated by the outer mitochondrial membrane PTEN-induced putative kinase 1 (PINK1) and the E3-ubiquitin ligase Parkin (also known as PARK2) whose mutations are associated with Parkinson's disease (PD) (Fig. 2). Parkin translocates from the cytoplasm to defective mitochondria, which is associated with mitophagy. PINK1 overexpression is sufficient to translocate Parkin even in the absence of mitochondrial stress (148). It has been shown that PINK1 directly interacts with Parkin, resulting in Parkin translocation to mitochondria and the activation of Parkin to ubiquitinated mitochondrial substrates, including mitofusins (Mfn1 and Mfn2) and the mitochondrial outer membrane–voltage-dependent anion channel 1 (VDAC1). Polyubiquitination of mitofusins and their proteasomal degradation is required for proper mitophagy. Polyubiquitinated VDAC1 (Lys 27 chains) recruits the autophagy receptor p62, which recognizes mitochondria *via* its UBA domain and the LIR motif (109) (Fig. 2). However, mitophagy can occur even in the absence of p62 localization at the mitochondria (107). The removal of mitochondria during development is a programmed mechanism for the elimination of ''healthy'' mitochondria. Mitophagy in

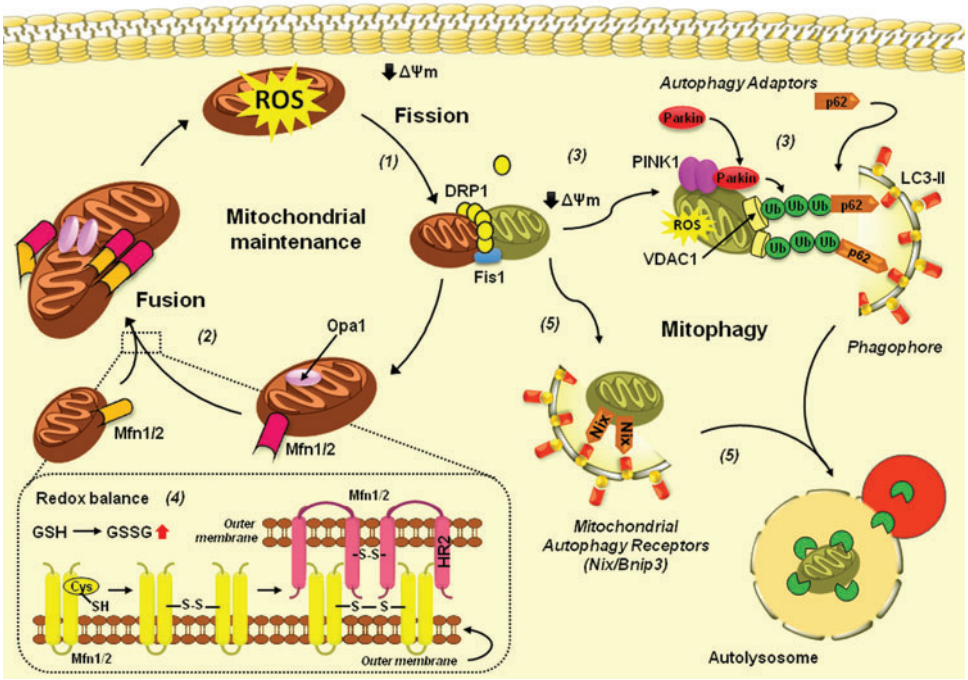

FIG. 2. Mitochondrial fusion, fission, and mitophagy. Mitochondrial maintenance is a dynamic process undergoing continuous events of fission and fusion to preserve proper mitochondrial functions. (1) Fission requires local organization of Fis1 and recruitment of the GTPase DRP1 for assembly of the fission machinery that subsequently leads to membrane scission. (2) Fusion is mediated by the dynamin GTPases Mfn1/2 at the outer membrane and the optic atrophy protein Opa1 at the inner membrane that tether adjacent mitochondria together. In order to transform normally elongated mitochondria into a form suitable for engulfment, mitochondrial fission can precede mitophagy. In addition, on oxidative stress, mitophagy can decrease mitochondria-derived ROS formation, *via* degradation of damaged mitochondria. (3) After fission, reduced mitochondrial membrane potential ($\Delta\Psi$m) leads to the translocation of PINK1and Parkin to the mitochondria, where it promotes the ubiquitination of proteins in the mitochondrial membrane such as VDAC1, which recruit the autophagy receptor p62 that targets mitochondria for removal. (4) GSSG accumulation has been demonstrated to mediate the oxidation of cysteines within Mfns by disulfide bond formation, causing a conformational change that aids in the tethering of Mfns (*via* the heptad repeat domain, HR) to enhance membrane fusion. (5) Nix-dependent autophagy is involved in the removal of ''healthy'' mitochondria during development. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

this situation seems to depend on Nix (Bnip3). Nix directly interacts with LC3/GABARAP *via* the LIR on the N-terminal part of Nix, a WxxL tetrapeptide motif found in autophagic cargo recognition receptors (Fig. 2). Nix-independent mitophagy pathway is induced by mitochondria depolarization and BH3 mimetics. Atg7 and ULK1 proteins are also involved in programmed mitochondrial clearance. The Nix homolog Bnip3 has similar mitochondrial localization and an interaction pattern with LC3/GABARAP (109).

Microautophagy

In microautophagy, portions of cytoplasm in mammalian cells are directly sequestered and, subsequently, engulfed by lysosomes. The formation of intralysosomal vesicles (also referred to as lysosomal wrapping mechanism) starts with the direct engulfment of cytoplasmic components by pre-existing primary or secondary lysosomes followed by the opposition of the extensions ends, leading to the sealing of the sequestered materials. Lysosomal enzymes are proposed to be directly acquired by degeneration of the inner membrane. Microautophagy in mammalian cells is unresponsive to amino-acid and glucagon deprivation (100).

Chaperone-mediated autophagy

CMA specializes in breaking down cytosolic proteins. Proteins degraded by CMA are identified by a chaperone that delivers them to the surface of the lysosomes, where substrate proteins unfold and cross the lysosomal membrane (Fig. 6). CMA is mediated by the presence of a CMA target sequence. All CMA-targeting motifs contain one or two lysine (K) or arginine (R) positively charged amino-acid residues; one or two isoleucine (I), leucine (L), valine (V), or phenylalanine (F) hydrophobic residues; one aspartate (D) or glutamate (E) negatively charged residue; and one glutamine (Q) on either side of the pentapeptide. CMA-targeting motifs can also be generated through post-translational modifications such as phosphorylation or acetylation. These sequences, located in the C, N terminus, or in the central region of the protein, are inaccessible in properly folded proteins, but become available when the protein is misfolded. About 30% of soluble cytosolic proteins contain a putative CMA-targeting motif. These motifs are recognized specifically by the cytosolic protein Hsc70 (heat shock cognate protein of 70 kDa). Hsc70 binding to KFERQ motifs has also been demonstrated to mediate endosomal microautophagy. Chaperone-targeted proteins for CMA bind to the lysosomal membrane *via* an interaction with LAMP-2A (lysosome-associated membrane protein type 2A). Multimerization of LAMP-2A is required for substrate translocation. Once the substrate has been released into the lysosomal lumen, LAMP-2A dissociates into monomers. Hsc70 also associates with the cytosolic side of the lysosomal membrane, where it has been proposed to contribute to protein unfolding and disassembly of the LAMP-2A translocation complex (72).

ROS/RNS Formation, from Redox Signaling to Oxidative Stress and Oxidative Damage

ROS and RNS formation

Several organelles within the cell have the ability to produce ROS. These include peroxisomes (129), the ER (93), autophagosomes/lysosomes (80), endosomes (91), and the nucleus (133). However, one of the main source of ROS are mitochondria (104), where superoxide ($O_2^{\bullet -}$) is produced in the matrix by one-electron reduction of O_2 through complex I (51), and in both the matrix and the inner membrane space (IMS) by complex III (17) of the electron transport chain (103) (Fig. 3). A second important source of ROS production is the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) family of enzymes that catalyze the production of $O_2^{\bullet -}$ from O_2 and NADPH (62). The most important cellular defenses against $O_2^{\bullet -}$ are superoxide dismutases (SODs). In mammals, there are three compartmentalized isoforms: manganese superoxide dismutase (MnSOD or *SOD2*) localized in the mitochondrial matrix (136); copper-zinc SOD (CuZnSOD or *SOD1*) in the IMS, peroxysomes, nucleus, or cytosol (110); and extracellular SOD (EcSOD or *SOD3*) anchored to the cell's surface. SODs generate hydrogen peroxide (H_2O_2) (Fig. 3), which can act as a second messenger due to its low reactivity, specificity for cysteine residues, and ability to diffuse across membranes (58). In addition, H_2O_2 reacts with several molecules or metals ions and produces hydroxyl radical ($OH^{\bullet}$) *via* Fenton reaction (25).

