



Contents lists available at ScienceDirect

Free Radical Biology & Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Reactive oxygen species, cellular redox systems, and apoptosis

Magdalena L. Circu, Tak Yee Aw*

Department of Molecular & Cellular Physiology, Louisiana University Health Sciences Center, Shreveport, LA 71130, USA

ARTICLE INFO

Article history:

Received 2 March 2009

Revised 21 December 2009

Accepted 27 December 2009

Available online xxx

Keywords:

ROS and apoptosis

GSH and thioredoxin redox systems

GSH redox signaling and apoptosis

Pyridine nucleotide redox couples

and apoptosis

Mitochondria and apoptosis

Redox control of caspases

ABSTRACT

Reactive oxygen species (ROS) are products of normal metabolism and xenobiotic exposure, and depending on their concentration, ROS can be beneficial or harmful to cells and tissues. At physiological low levels, ROS function as “redox messengers” in intracellular signaling and regulation, whereas excess ROS induce oxidative modification of cellular macromolecules, inhibit protein function, and promote cell death. Additionally, various redox systems, such as the glutathione, thioredoxin, and pyridine nucleotide redox couples, participate in cell signaling and modulation of cell function, including apoptotic cell death. Cell apoptosis is initiated by extracellular and intracellular signals via two main pathways, the death receptor- and the mitochondria-mediated pathways. Various pathologies can result from oxidative stress-induced apoptotic signaling that is consequent to ROS increases and/or antioxidant decreases, disruption of intracellular redox homeostasis, and irreversible oxidative modifications of lipid, protein, or DNA. In this review, we focus on several key aspects of ROS and redox mechanisms in apoptotic signaling and highlight the gaps in knowledge and potential avenues for further investigation. A full understanding of the redox control of apoptotic initiation and execution could underpin the development of therapeutic interventions targeted at oxidative stress-associated disorders.

© 2010 Elsevier Inc. All rights reserved.

Contents

Overview of reactive oxygen species and intracellular sources	0
Cellular redox systems	0
The glutathione redox system and its cellular compartmentation	0
The thioredoxin redox system	0
The pyridine nucleotide redox system	0
NADPH and antioxidant defense	0
NAD ⁺ and the function of sirtuin proteins	0
ROS and redox involvement in apoptosis	0
Overview of the death receptor and mitochondrial apoptotic pathways	0
Death receptor pathway	0
Mitochondrial pathway	0
ROS and JNK-mediated apoptotic signaling	0
GSH redox status and apoptotic signaling	0

Abbreviations: AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocase; Apaf-1, apoptotic protease activation factor-1; ASK, apoptosis signal-regulating kinase; cypD, cyclophilin D; DD, death domain; DISC, death-inducing signaling complex; ER, endoplasmic reticulum; FADD, Fas-associated death domain; FasL, Fas ligand; FLIP, FLICE inhibitory protein; FOXO, Forkhead box O; G6PDH, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSSG, glutathione disulfide; Grx, glutaredoxin; IAP, inhibitor of apoptosis protein; ICDH, isocitrate dehydrogenase; JNK, c-Jun N-terminal kinase; ME, malic enzyme; mtDNA, mitochondrial DNA; mtGSH/GSSG, mitochondrial GSH/GSSG; NAC, N-acetylcysteine; NNT, nicotinate amide nucleotide transhydrogenase; NADK, NAD kinase; Nox, NADPH oxidase; OGG1, 8-oxodG glycosylase; Prx, peroxiredoxin; PTP, permeability transition pore; RIP1, receptor-interacting protein 1; ROS, reactive oxygen species; Sirt, sirtuin protein; Smac/Diablo, second mitochondria-derived activator of caspases/direct IAP binding protein with low pI; tBid, truncated form of Bid; TRAF, TNF α receptor-associated factor; TRADD, TNFR-associated death domain; TRAIL, TNF-related apoptosis-inducing ligand; Trx, thioredoxin; VDAC, voltage-dependent anion channel; XIAP, X-linked inhibitor of apoptosis protein.

* Corresponding author. Fax: +1 318 675 4217.

E-mail address: taw@lsuhsc.edu (T.Y. Aw).

0891-5849/\$ – see front matter © 2010 Elsevier Inc. All rights reserved.

doi:10.1016/j.freeradbiomed.2009.12.022

Please cite this article as: Circu, M. L.; Aw, T. Y., Reactive oxygen species, cellular redox systems, and apoptosis, *Free Radic. Biol. Med.* (2010), doi:10.1016/j.freeradbiomed.2009.12.022

Modulators of initiation and execution of apoptosis	0
Mitochondrial modulators of apoptotic initiation	0
ROS and the mitochondrial permeability transition	0
Oxidative mitochondrial DNA damage	0
Cytochrome c and cardiolipin interaction	0
Redox modulation of apoptotic execution: control of caspase activity	0
Concluding remarks	0
Acknowledgments	0
References	0

Overview of reactive oxygen species and intracellular sources

Reactive oxygen species (ROS) is a collective term that broadly describes O_2 -derived free radicals such as superoxide anion ($O_2^{\bullet-}$), hydroxyl (HO^\bullet), peroxy (RO_2^\bullet), and alkoxy (RO^\bullet) radicals, as well as O_2 -derived nonradical species such as hydrogen peroxide (H_2O_2) [1]. The mitochondrion is a major intracellular source of ROS. Of total mitochondrial O_2 consumed, 1–2% is diverted to the formation of ROS, mainly at the level of complex I and complex III of the respiratory chain, and this diversion is believed to be tissue and species dependent [2,3]. Mitochondria-derived $O_2^{\bullet-}$ is dismutated to H_2O_2 by manganese superoxide dismutase, and, in the presence of metal ions, highly reactive HO^\bullet is generated via Fenton and/or Haber-Weiss reactions, inflicting significant damage on cellular proteins, lipids, and DNA. To date ~10 potential mitochondrial ROS-generating systems have been identified [4]. Among these, Krebs cycle enzyme complexes, such as α -ketoglutarate dehydrogenase (α -KGDH) and pyruvate dehydrogenase, have been implicated as significant mitochondrial $O_2^{\bullet-}$ and H_2O_2 sources [5]. Notably, increased nicotinamide adenine dinucleotide is linked to elevated H_2O_2 production by mitochondrial α -KGDH, and this elevated oxidant burden elicits further ROS production from mitochondrial complex I and accelerates cell death [6]. Other interesting mitochondrial ROS sources include $p66^{Shc}$, an intermembrane space enzyme [7]; and monoamine oxidase, an outer membrane enzyme [4]; and altered mitochondrial membrane potential [8] or matrix pH [9]. As major ROS generators, mitochondria are often targets of high ROS exposure with deleterious consequences, such as oxidative damage to mitochondrial DNA [10,11]. Although elevated $O_2^{\bullet-}$ and HO^\bullet associated with mtDNA damage have been implicated in cell apoptosis [12], the precise mechanism whereby mtDNA damage mediates apoptotic signaling is incompletely understood and should provide a fruitful avenue for future investigation.

Peroxisomes are sources of cytosolic H_2O_2 under physiological conditions, and peroxisomal H_2O_2 elimination is compartmentalized given its structural organization. Matrix H_2O_2 is removed by catalase [13], whereas urate oxidase-catalyzed H_2O_2 generation within the core is directly released into the cytosol via cristalloid core tubules [13]. Endoplasmic reticular (ER) monooxygenases (e.g., cytochrome P450) contribute to increased cellular H_2O_2 and $O_2^{\bullet-}$ that promote lipid peroxidation, altered calcium homeostasis, mitochondrial dysfunction, and cell apoptosis [14,15], whereas NADPH oxidase (Nox)-derived ROS at the plasma membrane function in cellular signaling [16]. In the extrinsic apoptotic pathway, ligand–death receptor engagement (Fas ligand–Fas receptor, TNF α –TNF receptor1) induces lipid raft formation and Nox recruitment/activation and ROS generation that signal acid sphingomyelinase activation, ceramide production, and receptor clustering. These combined processes constitute lipid raft-derived signaling platforms that mediate death receptor activation and induction of apoptosis [17,18]. The physiological relevance and quantitative significance of ROS-dependent receptor-mediated apoptosis compared to the classical receptor/ligand-induced apoptotic signaling are, at present, incompletely understood and warrant further investigation.

Cellular redox systems

The glutathione redox system and its cellular compartmentation

The centrality of glutathione (L- γ -glutamyl-L-cysteinylglycine; GSH) in cellular redox homeostasis is well recognized, and collective studies from our laboratory have established the importance of GSH redox in cell apoptosis in a variety of cell types [19–22]. GSH, as the most abundant free thiol in eukaryotic cells, maintains an optimal intracellular redox environment for proper function of cellular proteins. Reduced GSH is the biological active form that is oxidized to glutathione disulfide (GSSG) during oxidative stress; the ratio of GSH to GSSG thus offers a simple and convenient expression of cellular oxidative stress [23]. Typically, cells exhibit a highly reduced GSH-to-GSSG ratio, and greater than 90% of total GSH is maintained in the reduced form through cytosolic de novo GSH synthesis, enzymatic reduction of GSSG, and exogenous GSH uptake [24]. Because GSH participates in numerous redox reactions, an oft-asked question that remains unresolved is that of specificity of GSH redox in the control of cell signaling. Current evidence suggests that in part, specificity and targeted redox control are achieved through the existence of distinct intracellular redox compartments that exhibit a unique distribution of GSH and other redox couples [25].

Intracellular GSH is compartmentalized as distinct redox pools within the cellular compartments of cytosol, mitochondria, endoplasmic reticulum, and nucleus. Cytosolic GSH is highly reduced, with GSH concentrations ranging between 2 and 10 mM in most cell types. The cellular ratio of GSH to GSSG under physiological conditions highly favors reduced GSH (around 100 to 1 in liver) and is decreased during oxidative stress and apoptosis [26]. GSH within the ER is between 2 and 10 mM, and it maintains catalytic site thiols of protein disulfide isomerase (PDI) for protein folding [27,28] and buffers against ER-generated ROS [27,28]. Altered GSH redox state triggers the unfolding protein response and apoptosis [29]. Recent evidence suggests that the distribution of GSH and GSSG was closer to 5 to 1 [30], that is, more reduced than previously thought (ratio of ~1 to 1). The full implication of a more reducing redox environment for PDI-catalyzed protein folding is unclear at present and warrants reevaluation in the light of this new observation. An independent nuclear GSH pool functions to preserve nuclear proteins in a reducing environment and protects against oxidative and ionizing radiation-induced DNA damage [31]. Cytosol-to-nuclear GSH distribution is reportedly a dynamic process that correlates with cell cycle progression [32], with nuclear GSH being fourfold greater than cytosolic GSH during cell proliferation and equally distributed between the two compartments when cells reach confluency [31,32]. Cytosol-to-nuclear GSH import was suggested to occur by passive diffusion via nuclear pores [33] that is facilitated by the antiapoptotic protein Bcl-2 [34]. A distinct mitochondrial GSH (mtGSH) pool preserves the integrity of mitochondrial proteins and lipids and controls mitochondrial ROS generation. The pool size of mtGSH is cell-type specific, varying from 10–15% of the total GSH in the liver [35] to 15–30% of total GSH in the renal proximal tubule [36]. Matrix GSH is maintained through active transport from the cytosolic

compartment via inner membrane dicarboxylate and 2-oxoglutarate GSH carriers [37]. Bcl-2 reportedly functions in the preservation of GSH in the intermembrane space through interactions with GSH via its BH2 groove [38], thus contributing to a localized source of mtGSH at this site. Disruption of this interaction by apoptotic stimuli inhibits cytosol-to-mitochondria GSH transport, inducing mtGSH efflux and the apoptotic cascade [38].

The thioredoxin redox system

The function of the GSH/GSSG redox couple in cellular redox homeostasis occurs in conjunction with redox proteins. Thioredoxins (Trx's), which represent a pivotal partner with GSH/GSSG in redox regulation, are small ubiquitous proteins that possess two catalytic-site redox-active cysteines (Cys-XX-Cys) [39]. Trx's catalyze the reversible reduction of protein disulfide bonds, and Trx active-site cysteines are regenerated by Trx reductase and NADPH [39]. The Trx system collaborates with the glutaredoxin (Grx) system in the reduction of protein mixed disulfides. Like GSH, mammalian forms of Trx are compartmentalized within cells. Trx1 localizes within the cytosol and during oxidative stress translocates to the nucleus. However, cytosolic and nuclear Trx1 are regulated independent of each other and exhibit functional differences [40,41]. Trx2 resides exclusively in the mitochondria and functions in mitochondrial redox homeostasis [40,41]. The regulation of mitochondrial Trx2 is distinct from that of cytosolic Trx1 and mitochondrial GSH/GSSG [40,42]; thus, reduced and oxidized Trx (Trx-SH/Trx-SS) as well as GSH and GSSG are distinct and independently controlled redox systems.

Described as unique "redox control nodes or circuitry," the GSH and Trx redox systems reportedly function as rheostat on/off switches in the redox regulation of cellular proteins [43]. Based on a simple concept that the GSH/GSSG, Trx/Trx-SS, and cysteine/cystine redox couples are not at equilibrium in biologic systems, which is in part supported by experimental data, Jones and co-workers have presented a compelling argument that the existence of such unique compartments of redox pools could, in fact, afford an elegant mechanism for redox control of specific protein sets [41,44]. Thus, the mode of independent regulation of the redox status of specific protein sets could represent a crucial and generalized redox signaling mechanism in the control of redox-sensitive biological processes within mammalian cells. However, the full extent of the biological importance of metabolically distinct redox pools in redox regulation and the universality of such a regulatory redox mechanism in all tissue types remain to be established. In addition, much remains unknown about precise interactions of compartmental regulatory redox mechanisms, the extent of GSH/Trx cross talk and communication among the various redox compartments, and the quantitative impact of altered GSH/GSSG and Trx/Trx-SS status within one compartment, such as the mitochondria, on the threshold for redox signaling and gene expression in another compartment, such as the nucleus. A full discussion of the integration of redox compartments and communications and implications for redox biological processes is beyond the scope of this review. The reader is referred to several excellent reviews by Jones and co-workers [43, 44] and our recent review [25] for a more in-depth coverage of the concept of redox compartmentation in mammalian cells, nonequilibrium thiol/disulfide redox systems, and implications for redox signaling and regulation. Given the conceptual novelty that distinct redox nodes/circuitry could serve as a general paradigm of redox control of cell fate, vis-à-vis proliferation, differentiation, and apoptosis, it is anticipated that future research into this area of redox regulation will continue to grow.

Peroxiredoxins (Prx's) are a group of non-selenothiol-specific peroxidases that also contribute to cellular redox control via their ability to eliminate organic hydroperoxides and H₂O₂ [45]. All Prx's possess, within their catalytic site, peroxidic cysteines (fast reactive

cysteines) that are oxidizable to sulfenic acids (Cys-SOH) that rapidly form disulfide bonds with another cysteine at the C-terminal subunit. Cysteine regeneration is catalyzed by the Trx/TrxR system [46]. The six isoforms of Prx (PrxI to PrxVI) in mammalian cells are classified into typical 2-Cys Prx's, atypical 2-Cys Prx's, and 1-Cys Prx's. Among these, PrxI–II and PrxVI are cytosolic, PrxII is mitochondrial, and PrxIV is extracellular. PrxV is localized in the mitochondria and peroxisomes. The functional significance of redundancies of multiple Prx isoforms in the control of cellular oxidative stress, whether Prx's integrate with the GSH and Trx redox nodes, and whether Prx's, directly or indirectly, participate in redox regulation of apoptotic signaling are important unresolved questions that need to be addressed.

