Biochimica et Biophysica Acta 1863 (2016) 2977–2992

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Activation of apoptosis signalling pathways by reactive oxygen species

Maureen Redza-Dutordoir, Diana A. Averill-Bates *

Département des Sciences Biologiques (TOXEN, BIOMED), Université du Québec à Montréal, Montréal, Québec, Canada

ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form 12 September 2016
Accepted 15 September 2016
Available online 17 September 2016

Keywords:
Oxidative stress
Environmental stress
Apoptosis
Mitochondria
Death receptor
Endoplasmic reticulum

ABSTRACT

Reactive oxygen species (ROS) are short-lived and highly reactive molecules. The generation of ROS in cells exists in equilibrium with a variety of antioxidant defences. At low to modest doses, ROS are considered to be essential for regulation of normal physiological functions involved in development such as cell cycle progression and proliferation, differentiation, migration and cell death. ROS also play an important role in the immune system, maintenance of the redox balance and have been implicated in activation of various cellular signalling pathways. Excess cellular levels of ROS cause damage to proteins, nucleic acids, lipids, membranes and organelles, which can lead to activation of cell death processes such as apoptosis. Apoptosis is a highly regulated process that is essential for the development and survival of multicellular organisms. These organisms often need to discard cells that are superfluous or potentially harmful, having accumulated mutations or become infected by pathogens. Apoptosis features a characteristic set of morphological and biochemical features whereby cells undergo a cascade of self-destruction. Thus, proper regulation of apoptosis is essential for maintaining normal cellular homeostasis. ROS play a central role in cell signalling as well as in regulation of the main pathways of apoptosis mediated by mitochondria, death receptors and the endoplasmic reticulum (ER). This review focuses on current understanding of the role of ROS in each of these three main pathways of apoptosis. The role of ROS in the complex interplay and crosstalk between these different signalling pathways remains to be further unravelled during the coming years.

1. Introduction

1.1. Reactive oxygen species

Reactive oxygen species (ROS) are generally small molecules that are short-lived and highly reactive [1,2]. They can be oxygen-derived free radicals like superoxide anion ($O_2^{\bullet -}$) and the hydroxyl radical ($OH^{\bullet}$), or non-radical molecules such as hydrogen peroxide (H_2O_2) (Fig. 1). The generation of ROS in cells exists in equilibrium with a wide variety of antioxidant defences. These include enzymatic scavengers such as superoxide dismutases (SOD), catalase, glutathione peroxidase and peroxiredoxins, as well as non-enzymatic scavengers such as vitamins C and E, glutathione (GSH), lipoic acid, carotenoids and iron chelators [1].

At low to modest doses, ROS are considered to be essential for the regulation of normal physiological functions involved in development such as cell cycle progression and proliferation, differentiation, migration and cell death [3]. ROS also play an important role in the immune system and in maintenance of the redox balance [4]. ROS have been implicated in the activation of various cellular signalling pathways and transcription factors including phosphoinositide 3-kinase (PI3K)/Akt, mitogen-activated protein kinases (MAPK), nuclear factor (erythroid-derived 2)-like 2 (Nrf2)/Kelch like-ECH-associated protein 1 (Keap1), nuclear factor-κB (NF-κB) and the tumour suppressor p53, which can activate cell survival and/or cell death processes such as autophagy and apoptosis [3–6]. Redox-regulated signal transduction is often through reversible oxidation of thiol proteins. Nevertheless, understanding the physiological importance and mechanisms of redox signalling at the cellular level requires further clarification [2,4].

On the other hand, if the antioxidant detoxification systems fail to maintain low, tolerated levels of ROS, then excess cellular levels of ROS can be deleterious and trigger oxidative stress. Oxidative stress is defined as "a serious imbalance between the generation of ROS and antioxidant defences in favour of ROS, causing excessive oxidative damage" [1]. Excess cellular levels of ROS can cause damage to proteins, nucleic acids, lipids, membranes and organelles such as mitochondria [1]. Enhanced production of ROS has been associated with the development of pathologies such as cancer, diabetes, atherosclerosis, stroke, arthrosis, amyotrophic lateral sclerosis (ALS) and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases [7–9]. However, clinical evidence supports an association rather than a causative role of ROS with diseases, and the molecular mechanisms involved are yet not fully understood [8,9].

* Corresponding author at: Département des Sciences Biologiques, Université du Québec à Montréal, CP 8888, Succursale Centre-Ville, Montréal, Québec H3C 3P8, Canada.
E-mail address: averill.diana@uqam.ca (D.A. Averill-Bates).