Nitric oxide ($NO^{\bullet}$) is a hydrophobic molecule that diffuses freely across membranes and is generated from L-arginine by nitric oxide synthases (NOS) (99). $O_2^{\bullet -}$ reacts with $NO^{\bullet}$, leading to the production of peroxynitrite ($ONOO^-$), which is also able to cross membranes through anion channels in the anionic form, and by passive diffusion in its protonated form, peroxynitrous acid (ONOOH) (30) (Fig. 3). A number of cellular defenses exist against peroxides. Catalase mediates the decomposition of H_2O_2 and is primarily localized in the peroxisomes. Glutathione peroxidases (Gpx) are selenoproteins that reduce peroxides. Gpxs encoded by different genes vary in their cellular location and substrate specificity. Gpx1 is found primarily in the cytoplasm and preferably scavenges H_2O_2, while Gpx4 (PhGpx) hydrolyzes lipid hydroperoxides in both cytosolic and mitochondrial compartments. Peroxiredoxins (Prxs) are ubiquitous thiol peroxidases. Mammals have six Prxs, with Prx1, 2, and 6 found in the cytoplasm; Prx4 in the ER; Prx3 in the mitochondria; and Prx5 found in various compartments within the cell, including peroxisomes and mitochondria (54) (Fig. 3).

Other enzymatic reactions also mediate reactive species formation. Myeloperoxidases (MPO) produce hypochlorous acid (HOCl) from H_2O_2 and chloride anion (Cl^-) using heme as a cofactor (Fig. 3). MPO also oxidize tyrosine to tyrosyl radical using H_2O_2 as an oxidizing agent (141). Cyclooxygenases (COX) produce ROS as a by-product of arachidonic acid metabolism to prostaglandin G2 (PGG_2), utilizing two O_2 molecules and producing peroxyl radicals. COXs also possess a heme-containing active site that provides peroxidase activity, converting PGG_2 to prostaglandin H2 (PGH_2) by removing O_2, which is a source of oxygen radicals. In the presence of H_2O_2, the peroxide activity of COXs oxidizes various co-substrates such as NADH and GSH, which reduces O_2 to $O_2^{\bullet -}$ (61). Heme oxygenase (HO) catalyzes the first rate-limiting step in heme degradation, playing an important role in Fe^{2+} recycling. HO cleaves the carbon bridge of heme, resulting in decreased oxidative stress by the removal of heme (67).

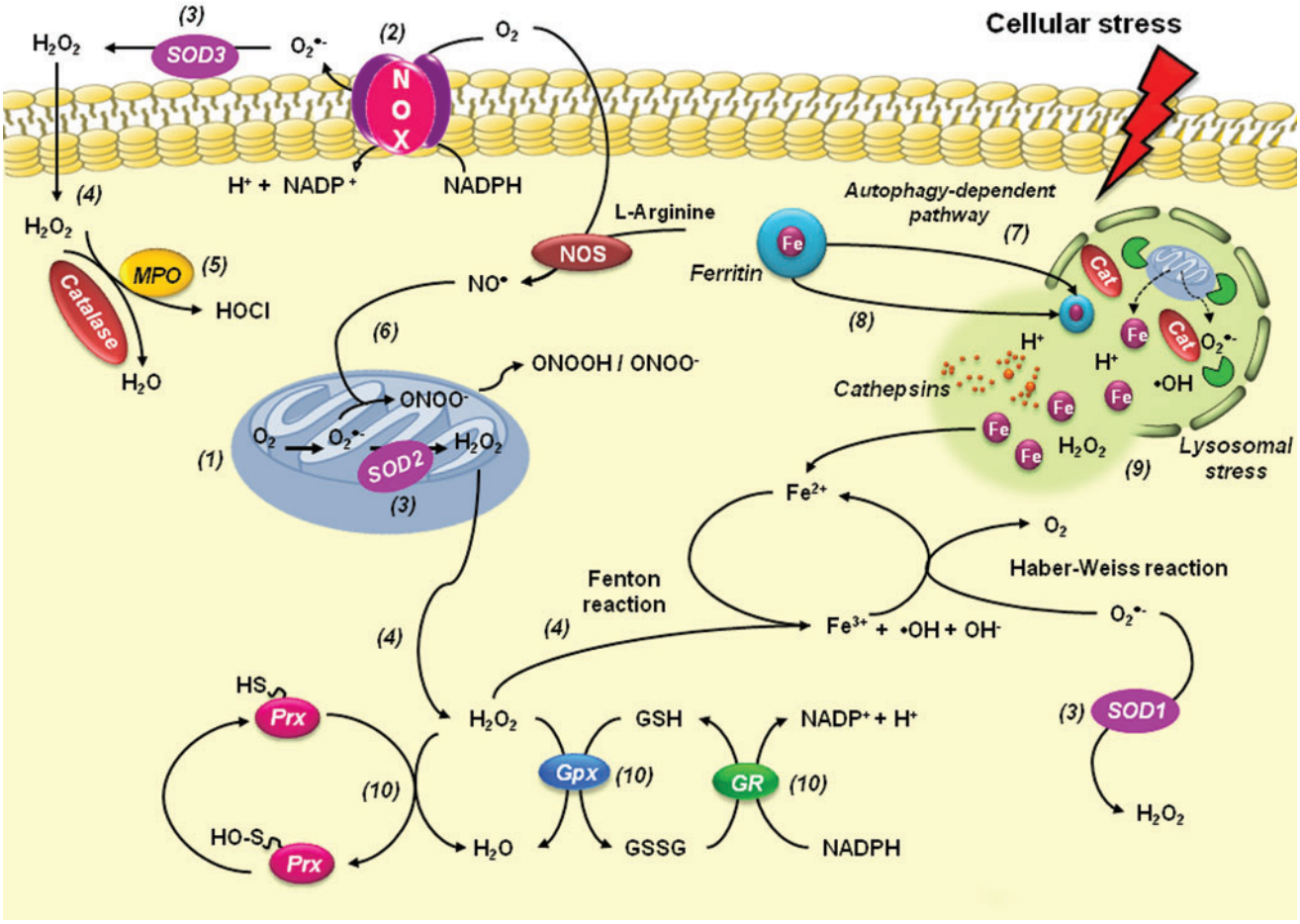

FIG. 3. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation. (1) Mitochondria are the primary source of ROS, where $O_2^{\bullet-}$ is produced primarily in the mitochondrial matrix. (2) NADPH oxidase (NOX) enzymes are also important sources for superoxide anion ($O_2^{\bullet-}$). (3) The most important cellular defenses against $O_2^{\bullet-}$ are superoxide dismutases (SODs), which generate hydrogen peroxide (H_2O_2) as a by-product. (4) H_2O_2 can diffuse across membranes and react with several molecules or metals to produce hydroxyl radical ($OH^\cdot$) by Fenton reaction. (5) Myeloperoxidases (MPO) produce hypochlorous acid (HOCl) from H_2O_2 and chloride anion (Cl^-). (6) Nitric oxide ($NO^\bullet$) generated by nitric oxide synthases (NOS) also has the ability to diffuse across membranes and react with $O_2^{\bullet-}$, leading to the production of peroxynitrite ($ONOO^-$). (7) Lysosomes are an important source of iron (Fe^{2+}) and ROS. Iron can be released from ferritin by targeting ferritin to the lysosome *via* a mechanism that involves autophagy under iron-depleted conditions. In contrast, (8) lysosomal targeting of ferritin in iron-rich conditions does not involve autophagy. (9) In response to cellular stress, lysosomal membrane permeabilization contributes to oxidative stress *via* the release of iron and ROS from damaged organelles. (10) A number of antioxidant defenses such as SODs, catalase, glutathione peroxidases (GPXs), and peroxiredoxins (Prxs) exist that contribute to the tight control of redox balance within the cell. GR, glutathione (GSH) reductase; GSSG, glutathione disulfide; NADP(H), nicotinamide adenine dinucleotide phosphate; ONOOH, peroxynitrous acid. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Oxidative stress, redox signaling, and oxidative damage

Both reversible and irreversible modifications participate in the signal transduction that is mediated by ROS/RNS. Redox signaling involves a targeted modification by reactive species through a chemically reversible reaction. In redox signaling, the reaction of ROS/RNS with the target molecule acts as an on-off switch signal (40). Oxidative damage in response to oxidative stress leads to irreversible oxidation of proteins lipids and nucleic acids. However, since amino-acid residues in proteins, fatty acids in lipids, and nucleic acid bases have different susceptibility to oxidative stress, ''mild'' oxidative stress appears to provide selectivity for a specifically targeted molecule and may constitute a signaling mechanism even when an irreversible modification is produced. Oxidative damage can be repaired to a certain extent, as evident in the diverse array of DNA repair systems. In addition, oxidized proteins can be effectively degraded and recycled by both the proteasome and autophagy systems. Proteasomal degradation of oxidatively modified proteins requires protein unfolding; thus, only mildly oxidized proteins are suitable proteasome substrates. During oxidative stress, the resulting cellular response and outcome is likely to involve both redox

signaling and oxidative damage, whose contribution will depend on the concentration and nature of the ROS/RNS involved (40). In contrast, since normal cellular homeostasis depends on redox signaling as well, oxidative stress may be redefined as the disruption of redox homeostasis (66). Thus, it is hard to differentiate clearly between oxidative stress and redox signaling.