The pyridine nucleotide redox system

The redox state of pyridine nucleotides is intricately tied to that of GSH and Trx in redox-dependent cellular processes. Indeed, NADPH, with a redox potential of ~400 mV, is the electron donor in the rejuvenation of GSH and Trx redox status. Pyridine nucleotides collectively comprise reduced and oxidized nicotinamide adenine dinucleotide (NADH/NAD⁺) and reduced and oxidized nicotinamide adenine dinucleotide phosphate (NADPH/NADP⁺), which are classically associated with ATP production and reductive biosynthesis, respectively. NADPH/NADP⁺ are also linked to oxidative stress defense and redox regulation. However, more versatile biological functions have been attributed to these molecules. For instance, NADH/NAD⁺ were shown to modulate gene transcription via the carboxy-terminal binding protein [47], Ca²⁺ signaling via cyclic ADP-ribose [48], cell death via poly(ADP-ribose) polymerase-1 [49], and sirtuin protein function (see NAD⁺ and the function of sirtuin proteins). NADPH/NADP⁺ were found to influence cellular signaling via nicotinic acid adenine dinucleotide phosphate [50] and cellular ROS production by electron transport chain or NADPH oxidases [51]. The specific contribution of pyridine nucleotides to redox regulation of cell apoptosis and their interactions with the GSH and Trx redox circuitry in cell signaling are poorly understood and should provide exciting and novel avenues for future research. Of relevance, NADPH/NADP⁺ participate in antioxidant defense in the control of cellular oxidative stress and GSH/GSSG redox balance. In addition to their classical role in mitochondrial energy production, novel roles for NADH/NAD⁺ in oxidative stress and apoptosis are underscored by their function in modulating activities of sirtuin proteins, a class of NAD⁺-dependent deacetylases and mono-ADP-ribosyl transferases. Aspects of NADPH/NADP⁺ and NADH/NAD⁺ redox functions pertinent to detoxification reactions and sirtuin activity are discussed in the following sections.

NADPH and antioxidant defense

NADPH is pivotal in GSSG and Trx-SS reduction. NADPH generation during oxidative stress is accomplished by mechanisms that involve either NADP⁺ reduction or NAD(H)-to-NADP(H) conversion. Consistent with a role for the pentose phosphate shunt in NADPH production, ROS-mediated increase in glucose-6-phosphate dehydrogenase (G6PDH) activity was associated with increased protection against oxidative stress-induced apoptosis [52,53]. Moreover, although dispensable for pentose synthesis, G6PDH was essential for NADPH-mediated antioxidant protection in mouse embryonic stem cells [54]. NADP⁺-linked dehydrogenases such as isocitrate dehydrogenase (ICDH) are major contributors to NADPH maintenance, and ICDH-mediated production of NADPH was correlated with increased protection against oxidative stress-induced cellular damage and apoptosis [55]. The two mammalian ICDH isoforms are localized to the mitochondria (mtICDH) and cytosol; the latter is capable of translocating to the peroxisomes [56]. Overexpression of mtICDH improved mitochondrial NADPH and GSH levels and protected human

neuroblastoma cells (SH-SY5Y) against cytochrome *c* release and ROS-mediated mitochondrial apoptotic signaling [57], whereas siRNA silencing of mtLCDH compromised mitochondrial redox status and enhanced HeLa cell apoptotic susceptibility to TNF α , anticancer drugs, or heat shock [58,59]. Interestingly, ICDH-mediated NADPH production did not seem to compensate for loss of NADPH supply by G6PDH deficiency; G6PDH-deficient mouse cells remained highly sensitive to oxidative stress [60]. Malic enzyme (ME) is another important enzymatic source of NADPH; of the three known mammalian MEs, NADP⁺- and NAD⁺-specific ME are mitochondrial, and NADP⁺-dependent ME is cytosolic [61]. In addition to NADPH generation, mitochondrial NADP⁺-specific ME reportedly participates in pyruvate recycling and maintenance of mtGSH [62]. The existence of multienzyme systems for NADPH production is consistent with the importance of its dual functions in reductive biosynthesis as well as detoxification and redox reactions. However, the biological significance of site-specific NADPH generation within the cytosolic and mitochondrial compartments in redox maintenance and drug detoxification remains to be better defined. Indeed, compared to the GSH and Trx redox systems, the questions of integration of compartmental pyridine nucleotide pools, communication among cellular compartments, and implications for redox biological processes are relatively unexplored.

An important aspect of antioxidant defense by pyridine nucleotides is underscored by the enzymatic NADH-to-NADPH conversion. Located in the inner membrane, the mitochondrial nicotinamide nucleotide transhydrogenase (NNT) coordinates proton translocation across the mitochondrial membrane and transfer of reducing equivalents between NADH and NADPH, representing a key mechanism for mitochondrial NADH-to-NADPH conversion. Under physiological conditions, NNT favors NADPH production that maintains intramitochondrial homeostatic redox state, but during oxidative stress, NNT-derived NADPH mediates the regeneration of GSH and protein thiols [63]. Within the cytosol, NAD(H)-to-NADP(H) conversion is mediated by cytosolic NAD kinases (NADK), a class of ubiquitous enzymes that catalyze the phosphorylation of NAD⁺. To date only a cytosolic mammalian NADK has been identified [64], and its importance in maintaining cellular NADPH for reductive biosynthesis in organogenesis is documented [64,65]. The relevance of NADK to NADPH-dependent protection against oxidative challenge is, however, less well understood. Pollak et al. found that NADK overexpression conferred only moderate protection against oxidative stress and that NADK-deficient cells exhibited a sensitivity to oxidative stress similar to that of NADK-sufficient cells [64], implying a relatively minor role for NADK in NADPH biogenesis. Moreover, whereas NADP⁺-linked dehydrogenases were activated by oxidative stress, NADK activity and expression remained unchanged [64], suggesting a lack of sensitivity to oxidative challenge. This notwithstanding, the importance of enzymatic conversion of NADH to NADPH and its relevance in antioxidant defense are underscored by recent studies demonstrating that during oxidative challenge, gluconeogenic and tricarboxylic acid cycle enzymes in NADH production were downregulated in favor of upregulation of NADPH-producing enzymes of the glyoxalate and glycolytic cycles [66,67]. Consequently, substrate flow was channeled away from NADH production toward increased NADPH generation. Significant diversion of reducing equivalents from mitochondrial energy production to NADPH generation for detoxification reactions is likely to have a marked impact on mitochondrial respiratory integrity. Furthermore, sustained compromise in mitochondrial respiration during oxidative challenge, particularly under conditions of decreased glucose, would have important biological implications for cell survival.

NAD⁺ and the function of sirtuin proteins

In recent years, much interest has focused on the function of sirtuin proteins, a conserved family of NAD⁺-dependent deacetylases

and mono-ADP-ribosyl transferases involved in cellular processes such as gene silencing, DNA repair, life-span extension, and cell apoptosis [68–71]. Sirtuins (Sirts) catalyze protein deacetylation, functioning as cellular rheostats that sense changes in the energy and redox status. For instance, an increase in the NAD⁺/NADH ratio enhanced protein deacetylation, whereas elevated NADH or nicotinamide inhibited Sirt activity [72], suggesting a close association between the cellular redox status of pyridine nucleotides and Sirt function. To date, seven mammalian Sirts (Sirt1–7) have been identified, each exhibiting a distinct subcellular localization. Although Sirt members reportedly control the functions of specific protein sets, the biological roles of most Sirt proteins have not been fully explored.

Sirt1 is among the best studied members, an NAD⁺ deacetylase with a predominant nuclear localization that is capable of nuclear-to-cytosol shuttling [73]. Sirt1 acts on numerous substrates that control cell senescence, proliferation, and apoptosis [74,75]. The association of Sirt1 with the tumor suppressor p53 and the transcription factor Forkhead box O (FOXO) is of relevance to oxidative stress-induced apoptosis. At low H₂O₂, Sirt1-mediated p53 deacetylation promoted p53 destruction via Mdm2-dependent ubiquitination [76,77], and Sirt1 overexpression inhibited p53-mediated nuclear transactivation and blocked oxidant-induced apoptosis [78]. However, Sirt1 could induce proapoptotic effects in certain cell types under oxidative conditions; at high ROS, hyperacetylated nuclear p53 promoted apoptosis through transactivation of the proapoptotic PUMA and Bax genes [79,80]. Interestingly, physiological levels of ROS can promote mitochondria-induced apoptosis in mouse embryonic cells via Sirt1-dependent mitochondrial translocation of p53 [81]. Sirt1 interacts with FOXO-1, -3a, and -4, wherein FOXO deacetylation confers cell resistance to oxidative stress and apoptosis [82]. During oxidative stress, Sirt1–FOXO3a interaction increased the transcription of stress-resistant genes and decreased the expression of FOXO3a-dependent proapoptotic genes [82]. In renal tubular cells, Sirt1 induced catalase downregulation and maintained steady-state ROS levels appropriate for cell signaling in the absence of oxidative stress [83], whereas increased H₂O₂ promoted Sirt1/FOXO3a-dependent catalase expression that protected against apoptosis [83]. Thus, it seems that Sirt1 controls renal tubular cell apoptosis by modulating intracellular ROS levels through bidirectional regulation of nuclear catalase expression and thus affords cytoprotection under physiological as well as oxidative conditions. In other examples, increased human cardiac fibroblast resistance to oxidative stress was associated with elevated FOXO3a and mitochondrial PrxIII expression [84], and blockade of caspase-3 and -7 that induced apoptosis in cancer epithelial cells was linked to Sirt1-mediated FOXO4 deacetylation [85]. The notion that Sirt1 may potentially be a key cellular target for prostate cancer therapy was suggested by the observation that Sirt1-dependent deacetylation of FOXO1 was associated with uncontrolled growth and proliferation of prostate cancer cells [86]. Much of the current Sirt1 research is in the area of cell proliferation and differentiation relevant to cancer, but a link to redox control is unknown. The findings that Sirt1 is sensitive to H₂O₂ and responsive to NAD⁺/NADH suggest a role for redox in the regulation of Sirt1 function, an exciting possibility that hitherto has not been rigorously explored, and one that warrants detailed study.

The functions of other Sirt members (Sirt2 to 6) are less studied. To date, Sirt3, 4, and 5 have been identified as mitochondrial proteins possessing NAD⁺ deacetylase (Sirt4), ADP-ribosyl transferase (Sirt5), or both enzyme activities (Sirt3) [87–89], suggesting that mitochondrial Sirts may control a broad spectrum of mitochondrial functions. In several studies, mitochondrial Sirt proteins were implicated in energy metabolism such as ATP generation, membrane potential regulation, and ROS production. For instance, Sirt3-mediated deacetylation of mitochondrial complex I engaged ATP production under normal homeostatic conditions, whereas decreased ATP generation during oxidant exposure resulted from Sirt3 dissociation and complex I

acetylation [90]. Sustained expression of Sirt3 induced thermogenesis in brown adipocytes through a decrease in mitochondrial membrane potential and enhanced ROS production [91]. Deacetylation by Sirt3 under caloric restriction activated glutamate dehydrogenase (GDH) [92], whereas ADP-ribosylation by Sirt4 repressed GDH activity and decreased ATP production due to reduced trafficking through tricarboxylic acid cycle intermediates [88]. Evidence of the contribution of mitochondrial Sirt proteins to redox modulation and cell apoptosis is sketchy. However, the notion that Sirts can contribute to mitochondrial redox control and apoptosis is supported by evidence that Sirt3 and 5 have roles in mitochondrial antioxidant defense and apoptotic signaling. NADP⁺-dependent ICDH (ICDH2) is a mitochondrial target of Sirt3, and protein deacetylation stimulated enzyme activity and NADPH regeneration [89]. Moreover, Sirt5 reportedly can translocate to the mitochondrial intermembrane space and deacetylate cytochrome *c* [89], but the precise impact of cytochrome *c* deacetylation on mitochondrial respiration and/or apoptosis is unknown. The current paradigm proposes an oxidant-induced disruption of the cardiolipin-cytochrome *c* complex in mitochondria-to-cytosol translocation of cytochrome *c* during apoptosis initiation (see [Mitochondrial modulators of apoptotic initiation](#)). The demonstration that cytochrome *c* deacetylation is mechanistically associated with mitochondrial cytochrome *c* release could constitute a novel paradigm shift.

Current research on Sirt2, 6, and 7 is scanty, and avenues for future investigations into the functions and redox control of these Sirt members are wide open. Evidence to date identifies Sirt2 as a cytosolic enzyme that exhibits deacetylase and ADP-ribosyl transferase activities and colocalizes with the microtubule network within the cytosol, causing the deacetylation of α -tubulin. Nuclear translocation of Sirt2 during the G₂/M phase of the cell cycle suggests a role in the control of the cell cycle checkpoint [93], but the mechanism of Sirt2-mediated cell cycle regulation is unclear. Beyond a reported involvement in maintaining genome integrity through activating base excision repair of oxidative damaged DNA or single-strand DNA breaks [94], the biological importance of Sirt6, a nucleolus-located deacetylase [95,96], is relatively unexplored. Similarly, despite documentation that Sirt7 can bind to heterochromatin regions within the nucleolus [95,96], a possible role for Sirt7 in promoting RNA polymerase 1-catalyzed gene transcription [96] remains to be defined. At present, the involvement of sirtuin proteins in the apoptotic process is unknown, and validation of an epigenetic control of apoptosis by sirtuin proteins should represent a fertile area for future research. One intriguing hypothesis is that acetylation/deacetylation functions in posttranslational regulation of cell apoptosis in a manner akin to glutathiolation/deglutathiolation and/or nitrosation/denitrosation.

ROS and redox involvement in apoptosis

Overview of the death receptor and mitochondrial apoptotic pathways

Death receptor pathway

The extrinsic pathway of apoptosis is mediated by death receptors in that ligand–receptor binding initiates protein–protein interactions at cell membranes that activate initiator caspases. Major known receptors include Fas (also called CD95 or APO-1), TNF receptor 1 (TNFR1), and TNF-related apoptosis-inducing ligand (TRAIL) receptor 1 (TRAIL-R1; also called DR4) and TRAIL receptor 2 (TRAIL-R2; also called DR5) [97]. TRAIL-R3, TRAIL-R4, and the soluble receptor osteoprotegerin lack functional cytosolic domains and are decoy receptors by which ligand binding does not transmit an apoptotic signal [98]. The death receptor comprises three functional extracellular ligand-binding, transmembrane, and intracellular domains. Ligands that activate death receptors belong to the TNF superfamily of cytokines; these include TNF α , Fas ligand (FasL), and TRAIL. Ligand

binding induces receptor trimerization and cross-linking via disulfide bond formation, a step that is necessary for receptor stabilization and activity [99]. Typically, apoptotic signaling is initiated by the association of death-domain-containing adaptor proteins within the death domain located at the C-terminal domain of the receptor. As discussed in the overview, newer evidence suggests possible direct roles for ROS in mediating death receptor activation and apoptotic induction through ROS-induced receptor clustering and formation of lipid-raft-derived signaling platforms. The pathophysiological scenarios whereby this would be a major mechanism of apoptotic signaling remain to be defined.

Fig. 1 summarizes the key players and mechanistic differences in the three major classical death receptor signaling pathways in cell apoptosis. The Fas/FasL system is one of the best studied systems in death-receptor-mediated apoptosis. Within minutes of Fas/FasL binding, Fas-associated DD (FADD) and procaspase-8 are recruited, and the resultant death-inducing signaling complex (DISC) is endocytosed [100]. The release of endosomal DISC from the receptor accumulates as cytosolic DISC to which additional FADD and procaspase-8 are recruited, resulting in activation of the initiator caspase-8 [101,102]. The extent of activated caspase-8 at the DISC determines Type 1 or Type 2 mechanisms; significant caspase-8 activation directly activates caspase-3 (Type 1), whereas low caspase-8 activation mediates caspase-3 activation through an amplification loop involving the mitochondria (Type 2) [103]. In Type 2 apoptosis, activated caspase-8 cleaves proapoptotic Bid, which induces outer mitochondrial membrane permeabilization through the interactions of tBid with Bax/Bak, resulting in the mitochondrial release of apoptogenic cytochrome *c*, second mitochondria-derived activator of caspases/direct IAP binding protein with low pI (Smac/Diablo), or apoptosis-inducing factor (AIF). Additionally, Fas-induced NADPH oxidase-dependent H₂O₂ and O₂^{•-} generation further down-regulates the antiapoptotic FLIP_L through FLIP_L ubiquitination/proteasomal degradation or through nitric oxide (NO) scavenging that prevents FLIP_L S-nitrosation and cytoprotection [104]. This newly described ROS–NO interaction in controlling FLIP_L downregulation was considered a key regulatory mechanism of Fas-induced apoptosis [104], which is likely to be significantly impacted under pathophysiological conditions of altered ROS and NO availability, such as occur during ischemia–reperfusion or chronic inflammation.