http://dx.doi.org/10.1016/j.bbamcr.2016.09.012
0167-4889/

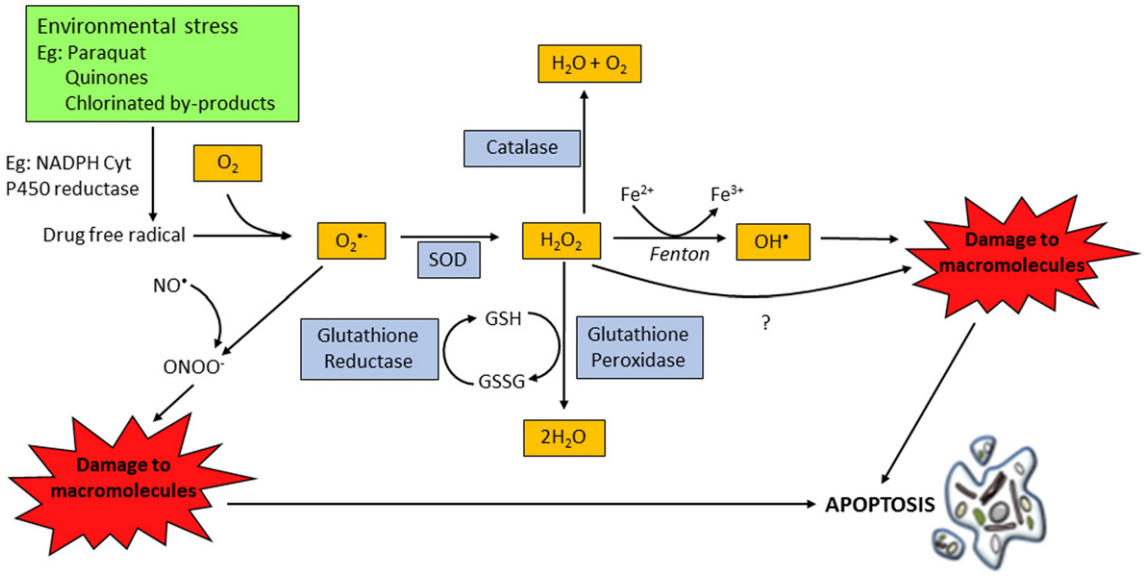

Fig. 1. Environmental stress generates ROS that cause cellular damage and apoptosis. Environmental stress attributed to toxic compounds (xenobiotics) can lead to generation of xenobiotic-derived free radicals through one-electron reduction catalysed by cytochrome P450 reductases. Xenobiotic-derived free radicals react rapidly with oxygen to produce superoxide ($O2^{\bullet -}$), which either reacts with nitric oxide ($NO^{\bullet}$) to produce peroxynitrite ($ONOO^-$), or undergoes dismutation to generate H_2O_2, catalysed by SOD. H_2O_2 can be either detoxified by antioxidants such as catalase and GSH/glutathione peroxidase, or can generate $OH^{\bullet}$ by the metal-catalysed Fenton reaction. $OH^{\bullet}$ and $ONOO^-$ cause damage cellular proteins, lipids and nucleic acids, which can lead to demise of the cell by apoptosis.

ROS can be generated in cells by both exogenous (see Section 1.2) and endogenous stimuli. Endogenous production of ROS, especially $O_2^{\bullet -}$, arises mainly from leaks during mitochondrial electron transport chain activity [10]. The two major sites for superoxide production are complexes I and III of the respiratory chain. Superoxide can also be produced by NADPH oxidases (Nox) localised at the membrane, as well as by xanthine oxidases and enzymatic activation of cytochrome P450 reductases [5]. $O_2^{\bullet -}$ can react with nitric oxide ($NO^{\bullet}$), a reactive nitrogen species (RNS), to generate the powerful oxidant peroxynitrite ($ONOO^-$) [2,10] (Fig. 1). Otherwise, $O_2^{\bullet -}$ is rapidly dismutated into O_2 and H_2O_2 by SOD. H_2O_2 is used in inflammatory processes or can be reduced by metal ions such as iron or copper to form $OH^{\bullet}$ through the Fenton reaction [8,11]. $OH^{\bullet}$ is highly reactive and harmful to biological macromolecules. To avoid the formation of $OH^{\bullet}$, H_2O_2 is detoxified by antioxidant enzymes such as catalase and glutathione peroxidases [12] (Fig. 1).

1.2. Environmental factors and oxidative stress

Living organisms are constantly exposed to variations in their environmental parameters, such as temperature, pH, oxygen pressure, metabolite concentrations, hormonal and immune signals. Moreover, they are continually assaulted by toxic components found in their environment [13,14], such as pesticides, aldehydes, chlorinated by-products, heavy metals, micro- or nano-particles, as well as UV and ionising radiation [15]. When the balance between environmental constraint and the capacity to sustain cell and tissue homeostasis is disturbed, a stress situation arises. Environmental stressors can cause more or less serious damage to cells and tissues, influence disease development, or even trigger death of cells, tissues or individual organisms [13]. Prolonged or acute exposure to environmental stressors can cause deleterious effects on health. Environmentally-related diseases include cancer, diabetes, immunosuppression, chronic lung disease and neurodegenerative disorders [14].

Numerous exogenous factors including environmental pollutants are able to generate pro-oxidant compounds such as ROS [8,14,15] (Fig. 1). These factors include UV and ionising radiation, quinone compounds, inflammatory cytokines, chemicals found in tobacco smoke, environmental toxins and various pharmaceutical agents. Many toxic xenobiotics including quinone compounds undergo redox cycling. This involves continuous generation of free radicals and ROS by one-electron transfer through successive oxidation and reduction reactions [8]. The initial one-electron reduction of a toxic compound to generate a drug-derived free radical is catalysed by flavoenzymes such as NADPH cytochrome P450 reductases. The drug-derived free radical subsequently reacts rapidly with oxygen to generate the superoxide free radical and other ROS such as H_2O_2 (Fig. 1). In the presence of metals, $OH^{\bullet}$ can be produced from H_2O_2 by the Fenton reaction [11].