Similar to autophagy, ROS/RNS formation has been linked to the regulation of both pro-survival and cell death pathways (Fig. 4). In general terms, basal levels of ROS/RNS formation and those induced by growth factor receptor activation are important to maintain proper cellular homeostasis and mediate cell proliferation by redox signaling (106). ROS/RNS-mediated redox signaling also regulates survival-promoting adaptive responses to cellular stress. Redox signaling, in general, occurs in the absence of an overall imbalance of pro-oxidants and antioxidants (66). In contrast, when antioxidant defenses are surpassed by ROS/RNS formation, and oxidative damage is not repaired by endogenous mechanisms, oxidative stress leads to cell demise. However, although oxidative damage to proteins, lipids, and nucleic acids is associated with the activation of programmed cell death, both pro-apoptotic and pro-survival signaling proteins are modulated by specific reversible oxidative modifications (1, 106).

Oxidative Stress-Induced Cell Death and Autophagy: Cell Death *Versus* Cell Survival

Cell death pathways are generally classified by biochemical and morphological criteria. Accordingly, three distinct types of pathways are generally recognized. These are apoptosis, necrosis, and autophagy, although there are numerous examples in which cell death displays mixed features (45, 77).

Apoptosis

Apoptosis is a ubiquitous homeostatic mechanism critical in the turnover of cells in tissues and during normal development and senescence. Dysregulation of apoptosis occurs as either a cause or consequence of distinct pathologies, including cancer, autoimmune diseases, and neurodegenerative disorders (36). Cell death by apoptosis is characterized by the sequential activation of defined signaling pathways conveying specific biochemical and morphological alterations, which include the activation of caspases and endonucleases, cell shrinkage, loss

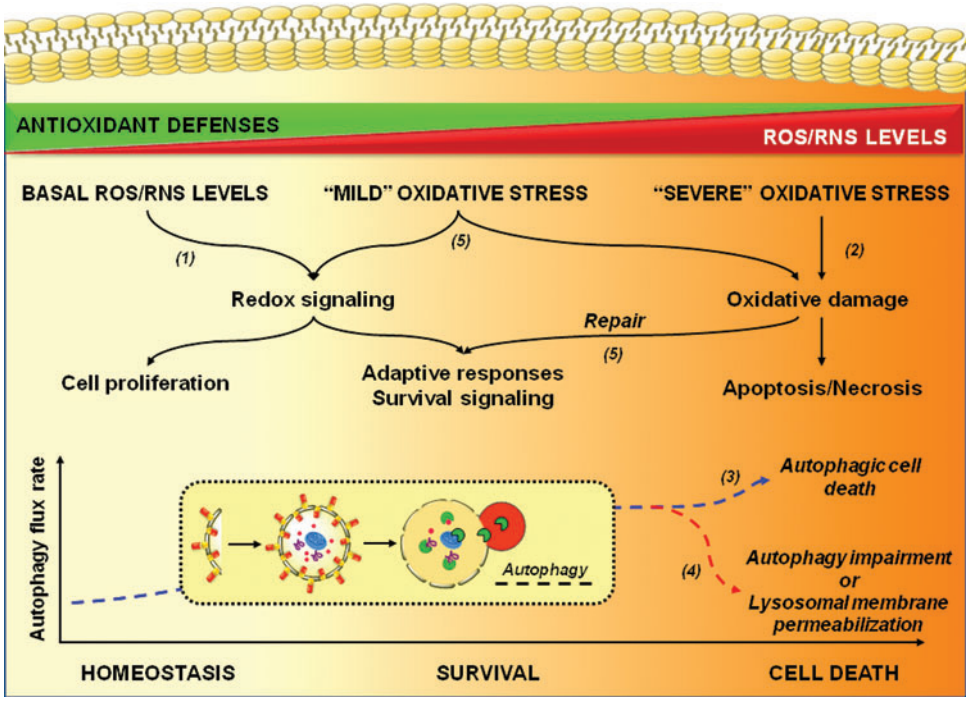

FIG. 4. Oxidative stress, redox signaling, and autophagy, cell death *versus* survival. (1) Basal or physiological levels of ROS/RNS play an important homeostatic role regulating signal transduction involved in proliferation and survival. (2) In contrast, when antioxidant defenses are surpassed by ROS/RNS formation, and oxidative damage is not repaired by endogenous mechanisms, oxidative stress leads to cell demise. (3) Under these pathological conditions, ''excessive'' autophagy might promote cell death through the degradation of important components within the cell *(dotted blue lines)*. In addition, (4) lysosomal membrane permeabilization induced by stress can also contribute to cell death *(dotted red lines)*. However, (5) ''mild'' oxidative stress can act as a signaling mechanism leading to adaptive stress responses. Oxidative damage can be repaired to a certain extent, and oxidized biomolecules, such as proteins, can be degraded and recycled by distinct processes, including autophagy. During oxidative stress, the resulting cellular response and outcome is likely to involve both redox signaling and oxidative damage, whose contribution will depend on the concentration and nature of the ROS/RNS involved, the duration of the stress response, as well as cell type or gender. A clear distinction between both oxidative stress and redox signaling is hard to define. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

of plasma membrane lipid asymmetry, chromatin condensation, apoptotic body formation, and ultimate cellular fragmentation (44).

Necrosis

Necroptosis, necrosis, and secondary necrosis after apoptosis have been recently described as different mechanisms of cell death that result in similar cellular morphological features (cytoplasmic swelling), particularly during the disintegration phase: oxidative burst, mitochondrial membrane hyperpolarization, lysosomal membrane, and plasma membrane permeabilization (56). Necrosis occurs as an accidental type of cell death in response to compromised cell integrity. Necrotic cell death can be finely regulated by specific signal transduction pathways and catabolic processes (necroptosis), which include the involvement of receptor interaction protein kinase 1 and 3 (RIP1 and RIP3) and can be specifically inhibited by necrostatins (147). Necrosis occurs in inflammatory and neurodegenerative disorders, heart disease, neuronal ischemia, muscular dystrophy, diabetes, infections, as well as in apoptotic cells that fail to be engulfed by phagocytosis (secondary necrosis) (77, 98).

Autophagy

Autophagy is generally considered a homeostatic mechanism for cell survival during stress conditions *via* the degradation of damaged cellular components and the recycling of cellular constituents. Autophagic cell death has been defined morphologically by massive autophagic vacuolization of the cytoplasm in the absence of chromatin condensation. Although dysregulation of autophagy has been associated with several pathologies, it is regarded primarily as a pro-survival mechanism, and there are only a limited number of cases where autophagy has been established as the bona fide cause of cell death (Fig. 4). Most of the literature out there proposing a role of autophagy as a mechanism to execute cell death has been based on pharmacological inhibitors with relatively poor selectivity (130). Current genetic tools can provide a more reliable approach to dissect the role of autophagy as a cell death pathway. However, it is important to note that in many instances distinct cell death mechanisms can be activated simultaneously and that the inhibition of one specific pathway may ''switch'' to another cell routine. It is well known that caspase inhibition can trigger necrosis (98). More recently, caspase inhibition has also been reported to induce autophagic cell death in mouse fibrosarcoma cells, which was blocked by Atg7 and Atg8 knockdown (149). We will next review the evidence supporting a role for autophagy as a cell death pathway or survival response upon oxidative stress.

Oxidative stress-induced autophagic cell death

A number of studies have reported the occurrence of both autophagy and oxidative stress in response to cell death stimuli. However, very little experimental evidence is found regarding a direct role for autophagic cell death in ROS/RNS or oxidative stress-mediated toxicity. For example, cell death induced by direct generation of $O_2^{\bullet -}$ (xanthine + xanthine oxidase + catalase system) was reported to induce cell death with both autophagic and necrotic features, and knockdown of Atg7 only delayed early cell death progression in primary murine cortical neurons (57). In contrast, increased H_2O_2 formation by MnSOD up-regulation was shown to mediate autophagic cell death in senescent keratinocytes as evidenced by its inhibition through Atg5 knockdown (32).