TNFR1 is a death receptor that mediates the major biological functions of TNF α . TNF α –TNFR1 binding elicits receptor trimerization, release of inhibitory silencer of death domain [105], and recruitment of TNFR1-associated death domain (TRADD) that results in complex I and II formation that activates distinct downstream survival or apoptotic signaling pathways [106]. At complex I, TRADD serves as a scaffold for the receptor-interacting protein 1 (RIP1) and TNF-receptor-associated factor 2 (TRAF2) in the recruitment of TGF- β -activated kinase 1 and activation of NF- κ B, p38, and Jun N-terminal protein kinase (JNK) [107]. NF- κ B activation is associated with induction of antiapoptotic proteins, FLIP_L, Bcl-xL, A1/Bfl-1, X-linked inhibitor of apoptosis (XIAP), and cellular inhibitors of apoptosis (c-IAP) 1 and 2 [108–110], and JNK activation is associated with ROS-induced activation of apoptosis signal-regulating kinase 1 (ASK1) and proteasomal degradation of FLIP_L [111,112]. Complex II comprises TRADD, FADD, and caspase-8 and is formed within the cytosol after TNFR1 receptorosome endocytosis [113]. The cellular status of FLIP_L and RIP1 seems to be an important checkpoint in determining whether TNFR1 induces apoptotic or survival signaling. High concentrations of FLIP_L competitively inhibit caspase-8 binding at complex II and prevent DISC formation [114], and complex I dissolution mediated by caspase-8 cleavage of RIP1 promotes complex II formation [115]. Given the similarity between the Fas and the TNFR1 systems, the activation of TNFR1-mediated apoptosis could similarly subscribe to modulation by ROS through clustering of receptors, signaling via lipid raft platforms, and interaction with NO, a suggestion that remains to be tested.

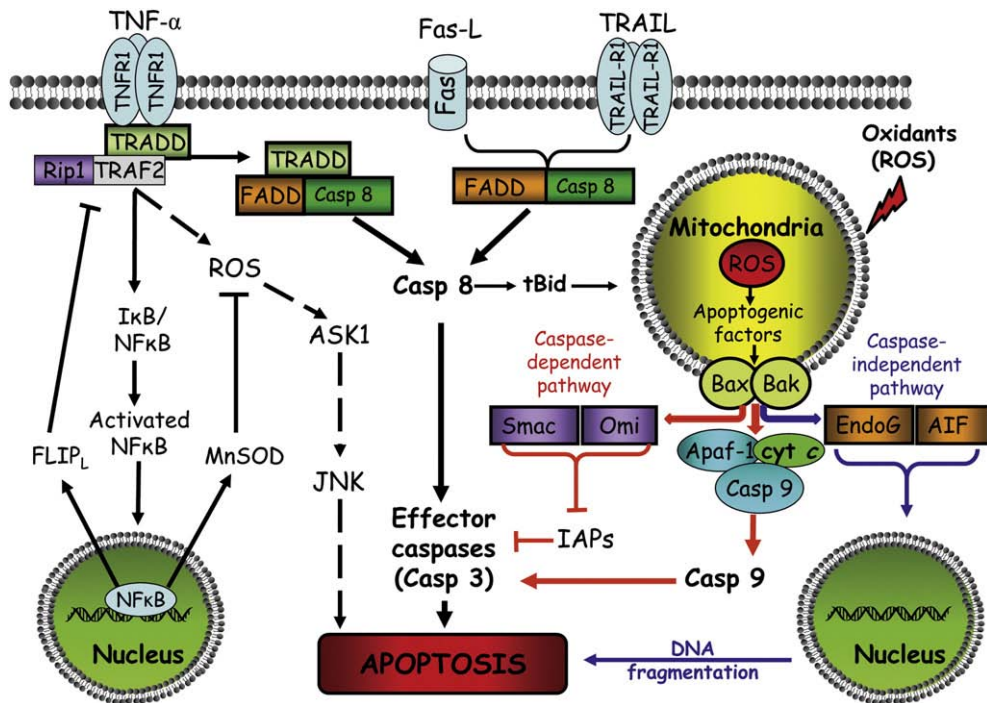


Fig. 1. Death-receptor-mediated and mitochondrial pathways of cell apoptosis. *Death receptor extrinsic pathway:* The major death receptor pathways include Fas/FasL, TNFR1/TNF α , and TRAIL-R1/TRAIL. Binding of ligands to their respective receptors activates downstream signaling and the formation of the death-inducing signaling complex. Activation of the NF- κ B survival pathway enhances transcription of antiapoptotic proteins such as FLIP_L or MnSOD and apoptosis blockade. At high ROS the failure to activate NF- κ B promotes ASK1/JNK activation that triggers cell apoptosis. Death receptor signaling is associated with caspase-8 activation that promotes apoptosis via activation of effector caspases (e.g., caspase-3) or engages mitochondrial apoptotic signaling via truncated Bid, leading to the release of apoptogenic factors such as cytochrome c into the cytosol. ASK1, apoptosis signal-regulating kinase 1; Apaf-1, apoptotic protease-activating factor-1; Bid, BH3-only proapoptotic protein; tBid, truncated form of Bid; Casp 8, 9, active forms of caspase-8 and -9; cyt c, cytochrome c; FLIP_L, FLICE inhibitory protein; FADD, Fas-associated death domain; FasL, Fas ligand; JNK, c-Jun N-terminal kinase; NF- κ B, nuclear transcription factor κ B; I κ B/NF- κ B, the inactive form of NF- κ B associated with its inhibitor; MnSOD, manganese superoxide dismutase; RIP1, receptor-interacting kinase 1; ROS, reactive oxygen species; TNF α , tumor necrosis factor- α ; TNFR1, TNF receptor-1; TRAIL, TNF-related apoptosis-inducing ligand; TRAIL-R1, Trail receptor-1; TRADD, TNF-receptor-associated death domain; TRAF-2, TNF-receptor-associated factor-2. *Mitochondrial intrinsic pathway:* Various apoptotic stimuli (e.g., ROS) mediate the permeabilization of the mitochondrial outer membrane and the release of proapoptotic proteins. Within the cytosol, cytochrome c together with Apaf-1 and dATP forms the apoptosome complex to which the initiator procaspase-9 is recruited and activated. Caspase-9-catalyzed activation of the effector caspase-3 executes the final steps of apoptosis. Caspase activation is further enhanced through neutralization of caspase inhibitors by apoptogenic proteins such as Smac/Diablo and Omi/HtrA2 that are released from the mitochondria. In addition, mitochondrial proteins such as AIF and Endo G promote caspase-independent apoptosis through nuclear translocation and by mediating genomic DNA fragmentation. Smac/Diablo, second mitochondria-derived activator of caspases/direct IAP binding protein of low pI; Omi/HtrA2, high-temperature requirement A2 serine protease; IAP, inhibitor of apoptosis protein; AIF, apoptosis-inducing factor; Endo G, endonuclease G; Bax/Bak, proapoptotic proteins.

The widely expressed TRAIL receptors (DR4 and DR5), unlike Fas and TNFR1, signal through Type 2 mechanisms [116,117] and are not dependent on internalization of the ligand-receptor complex for full activation of initiator caspases [118]. In addition, TRAIL receptor signals partner with decoy receptors that competitively bind TRAIL ligands. Because decoy receptor expression was reportedly lower in tumor cells, cancer cell susceptibility to TRAIL would probably be enhanced, a notion that requires validation and could be capitalized on in cancer therapy [119].

Mitochondrial pathway

Stimulation of the intrinsic mitochondrial apoptotic pathway by ROS and mitochondrial DNA damage promote outer membrane permeabilization and mitochondria-to-cytosol translocation of cytochrome c, AIF, or Smac/Diablo, which triggers caspase-dependent or caspase-independent cytosolic signaling events [120] (Fig. 1). In caspase-dependent signaling, cytochrome c forms the apoptosome complex with apoptotic protease-activating factor-1 (Apaf-1) and recruited procaspase-9, which induces cleavage of downstream effector caspases-3 and -7. Additionally, Smac/Diablo antagonizes the inhibitory effects of IAPs, which enhances caspase activation. AIF mediates caspase-independent signaling through cytosol-to-nuclear translocation and induction of nuclear chromatin condensation and DNA fragmentation [121].

Central to mitochondrial permeabilization and mitochondrial release of apoptogenic factors is the permeability transition pore

(PTP), a megapore spanning the inner and outer mitochondrial membranes. Of the three PTP component proteins, viz., cyclophilin D (cypD), voltage-dependent anion channel (VDAC), and the adenine nucleotide translocase (ANT), only cypD is a permanent constituent and modulator of PTP [122–124]. Key members of the antiapoptotic (Bcl-2, Bcl-X_L, and Bcl-w) and proapoptotic (Bax, Bak, Bad, Bim, and Bid) Bcl-2 superfamily of proteins are major players in mitochondrial outer membrane permeabilization and apoptotic susceptibility [125]. In the presence of an apoptotic stimulus, tBid promotes Bax/Bak oligomerization and membrane insertion that results in megapore formation, a highly orchestrated and active process [126,127]. Additionally, tBid/Bax-induced mitochondrial permeabilization was shown to be mediated through interaction with a functional outer membrane translocase, the TOM complex [128]. The pivotal contribution of tBid to apoptotic signaling in both the mitochondrial and the death receptor pathways is consistent with cross talk between intrinsic and extrinsic apoptotic signaling. However, the intracellular regulatory events and/or mechanisms that would preferentially trigger the engagement of the mitochondrial cascade after exposure to extrinsic Fas or TNF α signals remain to be determined.

ROS and JNK-mediated apoptotic signaling

ROS activation of JNK can induce extrinsic or intrinsic apoptotic signaling [129]. Upstream of JNK is the redox-sensitive MAPK kinase kinase, ASK1. ASK1 activity is inhibited by interactions with redox

proteins (Grx, Trx1), heat shock proteins (Hsp90, Hsp72), and 14-3-3 [130–132] and is stimulated by TRAF proteins, ASK1-interacting protein 1, Daxx, and JASP/JIP3 [133–135]. TNF α is a potent activator of the MAPK cascade, and the ASK1–JNK pathway plays an important role in TNFR1-mediated apoptotic signaling in various cell types [136]. Whether TNF α induces anti- or proapoptotic effects depends on the level and duration of JNK activation by ROS [137]. A transient and modest JNK activation mediates cell survival via NF- κ B-induced antiapoptotic gene expression, whereas a prolonged and robust JNK activation is associated with cell apoptosis via ASK1 signaling [138,139]. Thus, development of a strategy to manipulate the degree and duration of cellular JNK activation could provide a reasonable approach to specifically targeting cell survival or cell death such as in cancer therapy.

Fig. 2 summarizes our current understanding of the mechanism of ROS and redox modulation of ASK1/JNK signaling in cell apoptosis. This model proposes that ROS mediates the interaction of Trx1 and the N-terminal domain of ASK1, preventing ASK1 activation and downstream propagation of an apoptotic signal [130]. Only reduced Trx1 binds ASK1; the resultant Trx1/ASK1 complex, termed “ASK1 signalosome,” functions as a redox switch that senses cellular ROS and is activated under oxidizing conditions [140]. Elevated cellular ROS induce the dissociation of oxidized Trx1 from the complex and

permit ASK1 oligomerization through N-terminal coiled-coil domains and a gain of full ASK1 kinase activity [141]. Formation of a functional ASK1 signalosome complex is linked to the recruitment of TRAF2/6, which promotes ASK1 autophosphorylation and JNK activation [130,141]. ASK2, another member of the ASK family, binds and stabilizes ASK1 in the cytosol, nucleus, and mitochondria [142] and thus plays an important role in the regulation of ASK1/JNK signaling and cell apoptosis [143]. Current evidence shows that heterodimeric complexes of ASK1 and 2 stimulate JNK and p38 activity, whereas the absence of ASK2 diminishes apoptotic signaling [143]. ROS were shown to promote ASK1 activation by inducing the dissociation of the docking protein 14-3-3 [144] or by blocking the inhibitory effects of the protein phosphatases PP5 and PP2A [145]. Interestingly, the mitochondrial ASK1-dependent apoptotic signaling pathway reportedly activated both JNK-dependent and JNK-independent apoptosis [146]. Nuclear translocation of activated JNK promoted activator protein-1 (AP-1)-mediated expression of proapoptotic TNF α , FasL, and Bak [147], whereas mitochondrial JNK translocation promoted cytochrome *c* release [148]. Although the latter observations suggest cross talk among ASK1/JNK signaling and the classical mitochondrial and death receptor pathways in cell apoptosis, the extent of interaction and integration among these various apoptotic pathways is unclear, as is the universality and quantitative importance of ASK1/ASK2–JNK signaling in apoptosis of various cell types induced by intrinsic versus extrinsic signals.

GSH redox status and apoptotic signaling

ROS-mediated apoptotic signaling is associated with decreased cellular GSH levels and the loss of cellular redox balance [149,150]. Decreased cell GSH can occur through ROS-induced GSH oxidation or GSH export from cells; the resultant GSH reduction would enhance further ROS production during oxidative challenge [151]. It was demonstrated that GSH loss due to decreased de novo GSH synthesis triggered redox activation of protein kinase C (PKC δ) [152] and, through GSH efflux, induced JNK-dependent apoptosis [153]. The initiation of apoptosis through GSH efflux was a ROS-independent mechanism because apoptosis was attenuated by blockade of GSH export but not by antioxidants [154]. This suggestion is supported by our recent studies demonstrating that intestinal cell apoptosis induced by staurosporine (STP) was linked to GSH efflux without accompanying GSH/GSSG redox changes. This STP-induced export of cellular GSH was driven by γ -glutamyltransferase-catalyzed extracellular GSH hydrolysis [155] and was associated with caspase-3 activation independent of caspase-8 or -9 function. In other studies, FasL-induced GSH export was shown to be essential for the development of apoptosis in lymphoid cells; ROS production was merely a bystander phenomenon [154]. Collectively, the evidence to date has ruled out a major role for ROS signaling in GSH efflux-mediated cell apoptosis, and key challenges for future research will be delineating the mechanism(s) that couples the export of GSH to the triggering of apoptotic signals at cell membranes and the nature of these signals.