At sufficient doses, powerful oxidants such as $OH^{\bullet}$ and $ONOO^-$, and likely H_2O_2, cause severe damage to macromolecules [1]. This can lead to cell death by processes such as apoptosis and/or necrosis (Fig. 1) (see Section 4.2). Moreover, ROS such as $O_2^{\bullet -}$ and H_2O_2 can induce autophagy, which is mostly considered to be a cell survival response, although it can lead to cell death (see review [16]) (see Section 4.1). Induction of autophagy would most likely occur during exposure to modest doses of ROS.

2. Signalling pathways of apoptosis

2.1. General features

Apoptosis is a tightly regulated and highly conserved process of cell death during which a cell undergoes self-destruction [17]. It is an essential process in multicellular organisms that eliminates undesired or superfluous cells during development or neutralises potentially harmful cells with DNA damage, thus preventing carcinogenesis [15,18]. The regulation of apoptosis is crucial for maintaining normal cellular homeostasis. However, the deregulation of apoptosis has been linked to numerous pathologies such as chronic inflammation, atherosclerosis, cancer, respiratory disorders, and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [18].

Apoptosis is a well-characterised process presenting distinctive morphological and biochemical features [17]. It is distinguished by cell shrinking, membrane blebbing, chromatin condensation and nuclear fragmentation, followed by the formation of apoptotic bodies that are digested by phagocytosis by neighbouring cells or macrophages. This results in the elimination of dying cells with minimal damage to surrounding cells and tissues.

Apoptosis can be triggered by a variety of extrinsic and intrinsic signals. These include different stresses such as ROS, RNS, DNA-damaging agents (e.g. radiation), heat shock, serum deprivation, viral infection and hypoxia [15,18]. The exposure to xenobiotics such as pesticides, environmental pollutants and chemotherapeutic drugs can also trigger apoptosis, which is often mediated by ROS. Cell to cell variations are an important determinant of cell fate, as to whether cells will survive or die by apoptosis following a toxic insult [14]. These variations can result from differences in triggering signals and/or cellular state (e.g. genetic, phenotypic or stochastic fluctuations), cell cycle phase or cellular microenvironment [19]. The main mechanisms that regulate apoptosis are discussed in the following sections.

2.2. Main mechanisms of regulation of apoptosis

Most, but not all apoptotic pathways lead to the activation of cysteine-dependent aspartate-specific proteases (caspases) [20]. Procaspases exist as inactive zymogens that require cleavage to generate their active proteolytic forms. The latter are heterodimers composed of two small (~p10) and two large (~p20) subunits that contain two cysteine active sites. Certain initiator caspases can cleave other caspases, which leads to their activation further downstream in the apoptotic cascade [20]. Apoptotic caspases consist of upstream initiators like caspases-8, -10, -2 and -9 and downstream effectors such as caspases-3, -6 and -7. However, unlike the other initiator caspases, caspase-2 appears to play a role in both the initiation and execution phases of apoptosis, since it does not cleave other caspases [21,22]. Although they are classified as inflammatory caspases [23], caspases-4 and -12 are involved in apoptosis through the endoplasmic reticulum (ER) [24,25]. Caspases-11 and -13 are the murine and bovine orthologues of caspase-4 [20]. It should be noted that most caspases have subsequently been found to also possess non-apoptotic roles [19,20].

The proteolytic activity of caspases involves cleavage of their substrates at aspartate residues [26,27]. There are at least 1000 substrates of effector caspases such as caspase-3 and -7, which are located in the cytosol, cytoskeleton and nucleus. They include structural proteins such as actin, lamin A, gelsolin and fodrin, proteins involved in DNA repair like the poly(ADP-ribose)polymerase (PARP) and in cell cycle regulation such as p21, the E3 ubiquitin-protein ligase (or mouse double minute 2 homolog (Mdm2)) and the inhibitor of caspase-activated DNase (ICAD) [28]. During apoptosis, effector caspases can cleave the p75 subunit of complex I in the mitochondria, which disrupts the electron transport chain and hence the critical function of ATP production [29]. Furthermore, caspases cleave p21-activated kinase 2 (PAK2) and Rho-associated kinase-1 (ROCK-1), which regulate actin polymerization and actin–myosin contractility. This leads to abnormal reorganization of the actin cytoskeleton and membrane blebbing [30]. The activation of effector caspases leads to onset of the characteristic morphological features of apoptosis and ultimate dismantlement of the cell.

Caspases-1, -4, -5, -11 (in rodents) and -12 are involved in inflammatory processes. The functions of caspases-4 and -5 are not well known [20]. Inflammatory caspases such as caspase-1 and -5 are activated in platforms known as the inflammasome [30,31]. The inflammasome generally responds to inducers of inflammation as well as infectious agents such as viruses, bacteria and fungi. Caspase-1 promotes the secretion of two inflammatory cytokines, interleukin (IL) 1β and IL18. In addition, caspase-1 can cleave Bid and activate caspases-3 and -7 to stimulate apoptosis, although this type of cell death is generally referred to as pyroptosis. Caspase-12 appears to abrogate the inflammatory response by inhibiting caspase-1 [20]. The activation of caspase-11 is not entirely clear although it appears to be an upstream activator of caspase-1 [20]; in infected macrophages, pro-inflammatory caspase-11 produced caspase-1-dependent IL1β and IL18. On the contrary, caspase-11 induced cell death (lactate dehydrogenase (LDH) release) in a caspase-1-independent manner [31].