Impairment of mitochondrial function and concomitant ROS formation mediates autophagic cell death. For instance, knockdown/inhibition of the mitochondrial uncoupling protein 2 (UCP2) induces ROS-dependent autophagic cell death in human pancreatic adenocarcinoma cells (28). Autophagic cell death induced by inhibition of complex I (1-methyl-4-phenylpyridinium [MPP^+], rotenone) or II (2-thenoyltrifluoroacetone) has been reported to depend on $O_2^{\bullet -}$ formation (18) and is reduced by knockdown of Atg5, Atg7, and/or Beclin-1 in the transformed HEK 293, U87, HeLa, and SH-SY5Y cell lines (18, 151). However, research by our group and others has recently demonstrated that cell death by inhibition of complex I is largely independent of mitochondrial $O_2^{\bullet -}$ formation (12, 33, 39, 87, 105, 123); while impairment of autophagic flux potentiates MPP^+ and rotenone toxicity in human neuroblastoma cell lines and in primary ventral midbrain neurons from postnatal rats (29, 46, 94). Knockdown of Beclin-1, Atg5, or Atg7, and overexpression of dominant-negative Vps34 protects against H_2O_2-induced cell death, suggesting a role for autophagy in cell demise both *in vitro* and *in vivo* (13, 14, 19, 81). However, Atg5 deficiency in mouse embryonic fibroblasts ($Atg5^{-/-}$ MEFs) and Atg5 knockdown in HT22 neuronal cells induce compensatory pro-survival signaling, which might interfere with cell death induced by oxidative stress (116).

Autophagic cell death induced by oxidative stress has been reported to participate in a few pathological conditions. For example, *Mycobacterium tuberculosis*–induced macrophage death was reported to be mediated by NOX- and mitochondria-derived ROS, and it was also demonstrated to be inhibited by Beclin-1 and Atg5 knockdown (131). Dopaminergic cell death in PD has also been proposed to be mediated by autophagic cell death. Mitochondrial dysfunction observed in PD is modeled by using complex I inhibitors. As mentioned earlier, dopaminergic cell death induced by complex I inhibition has been associated with autophagy. In addition, iron (Fe^{2+})-induced toxicity in dopaminergic cells was also proposed to induce autophagic cell death (21). Similarly, dopaminergic cell death induced by exogenous addition of H_2O_2 was suggested to be mediated autophagy (13, 14, 23). However, in all these studies, the role of autophagic cell death was primarily determined by the use of pharmacological agents (21).

Autophagic cell death is defined by the ''excessive'' degradation of essential cellular components that are required for normal cell function. However, lysosomal membrane permeabilization in response to stress is another potential mechanism by which the autophagic machinery can contribute to cell death. The release of lysosomal proteases such as cathepsins has been demonstrated to mediate apoptosis induced by oxidative stress (68, 121, 122). In addition, since lysosomes are an important source of iron and ROS, they might contribute to the exacerbation of oxidative damage (5, 83, 85, 111) (Fig. 3).

Oxidative stress-induced cell death, a protective role for autophagy

A number of recent studies have demonstrated the protective effect of autophagy against oxidative stress-induced

cell death. In human glioma U251 cells, H_2O_2 induces autophagy, which is paralleled by increased Beclin-1 expression and mTOR signaling inhibition, and its blockage stimulates apoptosis (150). The protective effect of growth factor signaling against oxidative damage has been attributed to increased autophagy. For example, platelet-derived growth factor protects against oxidative-protein damage and cell death induced by 4-hydroxynonenal in vascular smooth muscle cells by increasing autophagy (125). Cardiomyocyte and neuronal cell death induced by mitochondrial ROS formation is either reduced by autophagy stimulators (35) or enhanced by pharmacological or genetic approaches that impair autophagic flux (48).

Autophagy provides cells with nutrients upon starvation. It has been shown that starvation-induced autophagy depends on mitochondrial $O_2^{\bullet -}$ formation, which also regulates adenosine monophosphate-dependent protein kinase (AMPK) activation in animal models and HeLa cells (16, 89). Similarly, in endothelial cells, autophagy induced by inhibition of glycolysis with 2-deoxy-D-glucose (2-DG) is mediated by AMPK activation *via* ROS formation (142). Hypoxia-reoxygenation-induced ROS also trigger autophagy, whose inhibition increases apoptotic cell death in primary hepatocytes (6).

$O_2^{\bullet -}$, but not H_2O_2, has been proposed to be the major ROS regulating autophagy (16). However, this conclusion was reached with the use of nonselective fluorescent probes to quantify $O_2^{\bullet -}$ and H_2O_2 levels (69). In cancer cells, cell death induced by chemotherapeutic agents such as cisplatin depends on ROS formation, and autophagy has been shown to exert a protective effect by decreasing ROS accumulation (70).

Regulation or Cellular Redox Homeostasis, ROS Formation, and Oxidative Stress/Damage by Autophagy

Autophagy regulates ROS formation and antioxidant response

Alterations in the autophagy flux/rate have been shown to regulate both redox balance and ROS formation under distinct circumstances. For example, autolysosomes have been proposed as sources for ROS formation in neuronal cells in response to excitotoxic glutamate concentrations (80). In addition, autophagy has been shown to mediate catalase degradation and excessive ROS accumulation, leading to nonapoptotic cell death of mouse fibrosarcoma cells (149). Ferritin is a cytosolic protein that stores iron and protects cells from iron toxicity. Ferritin-bound iron is utilized when cells become iron deficient. Autophagy-dependent and -independent pathways of ferritin delivery to lysosomes have been reported. The acidic environment of the lysosome mediates iron extraction from ferritin for its utilization by cells, and recently, ferritin was shown to be degraded in the lysosome under iron-depleted conditions. (2, 84) (Fig. 3).

The transcription of a variety of antioxidant genes through cis-acting sequences known as antioxidant response elements (ARE) in response to oxidative stress is mediated by the nuclear factor (erythroid-derived 2)-like 2 transcription factor (Nrf2). Nrf2 is sequestered in the cytoplasm by the kelch-like ECH-associated protein 1 (Keap1)-Cul3 complex and degraded in a ubiquitin-proteasome-dependent manner. Oxidant- or electrophile-induced modification of two reactive cysteine residues (Cys273 and Cys288) in Keap1 inhibits Nrf2 ubiquitination, enabling its translocation to the nucleus (7). Indirectly, Atg7/p62-dependent autophagic degradation of Keap1 has been shown to activate Nrf2 and protect against oxidative stress (4, 75, 86, 139). Transcriptional up-regulation of p62 by Nrf2, subsequently, creates a positive feedback loop (63) (Fig. 5).

Mitophagy regulates ROS formation

Mitophagy is induced by oxidative stress and is involved in the removal of dysfunctional mitochondria. Direct generation of mitochondrial ROS using a mitochondrial-targeted photosensitizer has been reported to induce mitophagy (143). In contrast, in cytochrome C- and mitochondrial mtDNA-deficient $\rho 0$ cells, STS-induced autophagy was not correlated with ROS formation and remained unaffected by antioxidant enzymes, suggesting that mitochondrial ROS are not required for mitophagy (64). Mild oxidative stress selectively triggers mitophagy in the absence of macroautophagy, which is observed to be dependent on the DRP1 mitochondrial fission protein in both mouse and human cells (43) (Fig. 2). Interestingly, both nonselective autophagy (*atg1*Δ)- and mitophagy (*atg32*Δ or *atg11*Δ)-deficient yeast cells are characterized by an enhanced accumulation of ROS on starvation (82, 137). However, the mechanisms mediating ROS accumulation are different. In nonselective autophagy-deficient cells, ROS accumulation on starvation is associated with a reduction in the cellular amino-acid pool and a reduction in the expression of mitochondrial respiratory and scavenger proteins (137). In contrast, in mitophagy-deficient cells, excess mitochondria are not degraded and produce excess ROS (82). Though autophagy has a clear role in regulating mitochondrial homeostasis, signaling cascades involved in autophagy can indirectly regulate mitochondrial function. For example, in skeletal muscle tissues and cells, mTOR inhibition with rapamycin decreases the expression of the peroxisome-proliferator-activated receptor coactivator (PGC)-1alpha whose transcriptional activity regulates mitochondrial gene expression and biogenesis, and, consequently, ROS formation (27).

Autophagy and the ubiquitin-proteasome system regulate oxidized protein turnover

Proteins can be damaged by ROS/RNS and its reactive metabolites. Depending on the balance between their oxidation and their degradation/repair systems, oxidized proteins can form oligomeric complexes, resulting in the formation of protein aggregates. Only a few repair/reduction mechanisms with regard to oxidative protein damage/modification have been demonstrated. A large variety of oxidative protein modifications can be induced either directly by free radicals or indirectly in a secondary reaction *via* by-products of oxidative stress. Thus, proteins containing irreversible oxidative modifications should be degraded in order to maintain proper cellular protein homeostasis. The proteasome is a multicatalytical protease that consists of a 20S core structure formed by two β-rings which possess the proteolytic activity, and two α-rings that function as regulators of substrate access. When the 20S proteasome binds two 19S regulators on opposite sides of the barrel, it forms the 26S proteasome that binds and degrades polyubiquitinated proteins in an ATP-dependent manner. Degradation by the 20S proteasome is ATP- and ubiquitin independent but still requires proteins to be in an unfolded state with exposed hydrophobic surface structures (115) (Fig. 6).