GSH oxidation is a major contributor to cell apoptosis mediated by oxidants. Accumulated evidence from our laboratory has consistently shown that an early spike in GSSG formation, typically within minutes of oxidant exposure, preceded oxidant-induced activation of mitochondrial apoptotic signaling and cell apoptosis hours later [20,21,156,157]. Importantly, postoxidant recovery of cellular GSH/GSSG redox status did not influence the apoptotic outcome, indicating that oxidant-induced apoptotic initiation occurred within an early and narrow window of GSH/GSSG redox shift. Indeed, whereas cell apoptosis was effectively blocked by NAC pretreatment, apoptosis was not prevented when the thiol antioxidant was administered after oxidant-induced GSH oxidation [21]. Collectively, our studies establish that this mode of early induction of cellular GSH redox imbalance

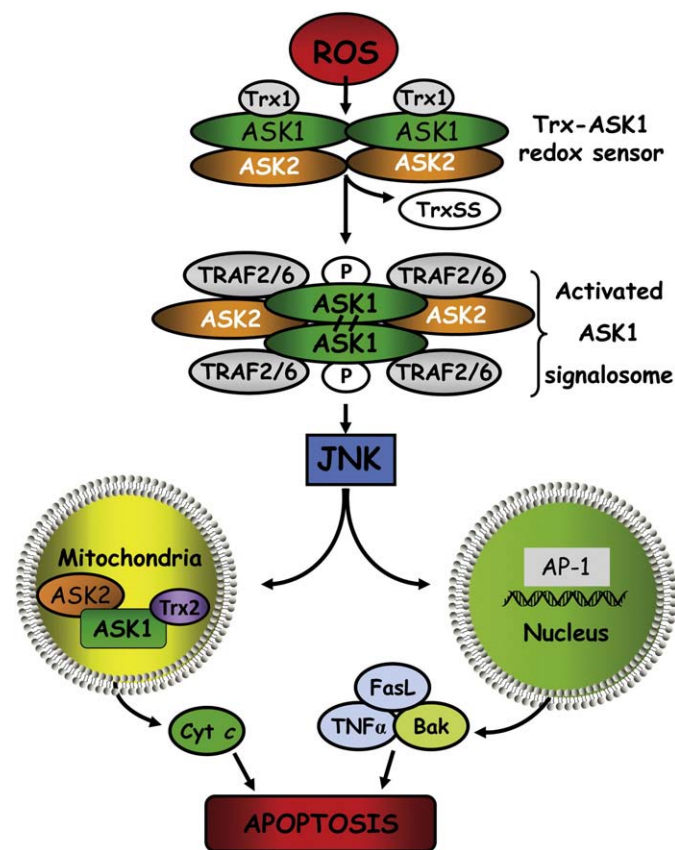


Fig. 2. ROS-induced ASK1/JNK signaling and apoptosis. Reactive oxygen species initiate Trx dissociation from the ASK1–Trx complex, the ASK1 signalosome, through oxidation of the Trx redox-active site. ASK1 undergoes autophosphorylation and covalent binding between its subunits, leading to the formation of an “activated signalosome” and recruitment of tumor necrosis factor receptor-associated factors 2 and 6 to the complex. Activated ASK1 signals downstream JNK activation and induces apoptosis either via mitochondrial signaling or via transcription of AP-1-dependent proapoptotic genes. Additionally, ROS-mediated disruption of the mitochondrial ASK1/ASK2/Trx2 complex induces cytochrome *c* release. ROS, reactive oxygen species; TRAF2/6, TNF α receptor-associated factor 2, 6; ASK1, 2, apoptosis signal-regulating kinase 1 and 2; JNK, c-Jun N-terminal kinase; Trx1, thioredoxin 1, reduced form; TrxSS, thioredoxin, oxidized form; Trx2, thioredoxin 2, mitochondrial enzyme; Bak, proapoptotic protein; Cyt *c*, cytochrome *c*; TNF α , tumor necrosis factor α ; FasL, Fas ligand.

in apoptotic redox signaling is common among diverse oxidants such as hydroperoxides (*tert*-butyl, lipid hydroperoxides), redox cycling menadione quinones (MQ), and methylglyoxal and in various intestinal (CaCo2, HT-29, NCM460) and neuronal (naïve and differentiated PC12) cell types [19,21,156,158–160]. We further show that oxidant-mediated apoptotic susceptibility is related to a cell's phenotype. The induction of PC12 cell differentiation generated a phenotype that was more resistant to *tert*-butylhydroperoxide; this oxidative resistance was associated with a reduced intracellular GSH redox environment and decreased Apaf-1 expression [20]. Attenuated cellular susceptibility of the differentiated PC12 phenotype was also observed for carbonyl stress [160] and hyperglycemic stress [22,159]. If validated, the paradigm of enhanced cellular vulnerability associated with a proliferative state should provide a strong cellular basis for targeting actively growing tumor cells for oxidative stress-induced cell killing.

The mitochondrial GSH/GSSG redox status is critical for preserving mitochondrial function during oxidative stress. Studies from our laboratory and others have documented a relationship between mitochondrial GSH (mtGSH) loss and cell apoptosis. Earlier studies demonstrated that decreased mtGSH correlated with apoptosis induced by exposure to aromatic hydrocarbons [161], hypoxia [162], *tert*-butylhydroperoxide [163], and ethanol [164–166]. Associated with decreased mtGSH were mitochondrial ROS production, loss of mitochondrial membrane potential, and mitochondria-to-cytosol release of cytochrome *c* [167]. Interestingly, moderate mtGSH decrease was insufficient to elicit apoptosis in hepatocytes during hypoxia, suggesting that achievement of a critical threshold of mtGSH loss is necessary to trigger mitochondrial apoptotic signaling [162]. Moreover, Ghosh et al. demonstrated that oxidation of mtGSH was requisite for mitochondrial ROS generation, membrane potential collapse, and caspase-9 and -3 activation in cardiomyocyte apoptosis caused by short-term diabetes [168]. We similarly showed that early ROS production and mtGSSG formation preceded mitochondrial dysfunction and apoptosis in intestinal cells exposed to menadione [19]. Given the oxidative vulnerability of the mtGSH pool and the dynamics of its maintenance, manipulation of the mitochondrial redox compartment can be capitalized to selectively sensitize cells to oxidative damage.

Modulators of initiation and execution of apoptosis

Mitochondrial modulators of apoptotic initiation

ROS and the mitochondrial permeability transition

ROS are known triggers of the intrinsic apoptotic cascade via interactions with proteins of the mitochondrial permeability transition complex [169]. Components of the PTP, viz., VDAC [170], ANT [171], and cypD [172], are targets of ROS, and oxidative modifications of PTP proteins will significantly impact mitochondrial anion fluxes. Indeed, a mere transient increase in mitochondrial membrane hyperpolarization after exposure to H₂O₂ initiated the collapse of the mitochondrial membrane potential ($\Delta\psi_m$) [173], mitochondrial translocation of Bax and Bad, and cytochrome *c* release [174]. Even nonoxidants such as cadmium and staurosporine can trigger intrinsic apoptotic signaling through induction of ROS production and associated mitochondrial permeability transition and cytochrome *c* translocation [175,176]. Significant mitochondrial loss of cytochrome *c* will lead to further ROS increase due to a disrupted electron transport chain [177].

Oxidative mitochondrial DNA damage

Mitochondrial DNA is a circular double-stranded DNA organized in nucleoids in proximity to the electron transport chain. mtDNA lacks introns and, being close to a ROS source, is prone to oxidative damage. Because mtDNA encodes 13 polypeptides of the respiratory chain, impaired mtRNA transcription would compromise mitochondrial ATP

production [178]. mtDNA damage-induced decreased respiratory function enhances ROS generation, thus eliciting a vicious cycle of ROS–mtDNA damage that ultimately triggers apoptosis [179,180]. A limited nucleotide excision DNA repair capacity coupled to a high mitochondrial ROS load further contributes to oxidative damage to mtDNA. Single-strand breaks and abasic sites formed during enhanced ROS generation can induce apoptotic signaling [181]. Interestingly, of the two sources of O₂^{•-} production elicited by angiotensin II exposure, viz., NADPH oxidase and mitochondria, only the latter resulted from mtDNA damage-induced impaired mitochondrial complex I activity. The subsequent collapse of the $\Delta\psi_m$, release of cytochrome *c*, and cell apoptosis were all prevented by inhibiting mtDNA damage [12]. Thus, the protection of mtDNA integrity is critical not only to bioenergetic homeostasis, but to cell survival as well. Recent findings by Rachek et al. [11] revealed that mtDNA damage was an initiating event in mitochondrial dysfunction and hepatotoxicity induced by pharmacologic levels of the diabetic drug troglitazone, thus ascribing potential clinical importance to mtDNA damage in drug toxicity.

Our recent studies demonstrated that mtGSH is a determinant of the extent of oxidant-induced DNA damage [10]. MQ-induced oxidative mtDNA damage paralleled the formation of mitochondrial GSSG and protein disulfide, which was blunted by NAC and exacerbated by inhibition of GSH synthesis in accordance with increased and decreased cellular GSH, respectively [182]. Significantly, mtDNA damage was potentiated by blockade of mtGSH transport and prevented by overexpression of the oxoglutarate mtGSH carrier, validating the link between mtGSH and mtDNA integrity. Moreover, post-MQ recovery of mtDNA was preceded by restored cellular GSH, suggesting that DNA repair may also be GSH dependent [182]. In previous studies, an inverse relationship between GSH and basal oxidative DNA damage [183] and an association between ROS-induced DNA deletions and genomic rearrangements with GSH depletion/oxidation have been documented [184–187]. Moreover, age-derived ROS-induced mtDNA damage was linked to mtGSH oxidation [188]. It remains to be established as to whether GSH functions in attenuating mtDNA damage or in stimulating mtDNA repair. Posttranslational phosphorylation of OGG1 [189] and acetylation/deacetylation of Ape1 [190] have been implicated in the control of DNA repair; the possibility that glutathiolation is another key posttranslational mechanism in the regulation of base-excision repair enzymes is an exciting notion that warrants investigation.

*Cytochrome *c* and cardiolipin interaction*

Cytochrome *c* is a water-soluble heme-containing protein bound to the outer leaflet of the mitochondrial inner membrane through interactions with the anionic phospholipid cardiolipin. Normally, cytochrome *c* participates in shuttling electrons between complex III and complex IV of the mitochondrial electron transport chain; its release from the mitochondria initiates the apoptotic cascade (Fig. 3). Mitochondria-to-cytosol release of cytochrome *c* sequentially occurs via detachment from cardiolipin and translocation through the mitochondrial outer membrane [191]. At low mitochondrial ROS, tightly bound cytochrome *c* exhibits increased peroxidase activity [192] that oxidizes cardiolipin and facilitates its detachment [193,194]. Oxidized cardiolipin is distributed to the outer leaflet of the mitochondrial membrane where it functions as a docking platform for tBid, enabling mitochondrial membrane permeabilization and cytochrome *c* movement across the outer membrane into the cytosol [195]. Because oxidative modification of cardiolipin is pivotal in mitochondrial cytochrome *c* loss and cell commitment to apoptosis, cardiolipin-bound cytochrome *c* could be viewed as a mitochondrial oxidative stress sensor and redox regulator of apoptosis. Therefore, the extent of cardiolipin peroxidation would probably be an important determinant of apoptotic susceptibility of different cell types possessing various cardiolipin species and fatty acyl side chains. A possible

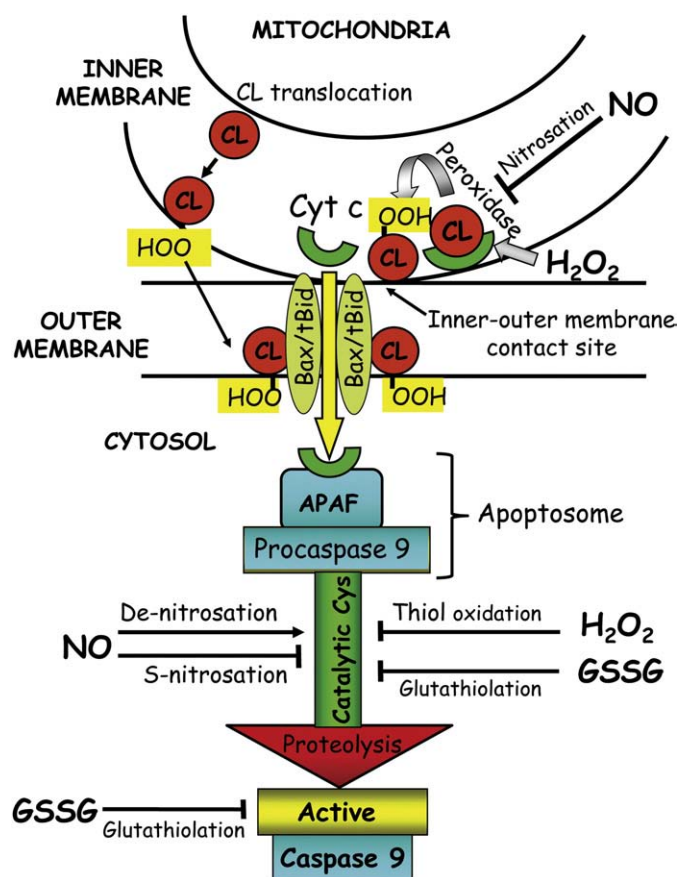


Fig. 3. Redox mediation of mitochondria-to-cytosol release of cytochrome *c* and activation/inactivation of caspase-9. Interaction with mitochondria-specific cardiolipin sequesters cytochrome *c* in the mitochondrial intermembrane space. Enhanced mitochondrial generation of H_2O_2 activates cytochrome *c* peroxidase activity and induces cardiolipin peroxidation and cytochrome *c* detachment. Oxidized cardiolipin is translocated to the mitochondrial outer membrane and, together with the proapoptotic proteins, Bid and Bax, forms a megapore channel that enables the mitochondria-to-cytosol transit of cytochrome *c*. Through a nitrosation reaction, the peroxidase activity of cytochrome *c* is inhibited by NO. Within the cytosol, cytochrome *c* interacts with Apaf-1 and dATP, forming the apoptosome complex to which procaspase-9 is recruited. Proteolytic activation of procaspase-9 is mediated by posttranslational modification of the catalytic site cysteines through thiol oxidation, nitrosation, or glutathiolation. H_2O_2 - and GSSG-mediated cysteine oxidation or S-glutathiolation, respectively, results in procaspase inactivation. NO-mediated S-nitrosation similarly inhibits caspase-9 activation, whereas denitrosation promotes proenzyme proteolysis and activation. Additionally, GSSG-dependent glutathiolation of active caspase-9 results in direct inhibition of enzyme activity. Apaf-1, apoptotic protease activation factor-1; CL, cardiolipin; CL-OOH, peroxidized cardiolipin; Cyt *c*, cytochrome *c*; Bid/Bax, proapoptotic proteins.

quantitative relationship between cardiolipin oxidation products and propensity for cell apoptosis has yet to be rigorously tested.

Nitric oxide reportedly modulates cytochrome *c* peroxidase function at the early stage of apoptotic initiation [196]. Physiological NO levels inhibit peroxidase activity and prevent cardiolipin oxidation, whereas elevated NO induces peroxynitrite-mediated nitration of Tyr-67 and enhances cytochrome *c* peroxidase activity [197,198]. However, the nitration of Tyr-74 after continuous peroxynitrite exposure prevented cytochrome *c*/apoptosome complex formation [199]. Interestingly, the oxidation/reduction state of cytochrome *c* per se has been implicated in mitochondrial apoptotic signaling [200], and cytochrome *c*-containing oxidized, but not reduced, heme was capable of caspase activation [201]. The redox state of cytochrome *c* within cells seems to be dependent on a nonapoptotic or apoptotic phenotype; generally, nonapoptotic cells favor cytochrome *c* reduction and cell survival, whereas apoptotic cells favor cytochrome *c* oxidation and apoptosis [202]. Precisely how the redox state of

cytochrome *c* contributes to the initiation of apoptosis is unknown, but it does not seem to involve direct effects on apoptosome formation or caspase-9 activation [200].

Redox modulation of apoptotic execution: control of caspase activity

Cellular caspases belong to a highly conserved family of cysteine proteases that cleave aspartate residues of caspase substrates and are the main players in the execution phase of apoptosis. The mammalian caspase family contains at least 14 members that are divided into initiator and executioner caspases [203]. Initiator or apical caspases are recruited at the death receptor via the death effector domain (caspase-8 and -10) or in the cytosol via the recruiting domain (caspase-2 and -9). Activated initiator caspases cleave executioner caspases such as caspase-3 and -7, which execute apoptosis through cleavage of protein substrates that include mediators and regulators of apoptosis, structural proteins, and DNA repair- and cell-cycle-related proteins [204]. Additionally, activated caspase-3 promotes caspase-2 and -6 activation in an amplification loop that enhances caspase-9 processing [205]. Caspases are constitutively expressed in the cytosol as inactive zymogen monomers and are activated by apoptotic signals such as ROS via proteolysis at internal sites [206]. Proteolytic cleavage of caspases at the N-terminal prodomain results in the generation of small p10 and large p20 active subunits, forming active p10/p20 tetramers. The activation of caspases is prevented by specific inhibitors belonging to the IAP family; members such as XIAP, c-IAP1, c-IAP2, and survivin bind and suppress enzyme catalytic activity [207]. During apoptotic signaling IAPs are antagonized by mitochondria-derived Smac/Diablo and Omi/HtrA2 proteins, allowing caspase-mediated execution [208,209].