Several newer caspases were identified although they are not well characterised. Caspases-15, -17 and -18 are absent in mammals, with the exception of caspase-16 [20]. Caspase-14 is present only in terrestrial mammals and its expression is limited to epidermal tissue and epithelia [20].

Apoptosis can be triggered by three main signalling pathways, upstream of caspase activation. These include an extrinsic pathway involving death receptors localised at the cell surface [30], or intrinsic pathways involving mitochondria or the ER [32,33].

2.2.1. Mitochondrial pathway

Mitochondria are essential organelles whose primary function is to convert nutrients into energy (ATP), which is subsequently exported and used throughout the cell [34]. This function is facilitated by distinct proprieties of the outer and inner mitochondrial membranes [35]. The outer mitochondrial membrane (OMM) is permeable to molecules up to 5 kDa, which allows the exchange of respiratory chain substrates and products between mitochondria and the cytosol. The inner mitochondrial membrane (IMM) is highly impermeable, an essential characteristic for generating the electrochemical potential necessary for oxidative phosphorylation and ATP production.

Mitochondria also have an important role in triggering and regulating apoptosis (see review, [36]). The regulation of IMM permeability appears to be critical in this process. Mitochondrial-dependent apoptosis appears to involve the mitochondrial permeability transition pore (MPTP) complex, whose molecular nature remains to be elucidated [37,38]. The MPTP complex is thought to be composed of cyclophilin D, a molecular chaperone in the mitochondrial matrix bound to the adenine–nucleotide–translocator (ANT) in the IMM, which interacts with the voltage dependent-anion-channel (VDAC) in the OMM [39]. VDAC and ANT form points of connection between the IMM and OMM. Under conditions of stress, the inner membrane permeability increases, which allow free passage of molecules of less than 1.5 kDa, including protons, into the mitochondrial matrix, leading to disruption of oxidative phosphorylation [38]. Moreover, this causes osmotic swelling of the mitochondrial matrix and compression of vesicles created by infolding of the intercristal space. Together with increased outer mitochondrial membrane permeabilisation (MOMP), this leads to release into the cytosol of pro-apoptotic proteins such as cytochrome *c*, apoptosis inducing factor (AIF), endonuclease G (endoG), and second mitochondria-derived activator of caspases/direct inhibitor of apoptosis (IAP)-binding protein with low pI (Smac/Diablo) (Fig. 2) [36]. This leads ultimately to apoptotic cell death by both caspase-dependent and -independent mechanisms [36]. Once in the cytosol, cytochrome *c* forms a complex with apoptosis activating factor-1 (Apaf-1) and procaspase-9, called the apoptosome, in the presence of dATP. This results in auto-activation of caspase-9, which in turn activates the executioner caspases-3, -6 and -7 (Fig. 2) [36]. Smac/Diablo can bind to inhibitor of apoptosis proteins (IAP), which are caspase inhibitors, thus preventing them from exerting their anti-apoptotic proprieties (Fig. 2). Furthermore, AIF and EndoG translocate into the nucleus and take part in caspase-independent apoptosis. The role of EndoG in cell death is still unclear, whereas AIF can bind directly to DNA, where its DNase activity causes chromatin condensation and DNA fragmentation [36].

The mitochondrial pathway of apoptosis, and particularly MOMP, is regulated by proteins belonging to the B-cell-lymphoma protein 2 (Bcl-2) family (Fig. 2). There are more than 30 proteins and related members of this family. These proteins contain up to four conserved Bcl-2 Homology domains (BH1-4) that are essential for their functions (see reviews, [40,41]). BH3 functions as a death domain. Bcl-2 family proteins are divided into two groups: anti-apoptotic survival proteins and pro-apoptotic proteins. The six anti-apoptotic members of this family include Bcl-2, Bcl-X_L, Bcl-W, Bcl-B, A1 and Mcl-1, which each have three or four BH domains. The pro-apoptotic proteins Bax, Bak and Bok possess two or three BH domains. Other pro-apoptotic members