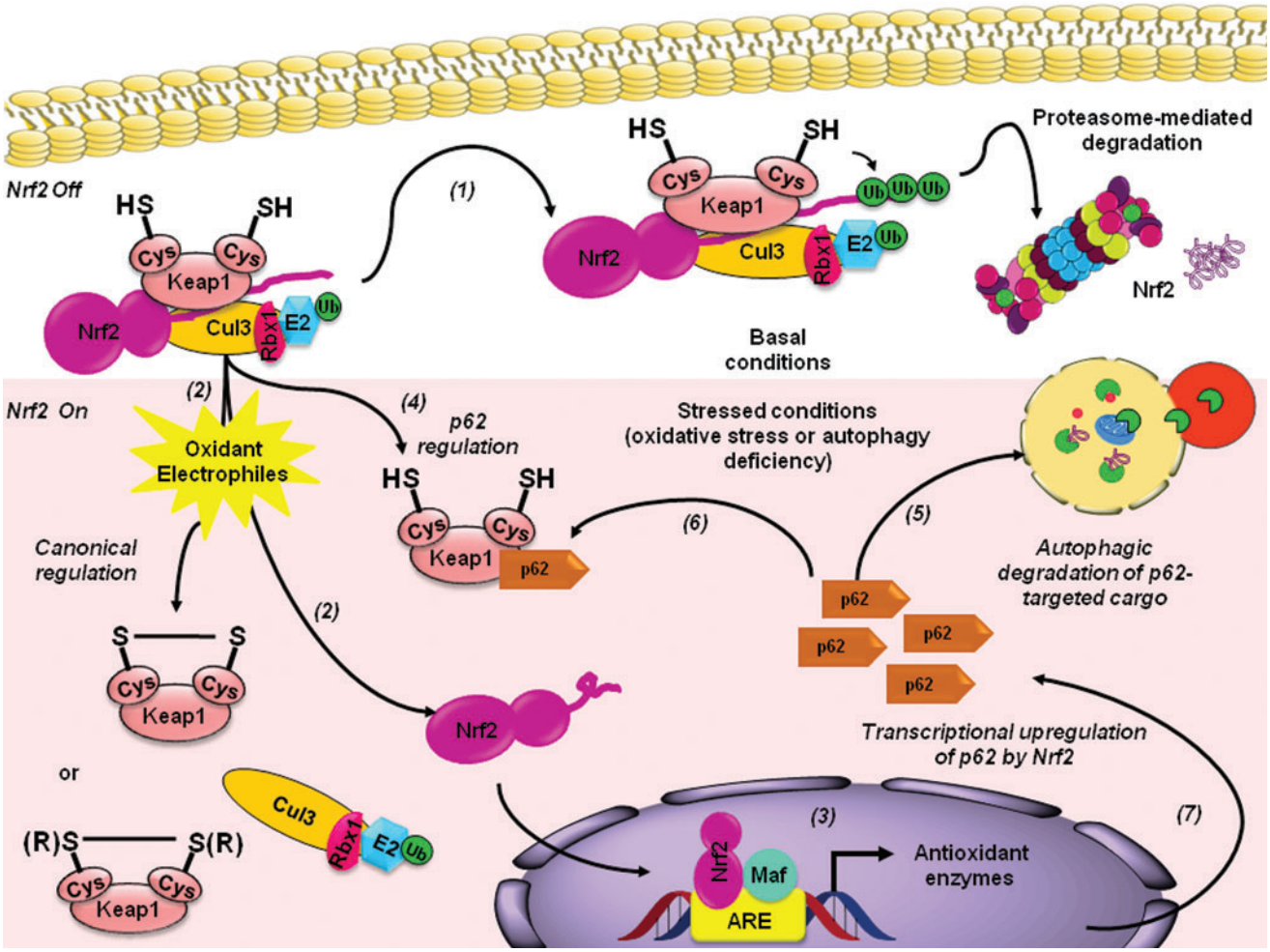

FIG. 5. Transcriptional regulation of antioxidant defenses by autophagy and the ubiquitin/proteasome system. Keap1 is a key regulator of the Nrf2-signaling pathway. (1) Under basal conditions (white background), the Nrf2 switch is in the off position, as Keap1 (reduced) functions as an E3 ubiquitin ligase, constantly targeting Nrf2 for ubiquitination and degradation. As a consequence, the constitutive levels of Nrf2 are very low. (2) Under stressed conditions (pink background), the Nrf2 switch is turned on when Keap1 is oxidized and restrains Nrf2 ubiquitination, enabling its translocation to the nucleus. (3) In the nucleus, Nrf2 forms a complex with the transcription factor Maf and activates the transcription of downstream target genes *via* promoters containing AREs (canonical regulation). (4) p62-dependent autophagic degradation of Keap1 has also been shown to activate Nrf2 and protect against oxidative stress. Thus, (5) under normal conditions, basal autophagy continuously degrades p62. However, (6) under oxidative stress conditions that produce autophagy deficiency, p62 accumulates in the cytoplasm and interacts with Keap1, disrupting the degradation of Nrf2 and enabling, in consequence, the transcription of Nrf2-targeted genes. (7) Transcriptional up-regulation of p62 by Nrf2, subsequently, creates a positive feedback loop. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Heat protein 70 (Hsp70) mediates the dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. This mechanism provides cells with immediate capacity to degrade oxidatively damaged proteins while preserving the 19S regulators in a bound complex (52). Ubiquitin-independent degradation by the 20S proteasome seems to mediate the degradation of the majority of oxidized proteins. However, a recent report demonstrates that chaperones and proteins involved in energy metabolism, cytoskeleton/intermediate filaments, and protein translation/ribosome biogenesis are specifically ubiquitinated in response to oxidative stress. The components of the ubiquitin/proteasome system are also targets of oxidative insults. Mild/transient oxidative stress has been reported to increase substrate availability and up-regulate the ubiquitin-conjugation systems. In contrast, sustained/chronic oxidative stress is reported to inactivate the proteasome without inhibiting the ubiquitination system, resulting in the accumulation of ubiquitin protein conjugates. Finally, extensive oxidative stress also inhibits the ubiquitination system, decreasing the levels of ubiquitin conjugates, and enabling protein misfolding/aggregation and the accumulation of oxidatively damaged proteins (76) (Fig. 6).

Covalent cross-links, disulfide bonds, hydrophobic interactions, and heavily oxidized stable proteins aggregates are not suitable for proteasomal degradation. However, these aggregates inhibit the proteasome. Recent evidence suggests that autophagy plays a major role in the degradation of oxidized protein aggregates by their incomplete degradation within the lysosomal compartment, which results in the