Our present understanding of the redox control of apoptotic execution is sketchy, but it is a growing area of research. Posttranslational modification of catalytic site cysteine residues has gained recognition as a potentially important redox mechanism in the control of caspase activity. Redox-active catalytic site cysteines of caspases are prone to oxidation, nitrosation, or glutathiolation (Fig. 3) [203]. Direct ROS effects on caspase activation have been documented; for instance, H_2O_2 derived from endogenous and exogenous sources was shown to induce reversible inactivation of caspase 3 and caspase 8 through oxidation of their catalytic site cysteines [210]. For caspase-9, H_2O_2 -induced enzyme inactivation was specifically mediated through iron-catalyzed oxidation of the procaspase-9 catalytic site cysteine [211]. Additionally, H_2O_2 -mediated redox-dependent intramitochondrial autoactivation of caspase-9 has been demonstrated in U937 cells in which procaspase-9 dimerization was induced by thiol–disulfide bond formation, a process that was inhibited by Trx [212]. Because mitochondrial procaspase-9 activation occurred during the preapoptotic phase before cytochrome *c* release, it was suggested that this mechanism could amplify the proapoptotic effect of cytochrome *c* [212].

Low levels of NO have been shown to exert antiapoptotic effects via S-nitrosation of a single cysteine residue at the catalytic site of caspases [213,214]. To date, the enzyme activity of seven members of the caspase family have been shown to be inhibited by redox modulation through this mechanism [215]. Evidence that caspase-3 is nitrosated with resultant inhibition of enzyme activity comes from studies in human umbilical vein endothelial cells. Using electron spin resonance spectroscopy of Myc-tagged p17 (a subunit of caspase 3), Rossig et al. found that S-nitrosation of Cys-163 prevented the caspase-3-mediated apoptotic cascade [216]. In hepatocytes, NO blocked Bid activation through S-nitrosation of caspase-8 and prevented TNF α -induced mitochondrial apoptotic signaling [217–219]. Additionally, NO donors were found to inhibit proper assembly of the Apaf-1/caspase-9 apoptosome complex and caspase-9 activation [217–219]. Denitrosation is reportedly proapoptotic. For instance, denitrosation of procaspase-3 and procaspase-9 has been shown to

be associated with proteolytic enzyme activation in Fas-mediated [220] and cytokine-induced apoptosis [221]. A role for Trx1 in procaspase-3 S-nitrosation has been documented, an interaction that involves a transnitrosation reaction between procaspase-3 and Trx1 [222]. The cellular sites of caspase nitrosation/denitrosation have not been fully investigated, but mitochondria are reportedly key locations for S-nitrosation reactions, judging by the preferred mitochondrial distribution of nitrosated caspases [223]. If this is the case, the intriguing question of how nitrosation/activation of matrix caspases mediates downstream cytosolic events in apoptotic execution warrants further study.

Apart from ROS and NO, other recent evidence implicates a direct role for cellular GSH in redox regulation of caspase activity, mediated by S-glutathiolation. Reportedly, S-glutathiolation of cysteine contributes to caspase stability and decreased accessibility for proteolytic cleavage, consistent with apoptotic resistance. Conversely, recent work by Pan and Berk demonstrated that deglutathiolation of caspase-3 increased caspase-3 activity and TNF α -induced endothelial cell apoptosis [224]. Grx-catalyzed reversible glutathiolation of caspase-3 has been suggested to represent a novel redox signaling mechanism in TNF α -mediated cell apoptosis; specifically, TNF α -induced Grx assisted in thiol transfer in caspase-3 deglutathiolation [225]. Recent findings in HL60 cells show that GSSG at physiological levels mediated cysteine glutathiolation of both caspase-3 subunits and inhibition of enzyme activity [226], linking S-glutathiolation with direct inhibition of caspase activity. Additional findings that procaspase-9 and -3 were targets of glutathiolation further suggests that proteolytic activation of caspases may also be under GSH redox control [226]. Although caspases are subject to individual modification by nitrosation and glutathiolation, unanswered questions remain as to whether or how individual posttranslational mechanisms interact and integrate with one another collectively to optimize caspase activities during cell apoptosis.

Concluding remarks

Apoptosis has long been appreciated as an important form of cell death in biological processes and various pathologies. Our current understanding of the regulation of apoptosis is incomplete despite decades of research. The recognition that ROS play a central role in cell signaling has spurred much recent interest in the role of redox mechanisms in apoptotic signaling and control. Evidence to date has implicated redox-dependent mechanisms in the mitochondria-to-cytosol release of cytochrome c, a central event in apoptotic initiation. Additionally, S-nitrosation and S-glutathiolation of catalytic-site cysteines were reportedly important posttranslational redox mechanisms in the reversible activation/inactivation of caspases in the control of apoptotic execution. Cellular redox systems, most notably the GSH/GSSG redox couple, often functioning in conjunction with the thioredoxin system, are central in redox regulation and cell apoptosis. Less well understood is the contribution of the pyridine nucleotide couples of NAD⁺/NADH and NADP⁺/NADPH. Apart from their classical roles in bioenergetic homeostasis and reductive biosynthesis, respectively, recent evidence suggests that pyridine nucleotides have broader biological functions, including controlling cell death. At present, the precise contribution of pyridine nucleotides and the extent to which they interact with the thiol redox systems of GSH/GSSG and thioredoxin in redox regulation of apoptosis are unclear and should provide fruitful avenues for future research.

Acknowledgments

Research in the authors' laboratory was supported by a grant from the National Institutes of Health, DK44510.

References

- [1] Halliwell, B.; Cross, C. E. Oxygen-derived species: their relation to human disease and environmental stress. *Environ. Health Perspect.* **102** (Suppl. 10):5–12; 1994.
- [2] Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **90**:7915–7922; 1993.
- [3] Turrens, J. F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* **552**: 335–344; 2003.
- [4] Andreyev, A. Y.; Kushnareva, Y. E.; Starkov, A. A. Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Moscow)* **70**:200–214; 2005.
- [5] Starkov, A. A.; Fiskum, G.; Chinopoulos, C.; Lorenz, B. J.; Browne, S. E.; Patel, M. S.; Beal, M. F. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *J. Neurosci.* **24**:7779–7788; 2004.
- [6] Tretter, L.; Adam-Vizi, V. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. *J. Neurosci.* **24**:7771–7778; 2004.
- [7] Migliaccio, E.; Giorgio, M.; Pelicci, P. G. Apoptosis and aging: role of p66Shc redox protein. *Antioxid. Redox Signaling* **8**:600–608; 2006.
- [8] Korshunov, S. S.; Skulachev, V. P.; Starkov, A. A. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett.* **416**:15–18; 1997.
- [9] Lambert, A. J.; Brand, M. D. Superoxide production by NADH:ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane. *Biochem. J.* **382**:511–517; 2004.
- [10] Circu, M. L.; Moyer, M. P.; Harrison, L.; Aw, T. Y. Contribution of glutathione status to oxidant-induced mitochondrial DNA damage in colonic epithelial cells. *Free Radic. Biol. Med.* **47**:1190–1198; 2009.
- [11] Racheh, L. I.; Yuzefovych, L. V.; Ledoux, S. P.; Julie, N. L.; Wilson, G. L. Troglitazone, but not rosiglitazone, damages mitochondrial DNA and induces mitochondrial dysfunction and cell death in human hepatocytes. *Toxicol. Appl. Pharmacol.* **240**: 348–354; 2009.
- [12] Ricci, C.; Pastukh, V.; Leonard, J.; Turrens, J.; Wilson, G.; Schaffer, D.; Schaffer, S. W. Mitochondrial DNA damage triggers mitochondrial-superoxide generation and apoptosis. *Am. J. Physiol. Cell Physiol.* **294**:C413–422; 2008.
- [13] Fritz, R.; Bol, J.; Hebling, U.; Angermuller, S.; Volkl, A.; Fahimi, H. D.; Mueller, S. Compartment-dependent management of H₂O₂ by peroxisomes. *Free Radic. Biol. Med.* **42**:1119–1129; 2007.
- [14] Zangar, R. C.; Davydov, D. R.; Verma, S. Mechanisms that regulate production of reactive oxygen species by cytochrome P450. *Toxicol. Appl. Pharmacol.* **199**: 316–331; 2004.
- [15] Caro, A. A.; Cederbaum, A. I. Role of cytochrome P450 in phospholipase A2- and arachidonic acid-mediated cytotoxicity. *Free Radic. Biol. Med.* **40**:364–375; 2006.
- [16] Dumitru, C. A.; Zhang, Y.; Li, X.; Gulbins, E. Ceramide: a novel player in reactive oxygen species-induced signaling? *Antioxid. Redox Signaling* **9**:1535–1540; 2007.
- [17] Zhang, A. Y.; Yi, F.; Zhang, G.; Gulbins, E.; Li, P. L. Lipid raft clustering and redox signaling platform formation in coronary arterial endothelial cells. *Hypertension* **47**:74–80; 2006.
- [18] Zhang, A. Y.; Yi, F.; Jin, S.; Xia, M.; Chen, Q. Z.; Gulbins, E.; Li, P. L. Acid sphingomyelinase and its redox amplification in formation of lipid raft redox signaling platforms in endothelial cells. *Antioxid. Redox Signaling* **9**:817–828; 2007.
- [19] Circu, M. L.; Rodriguez, C.; Maloney, R.; Moyer, M. P.; Aw, T. Y. Contribution of mitochondrial GSH transport to matrix GSH status and colonic epithelial cell apoptosis. *Free Radic. Biol. Med.* **44**:768–778; 2008.
- [20] Ekshyyan, O.; Aw, T. Y. Decreased susceptibility of differentiated PC12 cells to oxidative challenge: relationship to cellular redox and expression of apoptotic protease activator factor-1. *Cell Death Differ.* **12**:1066–1077; 2005.
- [21] Pias, E. K.; Ekshyyan, O. Y.; Rhoads, C. A.; Fuseler, J.; Harrison, L.; Aw, T. Y. Differential effects of superoxide dismutase isoform expression on hydroperoxide-induced apoptosis in PC-12 cells. *J. Biol. Chem.* **278**:13294–13301; 2003.
- [22] Okouchi, M.; Okayama, N.; Alexander, J. S.; Aw, T. Y. NRF2-dependent glutamate-L-cysteine catalytic subunit expression mediates insulin protection against hyperglycemia-induced brain endothelial cell apoptosis. *Curr. Neurovasc. Res.* **3**: 249–261; 2006.
- [23] Schafer, F. Q.; Buettner, G. R. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* **30**:1191–1212; 2001.
- [24] Meister, A.; Tate, S. S. Glutathione and related gamma-glutamyl compounds: biosynthesis and utilization. *Annu. Rev. Biochem.* **45**:559–604; 1976.
- [25] Circu, M. L.; Aw, T. Y. Glutathione and apoptosis. *Free Radic. Res.* **42**:689–706; 2008.
- [26] Meister, A.; Anderson, M. E. Glutathione. *Annu. Rev. Biochem.* **52**:711–760; 1983.
- [27] Chakravarthi, S.; Jessop, C. E.; Bulleid, N. J. The role of glutathione in disulfide bond formation and endoplasmic-reticulum-generated oxidative stress. *EMBO Rep.* **7**:271–275; 2006.
- [28] Jessop, C. E.; Bulleid, N. J. Glutathione directly reduces an oxidoreductase in the endoplasmic reticulum of mammalian cells. *J. Biol. Chem.* **279**:55341–55347; 2004.
- [29] Frand, A. R.; Kaiser, C. A. Two pairs of conserved cysteines are required for the oxidative activity of Ero1p in protein disulfide bond formation in the endoplasmic reticulum. *Mol. Biol. Cell* **11**:2833–2843; 2000.
- [30] Dixon, B. M.; Heath, S. H.; Kim, R.; Suh, J. H.; Hagen, T. M. Assessment of endoplasmic reticulum glutathione redox status is confounded by extensive *in vivo* oxidation. *Antioxid. Redox Signaling* **10**:963–972; 2008.
- [31] Chen, J.; Delannoy, M.; Odwin, S.; He, P.; Trush, M. A.; Yager, J. D. Enhanced mitochondrial gene transcript, ATP, bcl-2 protein levels, and altered glutathione