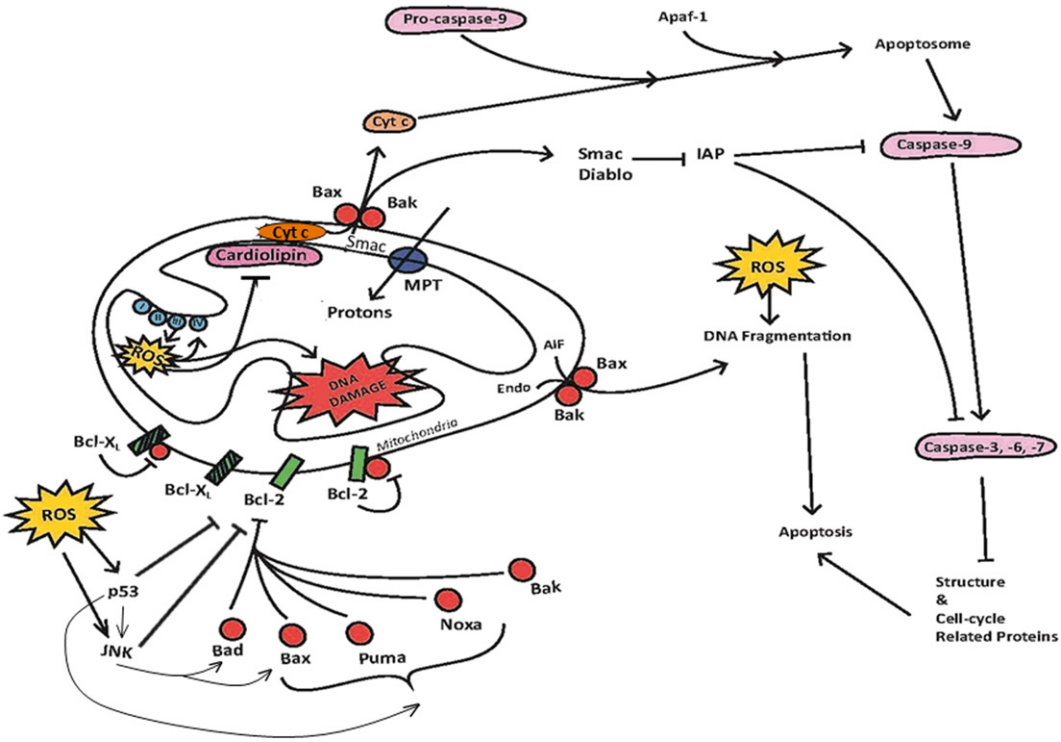

Fig. 2. Activation of the mitochondrial (intrinsic) pathway of apoptosis by ROS. ROS generated exogenously or endogenously can activate p53 and/or c-Jun N-terminal kinase (JNK), which activate pro-apoptotic Bcl-2 proteins (red circles) that can inhibit the functions of anti-apoptotic proteins (green oblongs). ROS cause oxidation of cardiolipin, which relinquishes cytochrome *c* (Cyt c), allowing its release into the cytosol. Moreover, ROS cause mitochondrial membrane depolarisation and/or opening of Bax/Bak channels on the OMM, which allows release of AIF, Endo G, cytochrome *c* and Smac/Diablo into the cytosol. Cyt c then forms the apoptosome complex in the cytosol together with Apaf-1 and procaspase-9, leading to caspase-9 activation. Caspase-9 then activates effector caspases such as caspase-3, resulting in cleavage of cellular proteins and cell demise by apoptosis. AIF and Endo G translocate to the nucleus and appear to be involved in DNA fragmentation. ROS can directly cause damage to nuclear and mitochondrial DNA.

of this family include the BH3-only proteins Bad, Bik, Bmf, Hrk, SOUL, Bid, Bim, Puma and Noxa. Most cells contain a variety of pro- and anti-apoptotic Bcl-2 family proteins. Regulation of the cellular balance between these pro- and anti-apoptotic Bcl-2 proteins is pivotal for the determination of cell fate, by either promoting survival or mitochondrial-mediated apoptosis. Under normal conditions, most pro-apoptotic Bcl-2 family proteins such as Bid, Bax and Bad are found in the cytosol. On the other hand, the anti-apoptotic Bcl-2 and Bcl-X_L proteins are localised mainly at the OMM, where they form heterodimers with Bax, Bim, Bak and Bad, thus inhibiting their pro-apoptotic proprieties. The function of Bok is not clear [40,42].

Under conditions of stress, relative expression of pro- and anti-apoptotic Bcl-2 proteins is modified (Fig. 2). BH3-only Bcl-2 proteins are activated either transcriptionally or post-transcriptionally for the initiation of apoptosis [42]. Stress due to heat shock caused an increase in cellular expression of pro-apoptotic relative to anti-apoptotic Bcl-2 proteins, creating a disequilibrium favouring apoptosis [43]. Stress conditions such as DNA damage and growth factor withdrawal caused targeting of the anti-apoptotic protein Mcl-1 for degradation by the ubiquitin-proteasome system [30]. Most pro-apoptotic Bcl-2 family proteins are activated under stress stimuli: Bid is cleaved into t-Bid by multiple proteases including caspases-2, -8, -10 and calpain; Bax and Bak form homo-oligomers; Bad is released from its sequestering protein, 14-3-3 [44]. Activated pro-apoptotic Bcl-2 proteins then translocate to the OMM. Some activated BH3-only members, such as Bad, Bik, Bmf, Hrk and SOUL, only form heterodimers with anti-apoptotic proteins like Bcl-2, Bcl-X_L and Bcl-W, but not with A1 and Mcl-1 [45]. Other BH3-only proteins such as Bim, Puma and t-Bid bind with high affinity and inhibit all of the anti-apoptotic Bcl-2 proteins [46]. Noxa antagonises only Mcl-1 and A1 [47]. The binding of these BH3-only proteins to anti-apoptotic proteins liberates any complexed pro-apoptotic proteins so that they can activate the pro-apoptotic proteins Bax and Bak. Bax and Bak then transition from inactive monomers to high molecular weight homo-oligomers. This process facilitates MOMP by forming channels large enough to allow leakage of pro-apoptotic factors such as cytochrome *c* into the cytosol (Fig. 2) [48].