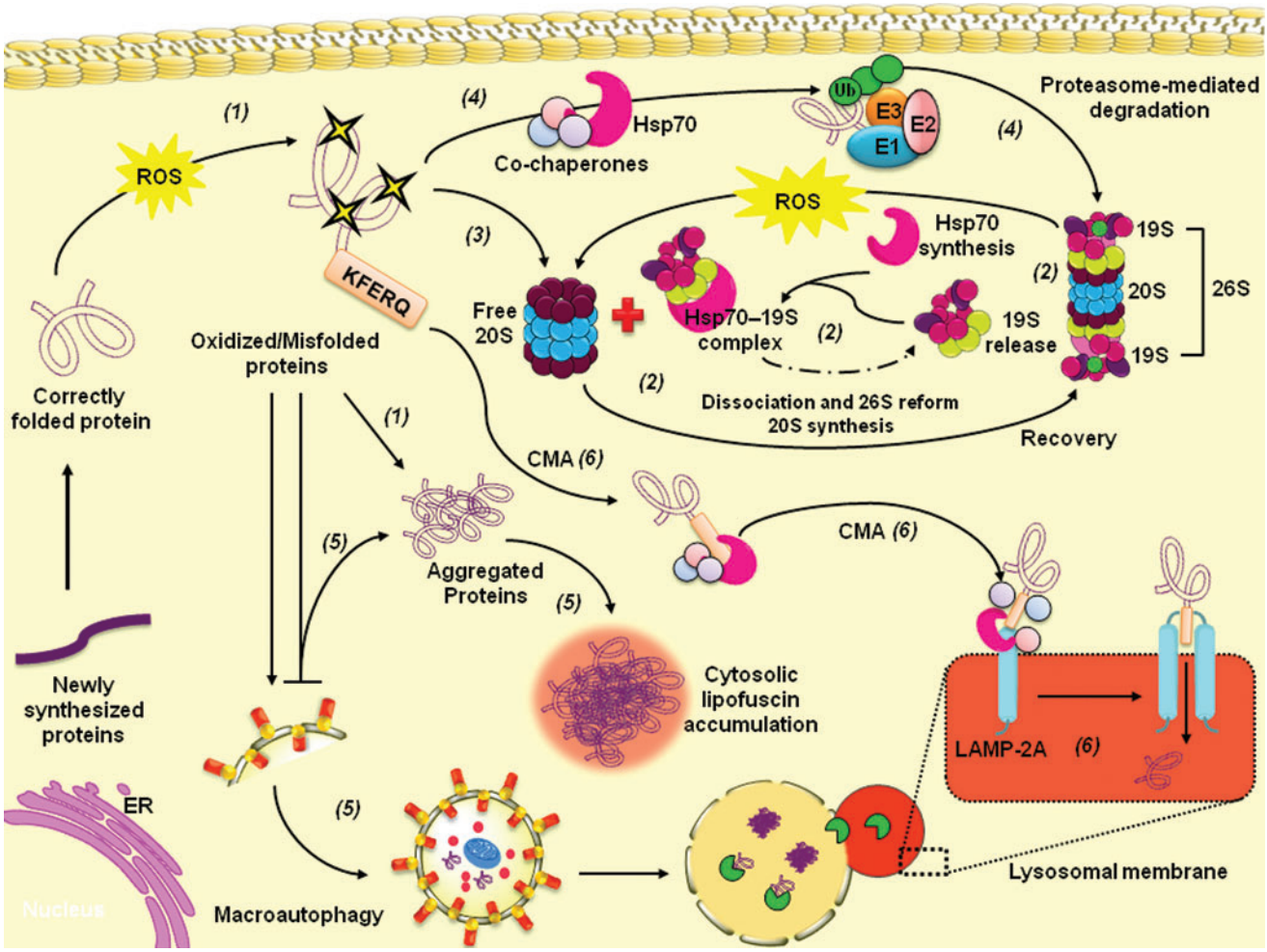

FIG. 6. Chaperone-mediated autophagy, macroautophagy, and the ubiquitin/proteasome system are involved in the degradation of oxidized proteins. (1) Proteins are important targets for oxidative damage, and they can form oligomeric complexes, resulting in the formation of protein aggregates. (2) The proteasome consists of a 20S core that binds two 19S regulators forming the 26S proteasome involved in the degradation of polyubiquitinated proteins. Upon oxidative stress, Hsp70 mediates the dissociation and re-association of the 26S proteasome, providing the immediate capacity to degrade oxidatively damaged proteins. (3) While ubiquitin-independent degradation by the 20S proteasome mediates the degradation of the majority of oxidized proteins, (4) ubiquitination has been reported to target chaperones primarily in response to oxidative stress. Mild/transient oxidative stress increases the availability of ubiquitin and up-regulates the ubiquitin-conjugation systems. Sustained/chronic oxidative inactivates the proteasome, while extensive oxidative stress also inhibits the ubiquitination system, decreasing the levels of ubiquitin conjugates and inducing the accumulation of oxidatively damaged proteins and protein misfolding/aggregation. (5) Autophagy plays a major role in the clearance of heavily oxidized stable proteins aggregates, which are incompletely degraded within the lysosomal compartment, resulting in the accumulation of polymerized lipofuscin aggregates. Autophagy dysfunction leads to the accumulation of lipofuscin in the cytosol, suggesting that its formation is independent from lysosomal hydrolases. (6) Finally, chaperone-mediated autophagy (CMA) has also been demonstrated to be involved in the turnover of oxidized proteins, which are identified by a chaperone that, subsequently, delivers them to the surface of the lysosomes. Targeted proteins unfold and cross the lysosomal membrane *via* the interaction with LAMP-2A, which is required for substrate translocation into the lysosomal lumen. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

formation of polymerized lipofuscin-like aggregates (24, 34). Lipofuscin consists of oxidized proteins (30%–70%), lipids (20%–50%), and metals (2%). Lipofuscin accumulation is observed on knockdown of Atg7, Atg12, or LAMP-2A (59, 71). However, lipofuscin can also be formed in the cytosol independent from macroautophagy and lysosomal activity (59).

CMA has been demonstrated to be involved in the turnover of oxidized proteins (73). High levels of several components of the lysosomal translocation complex, such as the chaperoning activities of Hsp70 and Hsc70, as well as transcriptional up-regulation of LAMP-2A, are induced under oxidative stress (10, 73). The impairment of CMA up-regulates macroautophagy in order to maintain normal rates of long-lived protein degradation. Interestingly, up-regulation of macroautophagy is unable to compensate for the increased sensitivity of CMA-deficient mouse fibroblasts to oxidative stress (97) (Fig. 6).

Redox Signaling and Autophagy

To understand the exact regulatory role of oxidative stress in the signal transduction events regulating autophagy, we should determine the substrate specificity and biomolecular alterations caused by ROS/RNS. Protein cysteines (protein thiols) mediate redox signaling processes in response to oxidative stress. Cysteines can act as sites of post-translational modifications, which are utilized for targeting proteins to membranes and/or influence protein activity, localization, and/or protein–protein interactions (38). Redox-sensitive cysteines undergo reversible and irreversible thiol modifications in response to ROS or RNS. Almost all physiological oxidants react with thiols (145). $O_2^{\bullet -}$ and peroxides (H_2O_2, and $ONOO^-$) mediate one- and two-electron oxidation of protein cysteines respectively, leading to the formation of the reactive intermediates protein sulfenic acids (PSOH) and protein thiyl radicals (PS•), respectively. PSOH can lead to the formation of additional oxidative modifications that act as signaling events regulating protein function. The reaction of PSOH with either another protein cysteine or GSH will generate a disulfide bond or a glutathionylated residue (PSSG). PSSG is considered a protective modification against irreversible cysteine oxidation. PSOH can also undergo a further reaction with H_2O_2 and irreversibly generate protein sulfinic (PSO_2H) and sulfonic (PSO_3H) acids. We have observed that upon oxidative stress, autophagy and the ubiquitin/proteasome system mediate the degradation of PSOHs but not PSSGs (*unpublished data*). The reversible covalent adduction of a nitroso group (NO) to a protein cysteine is referred to as protein nitros(yl)ation (PSNO). PSNO occurs by endogenous NO-mediated nitros(yl)ating agents such as dinitrogen trioxide (N_2O_3) or by transition metal-catalyzed addition of NO. The transfer of NO groups between PSNO and GSNO (transnitros[yl]ation) is one of the major mechanisms mediating PSNO. GSNO is formed during the oxidation of NO• in the presence of GSH, or as a by-product from the oxidation of GSH by $ONOO^-$ (41).

Very few studies have determined the role of redox signaling by oxidative cysteine modification in autophagy. Reversible conjugation of the Atg8 family of proteins to the autophagosomal membrane is a hallmark event in the autophagic process. All Atg8 homologues (including LC3) are substrates for the Atg4 family of cysteine proteases. Atg4s cleave Atg8 near the C-terminus, downstream of a conserved glycine, enabling its conjugation to PE. Atg4 further cleaves Atg8 (LC3)-PE, releasing it from the membrane (Fig. 7). Thus, after the initial cleavage of Atg8(LC3)-like proteins, Atg4 should be inactivated to ensure the conjugation of Atg8 (LC3) to the autophagosomal membrane, and after the autophagosome fuses with the lysosome, Atg4 is re-activated in order to delipidate and recycle Atg8 (LC3). Recently, it was demonstrated that upon starvation, increased generation of mitochondrial H_2O_2 oxidizes and inactivates Atg4 after the initial cleavage of LC3, ensuring the structural integrity of the mature (128) (Fig. 7).

A number of signaling molecules regulating apoptosis have been reported to be regulated by oxidative cysteine modifications. For example, glutathionylation (PSSG) of nuclear factor-kappa B (NF-κB) (117), and caspases (113), has been reported to regulate apoptotic cell death. Similarly, caspases and the anti-apoptotic Bcl-2 protein have been shown to be nitros(yl)ated (PSNO) under basal conditions in human lung epithelial cancer cells, and their denitros(yl)ation is required for their activation during apoptosis (3, 96) (Fig. 7). Both apoptosis and autophagy are simultaneously activated by distinct stressors. Cross-talk between both signaling pathways has been evidenced primarily by 1) the interaction of Bcl-2 or Bcl-xl with Beclin-1, which inhibits autophagy; and 2) the cleavage/degradation of Beclin-1 by caspases (47). Thus, both glutathionylation and nitrosylation might exert regulatory roles in autophagy by indirect regulation of Bcl-2 and caspase activity (3, 96, 113). Protein nitros(yl)ation exerts inhibitory effects on autophagy. Nitros(yl)ation and inhibition of JNK1 and IKKβ signaling pathways has also been reported to inhibit autophagy by increased Bcl-2-Beclin-1 interaction and decreased AMPK phosphorylation (114, 120, 126) (Fig. 7).