- distribution in ethinyl estradiol-treated cultured female rat hepatocytes. *Toxicol. Sci.* **75**:271–278; 2003.
- [32] Markovic, J.; Borrás, C.; Ortega, A.; Sastre, J.; Vina, J.; Pallardo, F. V. Glutathione is recruited into the nucleus in early phases of cell proliferation. *J. Biol. Chem.* **282**: 20416–20424; 2007.
- [33] Ho, Y. F.; Guenther, T. M. Isolation of liver nuclei that retain functional transmembrane transport. *J. Pharmacol. Toxicol. Methods* **38**:163–168; 1997.
- [34] Voehringer, D. W.; McConkey, D. J.; McDonnell, T. J.; Brisbay, S.; Meyn, R. E. Bcl-2 expression causes redistribution of glutathione to the nucleus. *Proc. Natl. Acad. Sci. USA* **95**:2956–2960; 1998.
- [35] Jocelyn, P. C.; Kamminga, A. The non-protein thiol of rat liver mitochondria. *Biochim. Biophys. Acta* **343**:356–362; 1974.
- [36] Schnellmann, R. G. Renal mitochondrial glutathione transport. *Life Sci.* **49**: 393–398; 1991.
- [37] Soderdahl, T.; Enoksson, M.; Lundberg, M.; Holmgren, A.; Ottersen, O. P.; Orrenius, S.; Bolcsfoldi, G.; Cotgreave, I. A. Visualization of the compartmentalization of glutathione and protein–glutathione mixed disulfides in cultured cells. *FASEB J.* **17**:124–126; 2003.
- [38] Zimmermann, A. K.; Loucks, F. A.; Schroeder, E. K.; Bouchard, R. J.; Tyler, K. L.; Linseman, D. A. Glutathione binding to the Bcl-2 homology-3 domain groove: a molecular basis for Bcl-2 antioxidant function at mitochondria. *J. Biol. Chem.* **282**: 29296–29304; 2007.
- [39] Nakamura, H.; Nakamura, K.; Yodoi, J. Redox regulation of cellular activation. *Annu. Rev. Immunol.* **15**:351–369; 1997.
- [40] Watson, W. H.; Jones, D. P. Oxidation of nuclear thioredoxin during oxidative stress. *FEBS Lett.* **543**:144–147; 2003.
- [41] Hansen, J. M.; Go, Y. M.; Jones, D. P. Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. *Annu. Rev. Pharmacol. Toxicol.* **46**: 215–234; 2006.
- [42] Hansen, J. M.; Zhang, H.; Jones, D. P. Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. *Free Radic. Biol. Med.* **40**:138–145; 2006.
- [43] Kemp, M.; Go, Y. M.; Jones, D. P. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. *Free Radic. Biol. Med.* **44**:921–937; 2008.
- [44] Go, Y. M.; Jones, D. P. Redox compartmentalization in eukaryotic cells. *Biochim. Biophys. Acta* **1780**:1273–1290; 2008.
- [45] Rhee, S. G.; Chae, H. Z.; Kim, K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic. Biol. Med.* **38**:1543–1552; 2005.
- [46] Wood, Z. A.; Schroder, E.; Robin Harris, J.; Poole, L. B. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem. Sci.* **28**:32–40; 2003.
- [47] Zhang, Q.; Piston, D. W.; Goodman, R. H. Regulation of corepressor function by nuclear NADH. *Science* **295**:1895–1897; 2002.
- [48] Guse, A. H. Second messenger function and the structure–activity relationship of cyclic adenosine diphosphoribose (cADPR). *FEBS J.* **272**:4590–4597; 2005.
- [49] Virag, L.; Szabo, C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol. Rev.* **54**:375–429; 2002.
- [50] Lee, H. C. Physiological functions of cyclic ADP-ribose and NAADP as calcium messengers. *Annu. Rev. Pharmacol. Toxicol.* **41**:317–345; 2001.
- [51] Bedard, K.; Krause, K. H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* **87**:245–313; 2007.
- [52] Bolanos, J. P.; Delgado-Esteban, M.; Herrero-Mendez, A.; Fernandez-Fernandez, S.; Almeida, A. Regulation of glycolysis and pentose–phosphate pathway by nitric oxide: impact on neuronal survival. *Biochim. Biophys. Acta* **1777**:789–793; 2008.
- [53] Scott, M. D.; Zuo, L.; Lubin, B. H.; Chiu, D. T. NADPH, not glutathione, status modulates oxidant sensitivity in normal and glucose-6-phosphate dehydrogenase-deficient erythrocytes. *Blood* **77**:2059–2064; 1991.
- [54] Pandolfi, P. P.; Sonati, F.; Rivi, R.; Mason, P.; Grosveld, F.; Luzzatto, L. Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. *EMBO J.* **14**:5209–5215; 1995.
- [55] Kim, H. J.; Kang, B. S.; Park, J. W. Cellular defense against heat shock-induced oxidative damage by mitochondrial NADP⁺-dependent isocitrate dehydrogenase. *Free Radic. Res.* **39**:441–448; 2005.
- [56] Yoshihara, T.; Hamamoto, T.; Munakata, R.; Tajiri, R.; Ohsumi, M.; Yokota, S. Localization of cytosolic NADP-dependent isocitrate dehydrogenase in the peroxisomes of rat liver cells: biochemical and immunocytochemical studies. *J. Histochem. Cytochem.* **49**:1123–1131; 2001.
- [57] Kim, S. J.; Yune, T. Y.; Han, C. T.; Kim, Y. C.; Oh, Y. J.; Markelonis, G. J.; Oh, T. H. Mitochondrial isocitrate dehydrogenase protects human neuroblastoma SH-SY5Y cells against oxidative stress. *J. Neurosci. Res.* **85**:139–152; 2007.
- [58] Shin, S. W.; Kil, I. S.; Park, J. W. Silencing of mitochondrial NADP⁺-dependent isocitrate dehydrogenase by small interfering RNA enhances heat shock-induced apoptosis. *Biochem. Biophys. Res. Commun.* **366**:1012–1018; 2008.
- [59] Kil, I. S.; Kim, S. Y.; Lee, S. J.; Park, J. W. Small interfering RNA-mediated silencing of mitochondrial NADP⁺-dependent isocitrate dehydrogenase enhances the sensitivity of HeLa cells toward tumor necrosis factor- α and anticancer drugs. *Free Radic. Biol. Med.* **43**:1197–1207; 2007.
- [60] Filosa, S.; Fico, A.; Paglialonga, F.; Balestrieri, M.; Crooke, A.; Verde, P.; Abrescia, P.; Bautista, J. M.; Martini, G. Failure to increase glucose consumption through the pentose-phosphate pathway results in the death of glucose-6-phosphate dehydrogenase gene-deleted mouse embryonic stem cells subjected to oxidative stress. *Biochem. J.* **370**:935–943; 2003.
- [61] MacDonald, M. J. Evidence for the malate aspartate shuttle in pancreatic islets. *Arch. Biochem. Biophys.* **213**:643–649; 1982.
- [62] McKenna, M. C.; Stevenson, J. H.; Huang, X.; Tildon, J. T.; Zielke, C. L.; Hopkins, I. B. Mitochondrial malic enzyme activity is much higher in mitochondria from cortical synaptic terminals compared with mitochondria from primary cultures of cortical neurons or cerebellar granule cells. *Neurochem. Int.* **36**:451–459; 2000.
- [63] Vogel, R.; Wiesinger, H.; Hamprecht, B.; Dringen, R. The regeneration of reduced glutathione in rat forebrain mitochondria identifies metabolic pathways providing the NADPH required. *Neurosci. Lett.* **275**:97–100; 1999.
- [64] Pollak, N.; Niere, M.; Ziegler, M. NAD kinase levels control the NADPH concentration in human cells. *J. Biol. Chem.* **282**:33562–33571; 2007.
- [65] Akella, S. S.; Harris, C. Developmental ontogeny of NAD⁺ kinase in the rat conceptus. *Toxicol. Appl. Pharmacol.* **170**:124–129; 2001.
- [66] Singh, R.; Lemire, J.; Mailloux, R. J.; Appanna, V. D. A novel strategy involved anti-oxidative defense: the conversion of NADH into NADPH by a metabolic network. *PLoS ONE* **3**:e2682; 2008.
- [67] Singh, R.; Mailloux, R. J.; Puiseux-Dao, S.; Appanna, V. D. Oxidative stress evokes a metabolic adaptation that favors increased NADPH synthesis and decreased NADH production in *Pseudomonas fluorescens*. *J. Bacteriol.* **189**:6665–6675; 2007.
- [68] Murayama, A.; Ohmori, K.; Fujimura, A.; Minami, H.; Yasuzawa-Tanaka, K.; Kuroda, T.; Oie, S.; Daitoku, H.; Okuwaki, M.; Nagata, K.; Fukamizu, A.; Kimura, K.; Shimizu, T.; Yanagisawa, J. Epigenetic control of rDNA loci in response to intracellular energy status. *Cell* **133**:627–639; 2008.
- [69] Sundaresan, N. R.; Samant, S. A.; Pillai, V. B.; Rajamohan, S. B.; Gupta, M. P. SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. *Mol. Cell. Biol.* **28**: 6384–6401; 2008.
- [70] Rose, G.; Dato, S.; Altomare, K.; Bellizzi, D.; Garasto, S.; Greco, V.; Passarino, G.; Feraco, E.; Mari, V.; Barbi, C.; Bonafe, M.; Franceschi, C.; Tan, Q.; Boiko, S.; Yashin, A. I.; De Benedictis, G. Variability of the SIRT3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. *Exp. Gerontol.* **38**: 1065–1070; 2003.
- [71] Oberdoerffer, P.; Michan, S.; McVay, M.; Mostoslavsky, R.; Vann, J.; Park, S. K.; Hartlerode, A.; Stegmuller, J.; Hafner, A.; Loerch, P.; Wright, S. M.; Mills, K. D.; Bonni, A.; Yankner, B. A.; Scully, R.; Prolla, T. A.; Alt, F. W.; Sinclair, D. A. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* **135**:907–918; 2008.
- [72] Blander, G.; Guarente, L. The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.* **73**:417–435; 2004.
- [73] Tanno, M.; Sakamoto, J.; Miura, T.; Shimamoto, K.; Horio, Y. Nucleocytoplasmic shuttling of the NAD⁺-dependent histone deacetylase SIRT1. *J. Biol. Chem.* **282**: 6823–6832; 2007.
- [74] Yamamoto, H.; Schoonjans, K.; Auwerx, J. Sirtuin functions in health and disease. *Mol. Endocrinol.* **21**:1745–1755; 2007.
- [75] Smith, B. C.; Hallows, W. C.; Denu, J. M. Mechanisms and molecular probes of sirtuins. *Chem. Biol.* **15**:1002–1013; 2008.
- [76] Kume, S.; Haneda, M.; Kanasaki, K.; Sugimoto, T.; Araki, S.; Isono, M.; Isshiki, K.; Uzu, T.; Kashiwagi, A.; Koya, D. Silent information regulator 2 (SIRT1) attenuates oxidative stress-induced mesangial cell apoptosis via p53 deacetylation. *Free Radic. Biol. Med.* **40**:2175–2182; 2006.
- [77] Li, M.; Luo, J.; Brooks, C. L.; Gu, W. Acetylation of p53 inhibits its ubiquitination by Mdm2. *J. Biol. Chem.* **277**:50607–50611; 2002.
- [78] Luo, J.; Nikolaev, A. Y.; Imai, S.; Chen, D.; Su, F.; Shiloh, A.; Guarente, L.; Gu, W. Negative control of p53 by Sir2 α promotes cell survival under stress. *Cell* **107**:137–148; 2001.
- [79] Nakano, K.; Vousden, K. H. PUMA, a novel proapoptotic gene, is induced by p53. *Mol. Cell* **7**:683–694; 2001.
- [80] Macip, S.; Igarashi, M.; Berggren, P.; Yu, J.; Lee, S. W.; Aaronson, S. A. Influence of induced reactive oxygen species in p53-mediated cell fate decisions. *Mol. Cell. Biol.* **23**:8576–8585; 2003.
- [81] Han, M. K.; Song, E. K.; Guo, Y.; Ou, X.; Mantel, C.; Broxmeyer, H. E. SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. *Cell Stem Cell* **2**:241–251; 2008.
- [82] Brunet, A.; Sweeney, L. B.; Sturgill, J. F.; Chua, K. F.; Greer, P. L.; Lin, Y.; Tran, H.; Ross, S. E.; Mostoslavsky, R.; Cohen, H. Y.; Hu, L. S.; Cheng, H. L.; Jedrychowski, M. P.; Gygi, S. P.; Sinclair, D. A.; Alt, F. W.; Greenberg, M. E. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303**:2011–2015; 2004.
- [83] Hasegawa, K.; Wakino, S.; Yoshioka, K.; Tatsumatsu, S.; Hara, Y.; Minakuchi, H.; Washida, N.; Tokuyama, H.; Hayashi, K.; Itoh, H. Sirt1 protects against oxidative stress-induced renal tubular cell apoptosis by the bidirectional regulation of catalase expression. *Biochem. Biophys. Res. Commun.* **372**:51–56; 2008.
- [84] Chiribau, C. B.; Cheng, L.; Cucoranu, I. C.; Yu, Y. S.; Clempus, R. E.; Sorescu, D. FOXO3A regulates peroxiredoxin III expression in human cardiac fibroblasts. *J. Biol. Chem.* **283**:8211–8217; 2008.
- [85] Ford, J.; Jiang, M.; Milner, J. Cancer-specific functions of SIRT1 enable human epithelial cancer cell growth and survival. *Cancer Res.* **65**:10457–10463; 2005.
- [86] Jung-Hynes, B.; Nihal, M.; Zhong, W.; Ahmad, N. Role of sirtuin histone deacetylase Sirt1 in prostate cancer: a target for prostate cancer management via its inhibition? *J. Biol. Chem.* **284**:3823–3832; 2008.
- [87] Lombard, D. B.; Alt, F. W.; Cheng, H. L.; Bunkenborg, J.; Streeper, R. S.; Mostoslavsky, R.; Kim, J.; Yancopoulos, G.; Valenzuela, D.; Murphy, A.; Yang, Y.; Chen, Y.; Hirshey, M. D.; Bronson, R. T.; Haigis, M.; Guarente, L. P.; Farese Jr., R. V.; Weissman, S.; Verdin, E.; Schwer, B. Mammalian Sir2 homologue SIRT3 regulates global mitochondrial lysine acetylation. *Mol. Cell. Biol.* **27**:8807–8814; 2007.
- [88] Haigis, M. C.; Mostoslavsky, R.; Haigis, K. M.; Fahie, K.; Christodoulou, D. C.; Murphy, A. J.; Valenzuela, D. M.; Yancopoulos, G. D.; Karow, M.; Blander, G.;