2.2.1.1. Calcium and mitochondrial apoptosis. Another function of mitochondria is their pivotal role in calcium homeostasis. Under physiological conditions, the mitochondrial Ca^{2+} pool is small, but it can rise under pathological conditions. Mitochondria are located in proximity to the ER and the local concentration of Ca^{2+} released by the ER can reach high levels, which are taken up by mitochondria through a Ca^{2+} uniporter. The Ca^{2+} concentration within the matrix plays a critical role in stimulating Ca^{2+}-sensitive matrix dehydrogenases that provide NADH for oxidative phosphorylation and ATP production (see review [36]).

Ca^{2+} plays a key role in the initiation and effectuation of cell death by apoptosis [36]. Perturbation of intracellular Ca^{2+} homeostasis is also associated with cell death by necrosis and some forms autophagic cell death, which will not be discussed in detail in the present review (see review [36]) (see Sections 4.1 and 4.2).

Mitochondrial Ca^{2+} accumulation can regulate opening of the MPTP complex in the IMM [36]. This is accompanied by osmotic swelling and triggers rupture of the mitochondrial membrane, which leads to release of pro-apoptotic proteins such as cytochrome *c* into the cytosol. Cell death during Ca^{2+} overload appears to require binding of cyclophilin D to the *c* subunit of the F_0 ATP synthase for MPTP opening [49].

A link was suggested between calcium and Bcl-2 family proteins during apoptosis [36]. Calpain activation caused a decrease in Bcl-2 proteins and activation of mitochondrial apoptosis. Another role for Ca^{2+} in apoptosis is its ability to dephosphorylate Bad through Ca^{2+}-mediated activation of the protein phosphatase calcineurin [50]. This triggers dissociation of Bad from 14-3-3 in the cytosol and its translocation to

mitochondria where it can block the anti-apoptotic action of Bcl-X_L. Furthermore, cleavage of Bid by calpain triggered mitochondrial apoptosis mediated by tBid [36].

Moreover, Ca^{2+} overload in mitochondria can activate a calpain that is localised in the intermembrane space [36]. AIF is anchored in the IMM and undergoes cleavage by calpain, producing a fragment that is released from mitochondria. The AIF fragment translocates to the nucleus where it causes considerable DNA fragmentation and chromatin condensation. AIF appears to play an important role in apoptotic cell death of neurons and certain cancer cells.

2.2.2. Death receptor pathway

Apoptosis can be activated through an extrinsic pathway involving death receptors from the tumour necrosis factor receptor (TNF-R) super-family [15,42,51]. TNF-Rs implicated in apoptosis are located at the plasma membrane and contain an intracellular death domain (DD). These receptors include Death receptor 1 (DR1) (also known as TNF-R1, CD120a, p55), DR2 (Fas, CD95 or Apo-1), DR3 (Apo-3, TRAMP, LARD or TNFRSF25 (TNF receptor superfamily 25)), DR4 (TNF-related apoptosis-inducing ligand (TRAIL)-R1 or Apo-2), DR5 (TRAIL-R2 or TRICK2) and DR6 (TNF receptor superfamily member 21 (TNFRSF21)) [52].

DR2-mediated apoptosis is initiated by binding of the Fas ligand (FasL) to its specific transmembrane receptor Fas, which triggers receptor trimerisation (Fig. 3) [42]. This is a key step for recruitment of the adaptor molecule Fas-associated death domain (FADD) to the intracellular surface of the receptor, through interactions between their respective DD domains, and formation of the death-inducing signalling