AMPK is a master regulator of metabolism, particularly glycolysis (92). By regulation of ULK1 and mTORC1 complexes, AMPK has been demonstrated to regulate autophagy (11, 134). In HEK293 cells, H_2O_2 was recently demonstrated to oxidize cysteine residues of the α-(Cys299 and Cys304) and β-subunits of AMPK *via* glutathionylation, with a concomitant increase in its kinase activity (112). Hypoxia has been shown to activate AMPK *via* mitochondrial ROS formation independent from the AMP/ATP ratio in mitochondrial DNA-deficient cells (147). The ataxia-telangiectasia mutated (ATM) protein kinase is activated by DNA double-strand breaks (DSBs) to initiate DNA damage response. Cells lacking ATM are also hypersensitive to insults other than DSBs, particularly oxidative stress. The oxidation of ATM directly induces its activation in the absence of DNA damage *via* a disulfide-cross-linking dimerization (53). The activation of ATM by oxidative stress or genotoxic damage was recently reported to activate AMPK and the tuberous sclerosis complex 2 (TSC2), which participates in energy sensing and growth factor signaling to repress the kinase mTOR in the mTORC1 complex (79) (Fig. 7).

Mitochondria are dynamic organelles undergoing continuous events of fission and fusion to preserve proper mitochondrial function. A fine-tuned balance between fusion and fission states appears to exist. When mitochondrial fusion is reduced, mitochondria become fragmented; whereas when mitochondrial fission is reduced, mitochondria become elongated and excessively interconnected. Mitochondrial fission requires recruitment of the GTPase DRP1 *via* local organization of mitochondrial surface receptors (Fis1, Mfn, MiD49, and MiD51/MIEF1) for assembly of the fission machinery, subsequently leading to membrane scission. Mitochondrial fusion intermittently homogenizes mitochondrial contents, enabling mitochondria to act as a coherent population. In addition, mitochondrial fusion has been proposed to rescue moderately dysfunctional mitochondria. Fusion is mediated by dynamin GTPases Mfn1/2 at the outer membrane, and optic atrophy GTPase Opa1 at the inner membrane. Mfns form homo- and heteroligomers to tether adjacent mitochondria together, and Opa1 is essential for the fusion of the inner membranes of the organelle. It has been proposed that mitochondrial fission precedes mitophagy, in order to transform normally elongated mitochondria into a form suitable for engulfment. Mitochondrial hyperfusion is a cellular stress response that creates enlarged mitochondria and prevents mitophagy (15, 79). Recently, a redox-based

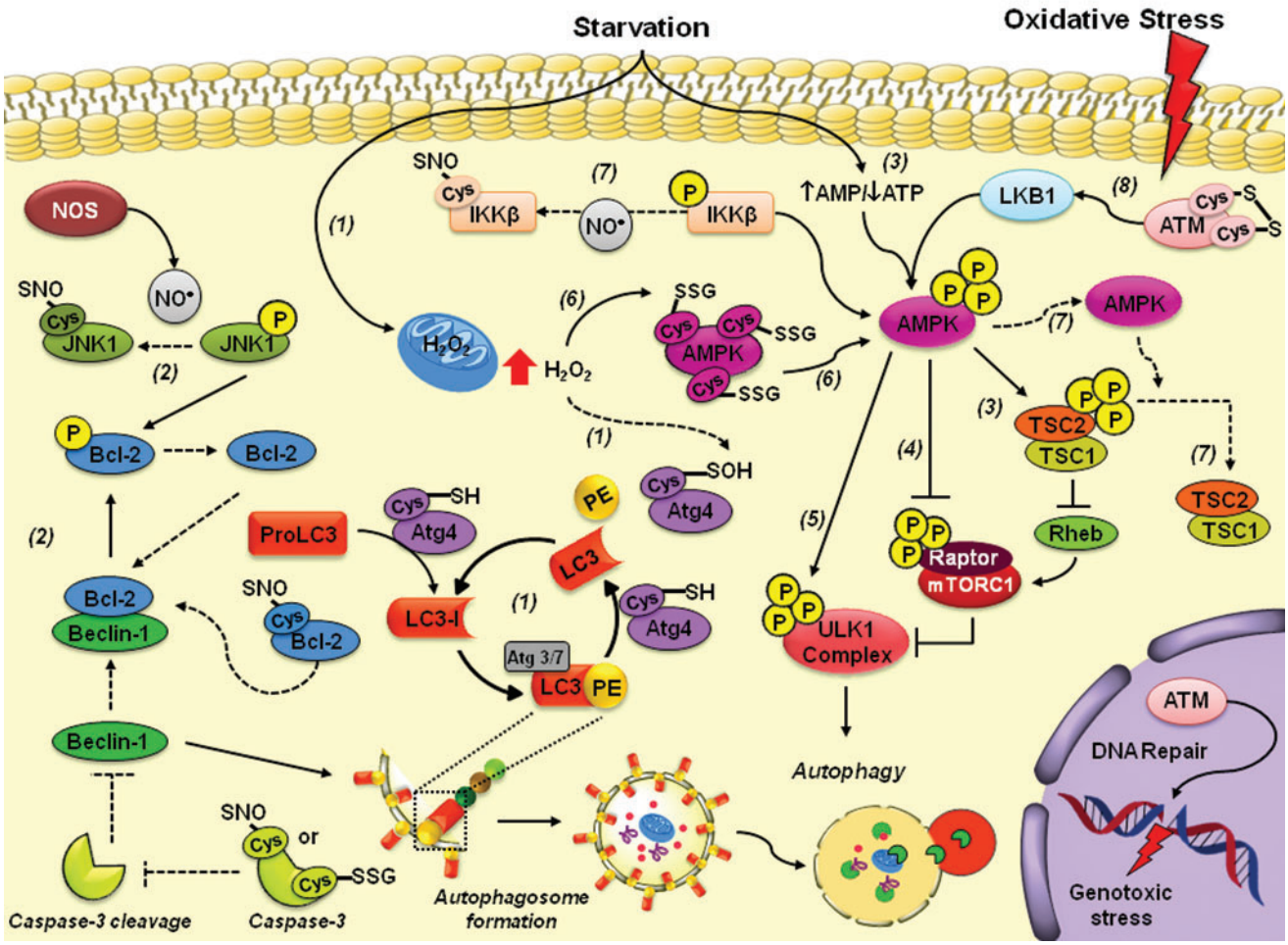

FIG. 7. Redox signaling and autophagy. The redox signaling events regulating autophagy remain largely elusive. (1) Starvation increases the generation of mitochondrial H_2O_2 that leads to the oxidation and inactivation of Atg4 after the initial cleavage of LC3, ensuring the structural integrity of the mature autophagosome and enhancing autophagy. Since autophagosome disassembly involves the deconjugation of LC3 from PE by Atg4, inactivation of Atg4 is needed to ensure the structural integrity of the mature autophagosome. (2) Bcl-2 normally inhibits autophagy by interacting with the autophagy protein Beclin-1, whereas Bcl-2 phosphorylation inhibits this interaction and stimulates autophagy. JNK1 induces autophagy by phosphorylation of Bcl-2 at multiple sites, and protein nitros(yl)ation inactivates JNK1, thereby reducing Bcl-2 phosphorylation (*dotted lines*) and autophagy. (3) ATP depletion activates AMPK, which further phosphorylates and activates TSC2 that, in turn, deactivates the Rheb GTPase. (4) In addition, AMPK can directly phosphorylate Raptor and inactivate mTORC1, or (5) directly activate ULK1. (6) AMPK has also been reported to be glutathionylated, which leads to an increase in its kinase activity. (7) Protein nitros(yl)ation has been reported to inhibit autophagy by inhibition of IKKβ activity, which decreases AMPK phosphorylation. This effect was associated to a decreased phosphorylation (and concomitant inactivation) of TSC2 (*dotted lines*), but the role of Raptor or ULK1 phosphorylation was not evaluated. (8) In contrast, the activation of ATM in response to oxidative stress or genotoxic damage stimulates AMPK phosphorylation and autophagy by phosphorylation of TSC2 and subsequent inhibition of mTOR. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

mechanism has been demonstrated to regulate mitochondrial fusion and possibly mitophagy. GSSG was shown to induce the generation of disulphide-mediated Mfns oligomers, causing a conformational change in the regions that aid in the tethering of Mfns (heptad repeat domain, HR) to enhance membrane fusion (124, 132) (Fig. 2).