- Wolberger, C.; Prolla, T. A.; Weindruch, R.; Alt, F. W.; Guarente, L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* **126**:941–954; 2006.
- [89] Schlicker, C.; Gertz, M.; Papatheodorou, P.; Kachholz, B.; Becker, C. F.; Steegborn, C. Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. *J. Mol. Biol.* **382**:790–801; 2008.
- [90] Ahn, B. H.; Kim, H. S.; Song, S.; Lee, I. H.; Liu, J.; Vassilopoulos, A.; Deng, C. X.; Finkel, T. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. *Proc. Natl. Acad. Sci. USA* **105**:14447–14452; 2008.
- [91] Shi, T.; Wang, F.; Stieren, E.; Tong, Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J. Biol. Chem.* **280**:13560–13567; 2005.
- [92] Guarente, L.; Picard, F. Calorie restriction—the SIR2 connection. *Cell* **120**:473–482; 2005.
- [93] North, B. J.; Marshall, B. L.; Borra, M. T.; Denu, J. M.; Verdin, E. The human Sir2 ortholog, SIRT2, is an NAD⁺-dependent tubulin deacetylase. *Mol. Cell* **11**:437–444; 2003.
- [94] Lombard, D. B.; Schwer, B.; Alt, F. W.; Mostoslavsky, R. SIRT6 in DNA repair, metabolism and ageing. *J. Intern. Med.* **263**:128–141; 2008.
- [95] Liszt, G.; Ford, E.; Kurtev, M.; Guarente, L. Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase. *J. Biol. Chem.* **280**:21313–21320; 2005.
- [96] Ford, E.; Voit, R.; Liszt, G.; Magin, C.; Grummt, I.; Guarente, L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev.* **20**:1075–1080; 2006.
- [97] Ashkenazi, A.; Dixit, V. M. Apoptosis control by death and decoy receptors. *Curr. Opin. Cell Biol.* **11**:255–260; 1999.
- [98] LeBlanc, H. N.; Ashkenazi, A. Apo2L/TRAIL and its death and decoy receptors. *Cell Death Differ.* **10**:66–75; 2003.
- [99] Berg, D.; Lehne, M.; Muller, N.; Siegmund, D.; Munkel, S.; Sebald, W.; Pfizenmaier, K.; Wajant, H. Enforced covalent trimerization increases the activity of the TNF ligand family members TRAIL and CD95L. *Cell Death Differ.* **14**:2021–2034; 2007.
- [100] Watanabe, N.; Kuriyama, H.; Sone, H.; Neda, H.; Yamauchi, N.; Maeda, M.; Niitsu, Y. Continuous internalization of tumor necrosis factor receptors in a human myosarcoma cell line. *J. Biol. Chem.* **263**:10262–10266; 1988.
- [101] Lee, K. H.; Feig, C.; Tchikov, V.; Schickel, R.; Hallas, C.; Schutze, S.; Peter, M. E.; Chan, A. C. The role of receptor internalization in CD95 signaling. *EMBO J.* **25**:1009–1023; 2006.
- [102] Huang, X.; Masselli, A.; Frisch, S. M.; Hunton, I. C.; Jiang, Y.; Wang, J. Y. Blockade of tumor necrosis factor-induced Bid cleavage by caspase-resistant Rb. *J. Biol. Chem.* **282**:29401–29413; 2007.
- [103] Barnhart, B. C.; Alappat, E. C.; Peter, M. E. The CD95 type I/type II model. *Semin. Immunol.* **15**:185–193; 2003.
- [104] Wang, L.; Azad, N.; Kongkanermit, L.; Chen, F.; Lu, Y.; Jiang, B. H.; Rojanasakul, Y. The Fas death signaling pathway connecting reactive oxygen species generation and FLICE inhibitory protein down-regulation. *J. Immunol.* **180**:3072–3080; 2008.
- [105] Jiang, Y.; Woronicz, J. D.; Liu, W.; Goeddel, D. V. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* **283**:543–546; 1999.
- [106] Hsu, H.; Xiong, J.; Goeddel, D. V. The TNF receptor 1-associated protein TRADD signals cell death and NF- κ B activation. *Cell* **81**:495–504; 1995.
- [107] Shim, J. H.; Xiao, C.; Paschal, A. E.; Bailey, S. T.; Rao, P.; Hayden, M. S.; Lee, K. Y.; Bussey, C.; Steckel, M.; Tanaka, N.; Yamada, G.; Akira, S.; Matsumoto, K.; Ghosh, S. TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways in vivo. *Genes Dev.* **19**:2668–2681; 2005.
- [108] Zong, W. X.; Edelstein, L. C.; Chen, C.; Bash, J.; Gelinas, C. The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF- κ B that blocks TNF α -induced apoptosis. *Genes Dev.* **13**:382–387; 1999.
- [109] Stehlik, C.; de Martin, R.; Kumabashiri, I.; Schmid, J. A.; Binder, B. R.; Lipp, J. Nuclear factor (NF)- κ B-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor α -induced apoptosis. *J. Exp. Med.* **188**:211–216; 1998.
- [110] Micheau, O.; Lens, S.; Gaide, O.; Alevizopoulos, K.; Tschopp, J. NF- κ B signals induce the expression of c-FLIP. *Mol. Cell. Biol.* **21**:5299–5305; 2001.
- [111] Chang, L.; Kamata, H.; Solinas, G.; Luo, J. L.; Maeda, S.; Venuprasad, K.; Liu, Y. C.; Karin, M. The E3 ubiquitin ligase itch couples JNK activation to TNF α -induced cell death by inducing c-FLIP(L) turnover. *Cell* **124**:601–613; 2006.
- [112] Pantano, C.; Shrivastava, P.; McElhinney, B.; Janssen-Heininger, Y. Hydrogen peroxide signaling through tumor necrosis factor receptor 1 leads to selective activation of c-Jun N-terminal kinase. *J. Biol. Chem.* **278**:44091–44096; 2003.
- [113] Schneider-Brachert, W.; Tchikov, V.; Neumeyer, J.; Jakob, M.; Winoto-Morbach, S.; Held-Feindt, J.; Heinrich, M.; Merkel, O.; Ehrenschwender, M.; Adam, D.; Mentlein, R.; Kabelitz, D.; Schutze, S. Compartmentalization of TNF receptor 1 signaling: internalized TNF receptors as death signaling vesicles. *Immunity* **21**:415–428; 2004.
- [114] Micheau, O.; Tschopp, J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* **114**:181–190; 2003.
- [115] Lin, Y.; Devin, A.; Rodriguez, Y.; Liu, Z. G. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. *Genes Dev.* **13**:2514–2526; 1999.
- [116] LeBlanc, H.; Lawrence, D.; Varfolomeev, E.; Totpal, K.; Morlan, J.; Schow, P.; Fong, S.; Schwall, R.; Sinicropi, D.; Ashkenazi, A. Tumor-cell resistance to death receptor-induced apoptosis through mutational inactivation of the proapoptotic Bcl-2 homolog Bax. *Nat. Med.* **8**:274–281; 2002.
- [117] Deng, Y.; Lin, Y.; Wu, X. TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. *Genes Dev.* **16**:33–45; 2002.
- [118] Jin, Z.; El-Deiry, W. S. Distinct signaling pathways in TRAIL- versus tumor necrosis factor-induced apoptosis. *Mol. Cell. Biol.* **26**:8136–8148; 2006.
- [119] Falschlehner, C.; Emmerich, C. H.; Gerlach, B.; Walczak, H. TRAIL signalling: decisions between life and death. *Int. J. Biochem. Cell Biol.* **39**:1462–1475; 2007.
- [120] Ryter, S. W.; Kim, H. P.; Hoetzel, A.; Park, J. W.; Nakahira, K.; Wang, X.; Choi, A. M. Mechanisms of cell death in oxidative stress. *Antioxid. Redox Signaling* **9**:49–89; 2007.
- [121] Susin, S. A.; Lorenzo, H. K.; Zamzami, M.; Marzo, I.; Snow, B. E.; Brothers, G. M.; Mangion, J.; Jacotot, E.; Costantini, P.; Loeffler, M.; Laroche, N.; Goodlett, D. R.; Aebbersold, R.; Siderovski, D. P.; Penninger, J. M.; Kroemer, G. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* **397**:441–446; 1999.
- [122] Baines, C. P.; Kaiser, R. A.; Sheiko, T.; Craigen, W. J.; Molkenin, J. D. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat. Cell Biol.* **9**:550–555; 2007.
- [123] Kokoszka, J. E.; Waymire, K. G.; Levy, S. E.; Sligh, J. E.; Cai, J.; Jones, D. P.; MacGregor, G. R.; Wallace, D. C. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* **427**:461–465; 2004.
- [124] Schinzel, A. C.; Takeuchi, O.; Huang, Z.; Fisher, J. K.; Zhou, Z.; Rubens, J.; Hetz, C.; Danial, N. N.; Moskowitz, M. A.; Korsmeyer, S. J. Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc. Natl. Acad. Sci. USA* **102**:12005–12010; 2005.
- [125] Kelekar, A.; Thompson, C. B. Bcl-2-family proteins: the role of the BH3 domain in apoptosis. *Trends Cell Biol.* **8**:324–330; 1998.
- [126] Eskes, R.; Desagher, S.; Antonsson, B.; Martinou, J. C. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. *Mol. Cell. Biol.* **20**:929–935; 2000.
- [127] Desagher, S.; Osen-Sand, A.; Nichols, A.; Eskes, R.; Montessuit, S.; Lauper, S.; Maundrell, K.; Antonsson, B.; Martinou, J. C. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J. Cell Biol.* **144**:891–901; 1999.
- [128] Ott, M.; Norberg, E.; Walter, K. M.; Schreiner, P.; Kemper, C.; Rapaport, D.; Zhivotovskiy, B.; Orrenius, S. The mitochondrial TOM complex is required for tBid/Bax-induced cytochrome c release. *J. Biol. Chem.* **282**:27633–27639; 2007.
- [129] Dhanasekaran, D. N.; Reddy, E. P. JNK signaling in apoptosis. *Oncogene* **27**:6245–6251; 2008.
- [130] Saitoh, M.; Nishitoh, H.; Fujii, M.; Takeda, K.; Tobiume, K.; Sawada, Y.; Kawabata, M.; Miyazono, K.; Ichijo, H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J.* **17**:2596–2606; 1998.
- [131] Song, J. J.; Rhee, J. G.; Suntharalingam, M.; Walsh, S. A.; Spitz, D. R.; Lee, Y. J. Role of glutaredoxin in metabolic oxidative stress: glutaredoxin as a sensor of oxidative stress mediated by H₂O₂. *J. Biol. Chem.* **277**:46566–46575; 2002.
- [132] Zhang, L.; Chen, J.; Fu, H. Suppression of apoptosis signal-regulating kinase 1-induced cell death by 14-3-3 proteins. *Proc. Natl. Acad. Sci. USA* **96**:8511–8515; 1999.
- [133] Zhang, H.; Zhang, R.; Luo, Y.; D'Alessio, A.; Pober, J. S.; Min, W. AIP1/DAB2IP, a novel member of the Ras-GAP family, transduces TRAF2-induced ASK1-JNK activation. *J. Biol. Chem.* **279**:44955–44965; 2004.
- [134] Chang, H. Y.; Nishitoh, H.; Yang, X.; Ichijo, H.; Baltimore, D. Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* **281**:1860–1863; 1998.
- [135] Matsuura, H.; Nishitoh, H.; Takeda, K.; Matsuzawa, A.; Amagasa, T.; Ito, M.; Yoshioka, K.; Ichijo, H. Phosphorylation-dependent scaffolding role of JSAP1/JIP3 in the ASK1-JNK signaling pathway: a new mode of regulation of the MAP kinase cascade. *J. Biol. Chem.* **277**:40703–40709; 2002.
- [136] Matsuzawa, A.; Ichijo, H. Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signaling. *Biochim. Biophys. Acta* **1708**:2008.
- [137] Kamata, H.; Honda, S.; Maeda, S.; Chang, L.; Hirata, H.; Karin, M. Reactive oxygen species promote TNF α -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* **120**:649–661; 2005.
- [138] Liu, H.; Lo, C. R.; Czaja, M. J. NF- κ B inhibition sensitizes hepatocytes to TNF-induced apoptosis through a sustained activation of JNK and c-Jun. *Hepatology* **35**:772–778; 2002.
- [139] Deng, Y.; Ren, X.; Yang, L.; Lin, Y.; Wu, X. A JNK-dependent pathway is required for TNF α -induced apoptosis. *Cell* **115**:61–70; 2003.
- [140] Fujino, G.; Noguchi, T.; Takeda, K.; Ichijo, H. Thioredoxin and protein kinases in redox signaling. *Semin. Cancer Biol.* **16**:427–435; 2006.
- [141] Fujino, G.; Noguchi, T.; Matsuzawa, A.; Yamauchi, S.; Saitoh, M.; Takeda, K.; Ichijo, H. Thioredoxin and TRAF family proteins regulate reactive oxygen species-dependent activation of ASK1 through reciprocal modulation of the N-terminal homophilic interaction of ASK1. *Mol. Cell. Biol.* **27**:8152–8163; 2007.
- [142] Wang, X. S.; Diener, K.; Tan, T. H.; Yao, Z. MAPKKK6, a novel mitogen-activated protein kinase kinase kinase, that associates with MAPKKK5. *Biochem. Biophys. Res. Commun.* **253**:33–37; 1998.
- [143] Ortner, E.; Moelling, K. Heteromeric complex formation of ASK2 and ASK1 regulates stress-induced signaling. *Biochem. Biophys. Res. Commun.* **362**:454–459; 2007.
- [144] Goldman, E. H.; Chen, L.; Fu, H. Activation of apoptosis signal-regulating kinase 1 by reactive oxygen species through dephosphorylation at serine 967 and 14-3-3 dissociation. *J. Biol. Chem.* **279**:10442–10449; 2004.
- [145] Chen, L.; Liu, L.; Huang, S. Cadmium activates the mitogen-activated protein kinase (MAPK) pathway via induction of reactive oxygen species and inhibition of protein phosphatases 2A and 5. *Free Radic. Biol. Med.* **45**:1035–1044; 2008.
- [146] Zhang, R.; Al-Lamki, R.; Bai, L.; Streb, J. W.; Miano, J. M.; Bradley, J.; Min, W. Thioredoxin-2 inhibits mitochondria-located ASK1-mediated apoptosis in a JNK-independent manner. *Circ. Res.* **94**:1483–1491; 2004.