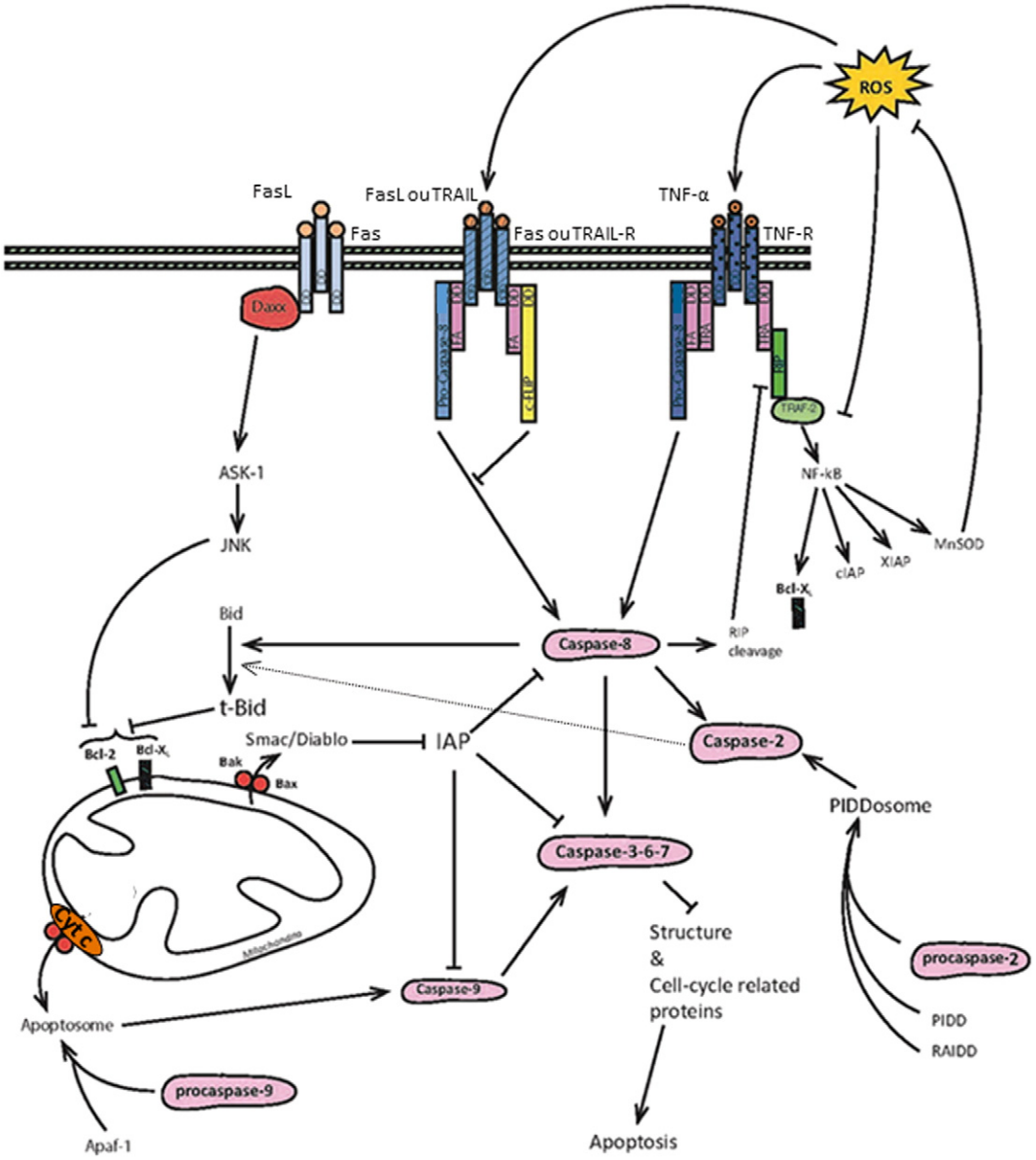

Fig. 3. Activation of the death receptor (extrinsic) pathway of apoptosis by ROS. Transmembrane death receptors such as Fas, TRAIL-R1/2 and TNF-R1 can be activated by ROS. Death receptor-mediated apoptosis involves recruitment of adaptor protein FADD and procaspase-8 or -10 to the cytoplasmic surface of the receptor to form the DISC. This results in activation of caspases-8/-10, which can directly activate caspases-3/-6/-7 and trigger apoptosis. Caspases-8/-10 can also cleave Bid to produce tBid, which activates a crosstalk pathway between death receptors and mitochondria. tBid translocates to mitochondria where it blocks anti-apoptotic activity of Bcl-2 and Bcl-X_L, and activates Bax and Bak. This leads to release of cytochrome *c* and Smac/Diablo and activation of the mitochondrial pathway of apoptosis. Caspase-2 undergoes activation either by caspase-8 or at the PIDDosome complex composed of procaspase-2, PIDD and RAIDD. Caspase-2 can activate the mitochondrial pathway through Bid cleavage. Daxx can interact with Fas, leading to activation of ASK-1 and JNK, and mitochondrial apoptosis (see Fig. 2). In addition to promoting apoptosis through caspase-8, TNF-R1 can activate survival pathways mediated by adaptor protein TRADD, RIP and Traf-2, leading to activation of survival genes by NF-κB.

complex (DISC). FADD then interacts with initiator procaspases-8 or -10 through their respective death effector domains (DED), leading in turn to their auto-activation (Fig. 3) [52]. This is followed by direct activation of downstream effectors such as caspases-3 and -7, a process that occurs in type I cells. However, in type II cells, once activated, caspase-8 or -10 can cleave BH3-only protein Bid to form truncated Bid (t-Bid). tBid then undergoes translocation to the OMM, where it triggers translocation and insertion of pro-apoptotic Bax into the OMM, activation of Bax/Bak and MOMP. Consequently, tBid contributes to amplification of mitochondrial apoptosis through modification of the balance between pro-apoptotic and anti-apoptotic members of the Bcl-2 family [53]. Apoptosis mediated by the death receptors TRAIL-R1 and -R2 is very similar to Fas-mediated apoptosis, but instead implies TRAIL as the death ligand (Fig. 3) [54,55].

The Fas and other death receptors are widely distributed on the surface of most cell types and are also important in control of the immune response [42]. The membrane-bound, rather than soluble form of FasL, appears to be a more potent inducer of apoptosis. Fas can also activate receptor-interacting protein (RIP) kinase-mediated non-apoptotic cell death by necroptosis when caspase-8 is inactivated or absent. Moreover, Fas can also activate other non-death signalling processes that lead to proliferation or differentiation.