Currently, a new issue that is being investigated regarding the role of autophagy in cell survival/death is the effect of the cellular ''gender,'' that is, the cell sex (referred to as XX and XY cells), which is a factor that largely influences health and human disease. Both women and men display important differences with regard to the pathogenic mechanisms, age of onset, progression, and prevalence of human diseases where autophagy and ROS play an important role. Similarly, cell sex exerts major differences in terms of ROS production and susceptibility to autophagy/apoptosis, mainly due to the presence of sex hormones, which can regulate autophagic pathways (92, 134). For example, the survival in some specific kinds of cancers is associated with their gender (female or male), and is related to the modulation of autophagic pathways that would affect the outcome of therapeutic strategies (92, 112). Likewise, neurons from men have been

reported to be more affected by starvation than female neurons, which is associated with a high autophagic adaptive response (92). Interestingly, in rat heart and liver tissues, the levels of autophagy markers Beclin-1, LC3-, and LAMP-1 were found to be higher in men than in women, and this was linked with increased levels of carbonyl groups (11). Vascular smooth muscle cells from female rats were reported to be more resistant to radiation-induced apoptosis (anoikis resistance) than cells from male origin, which was apparently linked to a higher capacity to undergo autophagy (135). Compared with male spleens, female spleens from senescence-accelerated prone mice 8 (SAMP8) demonstrated higher oxidative stress-related alterations in their immune response, and an up-regulation of autophagy pathways as an adaptive response to oxidative stress-induced apoptosis (9). The study of the gender/sex-associated differences regarding the mechanisms by which ROS, redox signaling, and autophagy regulate human disease progression is a new field of research that could provide pivotal information toward understanding the disparity observed between women and men to develop gender-specific-therapies.

Conclusions and Perspectives

Autophagy is a catabolic process that is involved in the degradation and recycling of damaged components within the cell. Recent research efforts have been directed toward understanding the role of autophagy in cell death and/or survival. Alterations in the autophagic cycle rate are commonly observed in response to stress, where the induction of autophagy seems to act primarily as a pro-survival mechanism, while very few examples exist where autophagic cell death regulates cell demise on oxidative stress.

A number of studies have reported the occurrence of autophagy in response to oxidative stress. Mitochondria-derived ROS have been proposed to regulate autophagy. However, the current evidence does not support a specific role for a particular ROS, as both $O_2^{\bullet -}$ and H_2O_2 have been reported to regulate autophagy. It is important to consider that the fate of a cell, either survival or death, depends on both the type of stress that it encounters and the degree/duration of its exposure. In addition, considering that cell signaling pathways are, in many cases, cell specific, the fate outcome will depend largely on the cell type (and its gender), and its particular environment, including nutrient, oxygen supply, and energy status. Some inconsistencies regarding the role of ROS in autophagy and cell survival/death discussed in this review might then relate to the experimental models used for their study.

Autophagy and the autophagy machinery regulate both redox balance and ROS formation under distinct circumstances. Autolysosomes have been proposed as sources for ROS. In addition, autophagy has been shown to mediate catalase degradation and delivery of ferritin to lysosomes for iron extraction. Autophagic degradation of Keap1 activates Nrf2 and protects against oxidative stress, while mitophagy regulates oxidative stress by degradation of ROS-producing mitochondria. Finally, macroautophagy and CMA play a major role in the degradation of oxidized proteins and aggregates.

Redox signaling involves a targeted modification by a reactive species through a chemically reversible reaction in the absence of an overall imbalance of pro-oxidants and antioxidants. To date, there is a good understanding of the signaling events regulating autophagy, as well as the signaling processes by which ROS/RNS mediate the activation/regulation of signaling cascades. Recent studies have begun to uncover the role of redox signaling and reversible oxidative post-translational modifications in protein cysteines in the regulation of specific signaling cascades that regulate autophagy. For example, mitochondrial H_2O_2 oxidizes and inactivates Atg4 stimulating autophagy by ensuring the structural integrity of matured autophagosomes. Apoptotic redox signaling mediated by glutathionylation and nitros(yl)ation has the potential to cross-talk with autophagy by the regulation of Beclin-1. In addition, nitros(yl)ation of JNK1 and IKKβ, and oxidation of ATM have been recently reported to inhibit autophagy *via* the Bcl-2-Beclin-1 and AMPK signaling pathways. Recently, GSSG was shown to promote the generation of disulfide-mediated mitofusin oligomers, enhancing membrane fusion and possibly mitophagy. All these findings have significantly contributed to our understanding of the mechanisms by which oxidative stress and autophagy regulate human diseases such as cancer and neurodegenerative disorders, and provide us with a starting point for designing novel therapeutic approaches. Our future research efforts should be focused on elucidating the events by which autophagy is fine tuned by oxidation/reduction events at the molecular level.

Acknowledgments

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Abbreviations Used

$\Delta\Psi$m = mitochondrial membrane potential
2-DG = 2-deoxy-D-glucose
Akt = protein kinase B (PKB), serine/threonine-specific protein kinase
Ambra1 = activating molecule in Beclin1-regulated autophagy
AMPK = 5′ adenosine monophosphate-activated protein kinase
ARE = antioxidant response element
Atg = autophagy-related gene
ATM = ataxia-telangiectasia mutated
Bcl-2 = B-cell lymphoma 2
Beclin-1 = Bcl-2 interacting myosin/moesin-like coiled-coil protein 1
Bif-1 = endophilin B1
CMA = chaperone-mediated autophagy
COX = cyclooxygenase
Cul3 = cullin-3
CuZnSOD = copper-zinc SOD (or *SOD1*)
DFCP1 = double FYVE domain-containing protein 1
DRP1 = dynamin-related protein 1
DSBs = double-strand breaks
EcSOD = extracellular SOD (or *SOD3*)
ER = endoplasmic reticulum
ESCRT = endosomal Sorting Complexes Required for Transport
FIP200 = ULK-interacting protein
Fis1 = fission Protein 1
Gpx = glutathione peroxidase
GR = glutathione reductase
GSH = glutathione
GSSG = glutathione disulfide
GSNO = S-Nitrosoglutathione
HO = heme oxygenase
HR = heptad repeat domain
Hsc70 = heat shock cognate protein of 70kDa
Hsp70 = heat shock protein of 70kDa
IKKβ = inhibitor of nuclear factor kappa-B kinase subunit beta
IMS = inner membrane space
JNK = c-Jun N-terminal kinase
Keap1 = kelch-like ECH-associated protein 1
LAMP = lysosome-associated membrane protein
LC3 = microtubule-associated protein light chain 3
LC3-I = soluble LC3 form
LC3-II = autophagic vesicle-associated or lipidated LC3 form
LIR = LC3-interacting region
LKB1 = Liver kinase B1 or Serine/threonine-protein kinase STK11
Mfn = dynamin GTPases mitofusins
MnSOD = manganese superoxide dismutase (or *SOD2*)
MPO = myeloperoxidase
MPP^+ = 1-methyl-4-phenylpyridinium
mTORC1 = mammalian target of rapamycin (mTOR) macromolecular complex 1
NADPH = nicotinamide adenine dinucleotide phosphate
NBR1 = neighbor of Brca1 gene
Nix/Bnip3 = BCL2/adenovirus E1B 19kDa protein-interacting protein 3
NOS = nitric oxide synthase enzyme
NOX = NADPH oxidase

Abbreviations Used (Cont.)

Nrf2 = nuclear factor erythroid-derived 2-like 2
Opa1 = optic atrophy GTPase
p150 protein = mammalian homolog of yeast Vps15
p62 protein = ubiquitin-binding scaffold protein also called sequestrome 1(SQSTM1)
Parkin = E3-ubiquitin ligase (also known as PARK2)
PD = Parkinson's disease
PE = phosphatidylethanolamine
PGG_2 = prostaglandin G2
PGH_2 = prostaglandin H2
PI3K = phosphatidylinositide 3-kinase
PI3KC3 = class III phosphatidylinositol 3-kinase
PI3P = phosphatidylinositol-3-phosphate
PINK1 = outer mitochondrial membrane PTEN-induced putative kinase 1
Prx = Peroxiredoxin
PS• = protein thiyl radical
PSNO = protein nitros(yl)ation
PSO_2H = protein sulfinic acids
PSO_3H = protein sulfonic acids
PSOH = protein sulfenic acids
PSSG = protein glutathionylated residue
Rab7 = member of the RAB family of RAS-related GTP-binding proteins
Rag 1/2 = Ras-related small GTPases
Raptor = regulatory-associated protein of mTOR
Rbx1 = E3 ubiquitin-protein ligase
Rheb = Ras homolog enriched in brain protein
RIP1/3 = receptor interaction protein kinase 1 and 3 (RIP1 and RIP3)
RNS = reactive nitrogen species
ROS = reactive oxygen species
RTKs = receptor tyrosine kinases
Rubicon = RUN domain protein as Beclin 1 interacting and cysteine-rich containing
SNARE = SNAP (Soluble NSF Attachment Protein) Receptor
SOD = superoxide dismutase
STS = staurosporine
TSC = tuberous sclerosis complex
UBA = Ub-associated domain
UCP2 = uncoupling protein 2
ULK = unc-51-like kinase
UVRAG = UV radiation resistance-associated gene
VDAC1 = voltage-dependent anion channel 1
VMP1 = vacuole membrane protein 1
Vps proteins = vacuolar proteins sorting
WIPI = WD-repeat protein interacting with phosphoinositides