- [147] Fan, M.; Goodwin, M. E.; Birrer, M. J.; Chambers, T. C. The c-Jun NH(2)-terminal protein kinase/AP-1 pathway is required for efficient apoptosis induced by vinblastine. *Cancer Res.* **61**:4450–4458; 2001.
- [148] Kharbanda, S.; Saxena, S.; Yoshida, K.; Pandey, P.; Kaneki, M.; Wang, Q.; Cheng, K.; Chen, Y. N.; Campbell, A.; Sudha, T.; Yuan, Z. M.; Narula, J.; Weichselbaum, R.; Nalin, C.; Kufe, D. Translocation of SAPK/JNK to mitochondria and interaction with Bcl-x(L) in response to DNA damage. *J. Biol. Chem.* **275**:322–327; 2000.
- [149] Merad-Boudia, M.; Nicole, A.; Santiard-Baron, D.; Saille, C.; Ceballos-Picot, I. Mitochondrial impairment as an early event in the process of apoptosis induced by glutathione depletion in neuronal cells: relevance to Parkinson's disease. *Biochem. Pharmacol.* **56**:645–655; 1998.
- [150] Lu, C.; Armstrong, J. S. Role of calcium and cyclophilin D in the regulation of mitochondrial permeabilization induced by glutathione depletion. *Biochem. Biophys. Res. Commun.* **363**:572–577; 2007.
- [151] D'Alessio, M.; Cerella, C.; De Nicola, M.; Bergamaschi, A.; Magrini, A.; Gualandi, G.; Alfonsi, A. M.; Ghibelli, L. Apoptotic GSH extrusion is associated with free radical generation. *Ann. N. Y. Acad. Sci.* **1010**:449–452; 2003.
- [152] Marengo, B.; De Ciucis, C.; Verzola, D.; Pistoia, V.; Raffaghello, L.; Patriarca, S.; Balbis, E.; Traverso, N.; Cottalasso, D.; Pronzato, M. A.; Marinari, U. M.; Domenicotti, C. Mechanisms of BSO (L-buthionine-S,R-sulfoximine)-induced cytotoxic effects in neuroblastoma. *Free Radic. Biol. Med.* **44**:474–482; 2008.
- [153] Meurette, O.; Lefeuvre-Orfila, L.; Rebillard, A.; Lagadic-Gossman, D.; Dimanche-Boitrel, M. T. Role of intracellular glutathione in cell sensitivity to the apoptosis induced by tumor necrosis factor α -related apoptosis-inducing ligand/anticancer drug combinations. *Clin. Cancer Res.* **11**:3075–3083; 2005.
- [154] Franco, R.; Panayiotidis, M. I.; Cidowski, J. A. Glutathione depletion is necessary for apoptosis in lymphoid cells independent of reactive oxygen species formation. *J. Biol. Chem.* **282**:30452–30465; 2007.
- [155] Circu, M. L.; Stringer, S.; Rhoads, C. A.; Moyer, M. P.; Aw, T. Y. The role of GSH efflux in staurosporine-induced apoptosis in colonic epithelial cells. *Biochem. Pharmacol.* **77**:76–85; 2009.
- [156] Wang, T. G.; Gotoh, Y.; Jennings, M. H.; Rhoads, C. A.; Aw, T. Y. Lipid hydroperoxide-induced apoptosis in human colonic CaCo-2 cells is associated with an early loss of cellular redox balance. *FASEB J.* **14**:1567–1576; 2000.
- [157] Pias, E. K.; Aw, T. Y. Early redox imbalance mediates hydroperoxide-induced apoptosis in mitotic competent undifferentiated PC-12 cells. *Cell Death Differ.* **9**:1007–1016; 2002.
- [158] Pias, E. K.; Aw, T. Y. Apoptosis in mitotic competent undifferentiated cells is induced by cellular redox imbalance independent of reactive oxygen species production. *FASEB J.* **16**:781–790; 2002.
- [159] Okouchi, M.; Okayama, N.; Aw, T. Y. Hyperglycemia potentiates carbonyl stress-induced apoptosis in naive PC-12 cells: relationship to cellular redox and activator protease factor-1 expression. *Curr. Neurovasc. Res.* **2**:375–386; 2005.
- [160] Okouchi, M.; Okayama, N.; Aw, T. Y. Differential susceptibility of naive and differentiated PC-12 cells to methylglyoxal-induced apoptosis: influence of cellular redox. *Curr. Neurovasc. Res.* **2**:13–22; 2005.
- [161] Hallberg, E.; Rydstrom, J. Selective oxidation of mitochondrial glutathione in cultured rat adrenal cells and its relation to polycyclic aromatic hydrocarbon-induced cytotoxicity. *Arch. Biochem. Biophys.* **270**:662–671; 1989.
- [162] Lluís, J. M.; Morales, A.; Blasco, C.; Colell, A.; Mari, M.; Garcia-Ruiz, C.; Fernandez-Checa, J. C. Critical role of mitochondrial glutathione in the survival of hepatocytes during hypoxia. *J. Biol. Chem.* **280**:3224–3232; 2005.
- [163] Olafsdottir, K.; Reed, D. J. Retention of oxidized glutathione by isolated rat liver mitochondria during hydroperoxide treatment. *Biochim. Biophys. Acta* **964**:377–382; 1988.
- [164] Fernandez-Checa, J. C.; Garcia-Ruiz, C.; Ookhtens, M.; Kaplowitz, N. Impaired uptake of glutathione by hepatic mitochondria from chronic ethanol-fed rats: tracer kinetic studies in vitro and in vivo and susceptibility to oxidant stress. *J. Clin. Invest.* **87**:397–405; 1991.
- [165] Garcia-Ruiz, C.; Fernandez-Checa, J. C. Mitochondrial glutathione: hepatocellular survival–death switch. *J. Gastroenterol. Hepatol.* **21** (Suppl. 3):S3–S6; 2006.
- [166] Lluís, J. M.; Colell, A.; Garcia-Ruiz, C.; Kaplowitz, N.; Fernandez-Checa, J. C. Acetaldehyde impairs mitochondrial glutathione transport in HepG2 cells through endoplasmic reticulum stress. *Gastroenterology* **124**:708–724; 2003.
- [167] Lluís, J. M.; Buricchi, F.; Chiarugi, P.; Morales, A.; Fernandez-Checa, J. C. Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor- κ B via c-SRC and oxidant-dependent cell death. *Cancer Res.* **67**:7368–7377; 2007.
- [168] Ghosh, S.; Pulinilkunnil, T.; Yuen, G.; Kewalramani, G.; An, D.; Qi, D.; Abrahami, A.; Rodrigues, B. Cardiomyocyte apoptosis induced by short-term diabetes requires mitochondrial GSH depletion. *Am. J. Physiol. Heart Circ. Physiol.* **289**:H768–H776; 2005.
- [169] Tsujimoto, Y.; Shimizu, S. Role of the mitochondrial membrane permeability transition in cell death. *Apoptosis* **12**:835–840; 2007.
- [170] Madesh, M.; Hajnoczky, G. VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. *J. Cell Biol.* **155**:1003–1015; 2001.
- [171] Giron-Calle, J.; Zwizinski, C. W.; Schmid, H. H. Peroxidative damage to cardiac mitochondria. II. Immunological analysis of modified adenine nucleotide translocase. *Arch. Biochem. Biophys.* **315**:1–7; 1994.
- [172] Baines, C. P.; Kaiser, R. A.; Purcell, N. H.; Blair, N. S.; Osinska, H.; Hambleton, M. A.; Brunskill, E. W.; Sayen, M. R.; Gottlieb, R. A.; Dorn, G. W.; Robbins, J.; Molkenstin, J. D. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**:658–662; 2005.
- [173] Skulachev, V. P. Bioenergetic aspects of apoptosis, necrosis and mitoptosis. *Apoptosis* **11**:473–485; 2006.
- [174] Cook, S. A.; Sugden, P. H.; Clerk, A. Regulation of bcl-2 family proteins during development and in response to oxidative stress in cardiac myocytes: association with changes in mitochondrial membrane potential. *Circ. Res.* **85**:940–949; 1999.
- [175] Santamaria, G.; Martinez-Diez, M.; Fabregat, I.; Cuezva, J. M. Efficient execution of cell death in non-glycolytic cells requires the generation of ROS controlled by the activity of mitochondrial H⁺-ATP synthase. *Carcinogenesis* **27**:925–935; 2006.
- [176] Oh, S. H.; Lim, S. C. A rapid and transient ROS generation by cadmium triggers apoptosis via caspase-dependent pathway in HepG2 cells and this is inhibited through N-acetylcysteine-mediated catalase upregulation. *Toxicol. Appl. Pharmacol.* **212**:212–223; 2006.
- [177] Chen, Q.; Lesnfsky, E. J. Depletion of cardiolipin and cytochrome c during ischemia increases hydrogen peroxide production from the electron transport chain. *Free Radic. Biol. Med.* **40**:976–982; 2006.
- [178] Clayton, D. A. Transcription of the mammalian mitochondrial genome. *Annu. Rev. Biochem.* **53**:573–594; 1984.
- [179] Lenaz, G.; Bovina, C.; D'Aurelio, M.; Fato, R.; Formigini, G.; Genova, M. L.; Giuliano, G.; Merlo Pich, M.; Paolucci, U.; Parenti Castelli, G.; Ventura, B. Role of mitochondria in oxidative stress and aging. *Ann. N. Y. Acad. Sci.* **959**:199–213; 2002.
- [180] Van Houten, B.; Woshner, V.; Santos, J. H. Role of mitochondrial DNA in toxic responses to oxidative stress. *DNA Repair (Amsterdam)* **5**:145–152; 2006.
- [181] Grishko, V.; Pastukh, V.; Solodushko, V.; Gillespie, M.; Azuma, J.; Schaffer, S. Apoptotic cascade initiated by angiotensin II in neonatal cardiomyocytes: role of DNA damage. *Am. J. Physiol. Heart Circ. Physiol.* **285**:H2364–H2372; 2003.
- [182] Circu, M. L.; Aw, T. Y. Menadione-induced mitochondrial DNA damage in colonic cells: influence of cellular GSH redox status and mitochondrial base excision repair (BER) capacity. Society for Free Radical Biology and Medicine 14th Annual Meeting, Washington, DC, p. S148; 2007.
- [183] Will, O.; Mahler, H. C.; Arrigo, A. P.; Epe, B. Influence of glutathione levels and heat-shock on the steady-state levels of oxidative DNA base modifications in mammalian cells. *Carcinogenesis* **20**:333–337; 1999.
- [184] Reliene, R.; Schiestl, R. H. Glutathione depletion by buthionine sulfoximine induces DNA deletions in mice. *Carcinogenesis* **27**:240–244; 2006.
- [185] Suliman, H. B.; Carraway, M. S.; Velsor, L. W.; Day, B. J.; Ghio, A. J.; Piantadosi, C. A. Rapid mtDNA deletion by oxidants in rat liver mitochondria after hemin exposure. *Free Radic. Biol. Med.* **32**:246–256; 2002.
- [186] Esteve, J. M.; Mompo, J.; Garcia de la Asuncion, J.; Sastre, J.; Asensi, M.; Boix, J.; Vina, J. R.; Vina, J. Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies in vivo and in vitro. *FASEB J.* **13**:1055–1064; 1999.
- [187] Hollins, D. L.; Suliman, H. B.; Piantadosi, C. A.; Carraway, M. S. Glutathione regulates susceptibility to oxidant-induced mitochondrial DNA damage in human lymphocytes. *Free Radic. Biol. Med.* **40**:1220–1226; 2006.
- [188] de la Asuncion, J. G.; Millan, A.; Pla, R.; Bruseghini, L.; Esteras, A.; Pallardo, F. V.; Sastre, J.; Vina, J. Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. *FASEB J.* **10**:333–338; 1996.
- [189] Dantzer, F.; Luna, L.; Bjoras, M.; Seeberg, E. Human OGG1 undergoes serine phosphorylation and associates with the nuclear matrix and mitotic chromatin in vivo. *Nucleic Acids Res.* **30**:2349–2357; 2002.
- [190] Yamamori, T.; Dericco, J.; Naqvi, A.; Hoffman, T.A.; Mattagajasingh, I.; Kasuno, K.; Jung, S.B.; Kim, C.S.; Irani, K. SIRT1 deacetylates APE1 and regulates cellular base excision repair. *Nucleic Acids Res.* Electronic publication ahead of print.
- [191] Ott, M.; Robertson, J. D.; Gogvadze, V.; Zhivotovskiy, B.; Orrenius, S. Cytochrome c release from mitochondria proceeds by a two-step process. *Proc. Natl. Acad. Sci. USA* **99**:1259–1263; 2002.
- [192] Kagan, V. E.; Tyurin, V. A.; Jiang, J.; Tyurina, Y. Y.; Ritov, V. B.; Amoscato, A. A.; Osipov, A. N.; Belikova, N. A.; Kapralov, A. A.; Kini, V.; Vlasova, I. I.; Zhao, Q.; Zou, M.; Di, P.; Svistunenko, D. A.; Kurnikov, I. V.; Borisenko, G. G. Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat. Chem. Biol.* **1**:223–232; 2005.
- [193] Kagan, V. E.; Borisenko, G. G.; Tyurina, Y. Y.; Tyurin, V. A.; Jiang, J.; Potapovich, A. I.; Kini, V.; Amoscato, A. A.; Fujii, Y. Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome c with cardiolipin and phosphatidylserine. *Free Radic. Biol. Med.* **37**:1963–1985; 2004.
- [194] Dejean, L. M.; Martinez-Caballero, S.; Kinnally, K. W. Is MAC the knife that cuts cytochrome c from mitochondria during apoptosis? *Cell Death Differ.* **13**:1387–1395; 2006.
- [195] Gonzalez, F.; Pariselli, F.; Dupaigne, P.; Budihardjo, I.; Lutter, M.; Antonsson, B.; Diolet, P.; Manon, S.; Martinou, J. C.; Gubern, M.; Wang, X.; Bernard, S.; Petit, P. X. tBid interaction with cardiolipin primarily orchestrates mitochondrial dysfunctions and subsequently activates Bax and Bak. *Cell Death Differ.* **12**:614–626; 2005.
- [196] Vlasova, I. I.; Tyurin, V. A.; Kapralov, A. A.; Kurnikov, I. V.; Osipov, A. N.; Potapovich, M. V.; Stoyanovsky, D. A.; Kagan, V. E. Nitric oxide inhibits peroxidase activity of cytochrome c-cardiolipin complex and blocks cardiolipin oxidation. *J. Biol. Chem.* **281**:14554–14562; 2006.
- [197] Jang, B.; Han, S. Biochemical properties of cytochrome c nitrated by peroxynitrite. *Biochimie* **88**:53–58; 2006.
- [198] Cassina, A. M.; Hodara, R.; Souza, J. M.; Thomson, L.; Castro, L.; Ischiropoulos, H.; Freeman, B. A.; Radi, R. Cytochrome c nitration by peroxynitrite. *J. Biol. Chem.* **275**:21409–21415; 2000.
- [199] Nakagawa, H.; Komai, N.; Takusagawa, M.; Miura, Y.; Toda, T.; Miyata, N.; Ozawa, T.; Ikota, N. Nitration of specific tyrosine residues of cytochrome C is associated with caspase-cascade inactivation. *Biol. Pharm. Bull.* **30**:15–20; 2007.

- [200] Brown, G. C.; Borutaite, V. Regulation of apoptosis by the redox state of cytochrome c. *Biochim. Biophys. Acta* **1777**:877–881; 2008.
- [201] Suto, D.; Sato, K.; Ohba, Y.; Yoshimura, T.; Fujii, J. Suppression of the proapoptotic function of cytochrome c by singlet oxygen via a haem redox state-independent mechanism. *Biochem. J.* **392**:399–406; 2005.
- [202] Borutaite, V.; Brown, G. C. Mitochondrial regulation of caspase activation by cytochrome oxidase and tetramethylphenylenediamine via cytosolic cytochrome c redox state. *J. Biol. Chem.* **282**:31124–31130; 2007.
- [203] Nicholson, D. W. Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ.* **6**:1028–1042; 1999.
- [204] Degtarev, A.; Boyce, M.; Yuan, J. A decade of caspases. *Oncogene* **22**:8543–8567; 2003.
- [205] Slee, E. A.; Harte, M. T.; Kluck, R. M.; Wolf, B. B.; Casiano, C. A.; Newmeyer, D. D.; Wang, H. G.; Reed, J. C.; Nicholson, D. W.; Alnemri, E. S.; Green, D. R.; Martin, S. J. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J. Cell Biol.* **144**:281–292; 1999.
- [206] Salvesen, G. S.; Abrams, J. M. Caspase activation—stepping on the gas or releasing the brakes? Lessons from humans and flies. *Oncogene* **23**:2774–2784; 2004.
- [207] Verhagen, A. M.; Coulson, E. J.; Vaux, D. L. Inhibitor of apoptosis proteins and their relatives: IAPs and other BIRPs. *Genome Biol.* **2** REVIEWS3009; 2001.
- [208] Du, C.; Fang, M.; Li, Y.; Li, L.; Wang, X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* **102**:33–42; 2000.
- [209] Suzuki, Y.; Takahashi-Niki, K.; Akagi, T.; Hashikawa, T.; Takahashi, R. Mitochondrial protease Omi/HtrA2 enhances caspase activation through multiple pathways. *Cell Death Differ.* **11**:208–216; 2004.
- [210] Borutaite, V.; Brown, G. C. Caspases are reversibly inactivated by hydrogen peroxide. *FEBS Lett.* **500**:114–118; 2001.
- [211] Barbouti, A.; Amorgianiotis, C.; Kolettas, E.; Kanavros, P.; Galaris, D. Hydrogen peroxide inhibits caspase-dependent apoptosis by inactivating procaspase-9 in an iron-dependent manner. *Free Radic. Biol. Med.* **43**:1377–1387; 2007.
- [212] Katoh, I.; Tomimori, Y.; Ikawa, Y.; Kurata, S. Dimerization and processing of procaspase-9 by redox stress in mitochondria. *J. Biol. Chem.* **279**:15515–15523; 2004.
- [213] Chung, H. T.; Pae, H. O.; Choi, B. M.; Billiar, T. R.; Kim, Y. M. Nitric oxide as a bioregulator of apoptosis. *Biochem. Biophys. Res. Commun.* **282**:1075–1079; 2001.
- [214] Kim, K. M.; Kim, P. K.; Kwon, Y. G.; Bai, S. K.; Nam, W. D.; Kim, Y. M. Regulation of apoptosis by nitrosative stress. *J. Biochem. Mol. Biol.* **35**:127–133; 2002.
- [215] Li, J.; Billiar, T. R.; Talanian, R. V.; Kim, Y. M. Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. *Biochem. Biophys. Res. Commun.* **240**:419–424; 1997.
- [216] Rossig, L.; Fichtlscherer, B.; Breitschopf, K.; Haendeler, J.; Zeiher, A. M.; Mulsch, A.; Dimmeler, S. Nitric oxide inhibits caspase-3 by S-nitrosation in vivo. *J. Biol. Chem.* **274**:6823–6826; 1999.
- [217] Kim, Y. M.; Kim, T. H.; Chung, H. T.; Talanian, R. V.; Yin, X. M.; Billiar, T. R. Nitric oxide prevents tumor necrosis factor alpha-induced rat hepatocyte apoptosis by the interruption of mitochondrial apoptotic signaling through S-nitrosylation of caspase-8. *Hepatology* **32**:770–778; 2000.
- [218] Tzeng, E.; Kim, Y. M.; Pitt, B. R.; Lizonova, A.; Kovesdi, I.; Billiar, T. R. Adenoviral transfer of the inducible nitric oxide synthase gene blocks endothelial cell apoptosis. *Surgery* **122**:255–263; 1997.
- [219] Zech, B.; Kohl, R.; von Knethen, A.; Brune, B. Nitric oxide donors inhibit formation of the Apaf-1/caspase-9 apoptosome and activation of caspases. *Biochem. J.* **371**:1055–1064; 2003.
- [220] Mannick, J. B.; Hausladen, A.; Liu, L.; Hess, D. T.; Zeng, M.; Miao, Q. X.; Kane, L. S.; Gow, A. J.; Stamler, J. S. Fas-induced caspase denitrosylation. *Science* **284**:651–654; 1999.
- [221] Kim, J. E.; Tannenbaum, S. R. S-Nitrosation regulates the activation of endogenous procaspase-9 in HT-29 human colon carcinoma cells. *J. Biol. Chem.* **279**:9758–9764; 2004.
- [222] Mitchell, D. A.; Morton, S. U.; Fernhoff, N. B.; Marletta, M. A. Thioredoxin is required for S-nitrosation of procaspase-3 and the inhibition of apoptosis in Jurkat cells. *Proc. Natl. Acad. Sci. USA* **104**:11609–11614; 2007.
- [223] Mannick, J. B.; Schonhoff, C.; Papeta, N.; Ghafourifar, P.; Szibor, M.; Fang, K.; Gaston, B. S-Nitrosylation of mitochondrial caspases. *J. Cell Biol.* **154**:1111–1116; 2001.
- [224] Pan, S.; Berk, B. C. Glutathiolation regulates tumor necrosis factor-alpha-induced caspase-3 cleavage and apoptosis: key role for glutaredoxin in the death pathway. *Circ. Res.* **100**:213–219; 2007.
- [225] Sykes, M. C.; Mowbray, A. L.; Jo, H. Reversible glutathiolation of caspase-3 by glutaredoxin as a novel redox signaling mechanism in tumor necrosis factor-alpha-induced cell death. *Circ. Res.* **100**:152–154; 2007.
- [226] Huang, Z.; Pinto, J. T.; Deng, H.; Richie Jr., J. P. Inhibition of caspase-3 activity and activation by protein glutathionylation. *Biochem. Pharmacol.* **75**:2234–2244; 2008.