The signalling mechanisms through the TNF-R1 transmembrane receptor are different from those for the Fas and TRAIL-R1/R2 receptors [52]. The binding of TNF-α to TNF-R1 causes trimerisation of TNF-R1, which results in recruitment of the TNF-R-associated adaptor protein with death domain (TRADD) to its cytoplasmic side (Fig. 3). This complex can promote either cell survival or cell death, depending on the other factors it associates with [52]. Indeed, recruitment of RIP and TNF-associated factor-2 (Traf-2) to TRADD leads to activation of transcription factors such as nuclear factor kappa B (NF-κB) [56]. This in turn can activate an inflammatory response, or factors that promote cell survival through up-regulation of antioxidants such as manganese superoxide dismutase (MnSOD) and anti-apoptotic proteins such as Bcl-X_L, X-linked inhibitor of apoptosis (XIAP) and cellular inhibitors of apoptosis (cIAP) 1 and 2 (Fig. 3) [56,57]. On the other hand, the association of TRADD with FADD and procaspase-8 leads to the activation of effector caspases-3, -6 and -7 and hence apoptosis, either directly, or through t-Bid-mediated amplification of the mitochondrial pathway (Fig. 3) [58].

The mechanisms regulating whether TNF-R1 binding will result in cell survival or cell death involve the FLICE inhibitory protein (FLIP), which is a transcriptional target of NF-κB [56,57,59,60]. The domain structure of FLIP is similar to that of caspase-8, but lacks a catalytic cysteine. Indeed, high concentrations of c-FLIP, a procaspase-8 competitive inhibitor that prevents it from associating with FADD, promote cell survival, whereas cleavage of RIP by caspase 8 promotes apoptosis [53]. Low concentrations of c-FLIP, however, promote caspase-8 recruitment and activation.

2.2.2.1. Role of caspase-2. Pioneering work about caspase-2 has been carried out by the group of Jürg Tschopp [61]. Caspase-2, the most conserved caspase identified, is thought to have an important role in regulation of apoptosis [22,61]. However, its function is not entirely clear and is controversial, compared to the other caspases [20]. As well as being located in the cytosol, caspase-2 contains a nuclear localisation signal (NLS) sequence and is transported into the nucleus, although its function in the nucleus is not presently known [62].

Caspase-2 activation occurs in response to various stress stimuli including DNA damage, metabolic imbalance, cytoskeletal disruption, ER stress, heat shock [63,64] and H_2O_2 [65]. Mechanisms responsible for caspase-2 activation are not entirely clear, although this caspase can associate with the p53-inducible death domain-containing protein (PIDD) and the adaptor molecule RIP-associated ICH-1 homologous protein with a death domain (RAIDD), forming the PIDDosome complex [20,61] (Fig. 3). PIDD and RAIDD interact in the PIDDosome via their respective DD domains. The PIDDosome appears important for caspase-2 processing and auto-activation in response to DNA damage [20,62]. This pathway links caspase-2 to p53-mediated cell death.

However, in certain experimental models, caspase-2 activation occurred in the absence of RAIDD and PIDD, suggesting the existence of an alternative platform for its activation. During p53-mediated apoptosis, caspase-2 was activated by recruitment to the Fas DISC and processing by caspase-8 [66]. Caspase-2 is also activated by caspase-3 [67]. Furthermore, DNA damage activates the Ataxia telangiectasia mutated/Ataxia telangiectasia and Rad3 related (ATM/ATR) pathway, which can induce caspase-2-dependent apoptosis when checkpoint kinase 1 (Chk1) is suppressed, thus circumventing the requirement for p53 [68].

Compared to the effector caspases-3 and -7, there are few known cleavage substrates of activated caspase-2 [21]. These include Mdm2, αII-spectrin, Bid, ROCK-2, protein kinase C δ (PKCδ), ICAD, PARP and plakin [20,21]. Most of these proteins are also substrates of calpains and other caspases. The cytoskeletal-associated protein desmoplakin appears to be specifically cleaved by caspase-2 and not by other caspases [21]. The cleavage of Bid by activated caspase-2 leads to MOMP and mitochondrial apoptosis [20], although this is much less efficient than caspase-8-mediated MOMP and apoptosis. Mdm2 binds to p53, targeting it for ubiquitination and degradation by the 26S proteasome, when it is no longer needed [15]. Hence, caspase-2-mediated cleavage of Mdm2 increases the stability of p53 and enhances its functions, such as growth arrest and induction of apoptosis [20,69]. Mdm2 cleavage results in a positive feedback loop: p53 induces PIDD upregulation, while PIDD stabilizes p53 through activation of caspase-2.

More recently, caspase 2 was reported to play a role in regulation of the stability of several cytoskeletal proteins during apoptosis [21]. These proteins include stathmin-1, tropomyosin, myotrophin and profilin. They are all associated with actin or tubulin filaments, and their function is to stabilise integrity of the cytoskeleton. They were degraded in a caspase-2–dependent manner during exposure to agents that cause DNA damage, ER stress or microtubule destabilisation [21]. These proteins were not directly cleaved by caspase-2 but were targeted for ubiquitination and proteasomal degradation in cells exposed to apoptotic stimuli.

Interestingly, the antioxidants SOD1 (CuZnSOD) and thioredoxin were among the cytosolic proteins that were affected by active caspase-2 and found to be less abundant [21]. This suggests that caspase-2 can inhibit antioxidants, which would promote ROS-mediated apoptosis.

Besides its putative role in apoptosis, caspase-2 has been shown to take part in non-apoptotic functions such as cell cycle regulation as a checkpoint protein, tumour suppression and cancer prevention, and DNA repair [20,62,63].

2.2.3. Endoplasmic reticulum pathway

2.2.3.1. UPR and ER stress. The ER is a vital organelle in the secretory pathway and is also involved in lipid biosynthesis and regulation of Ca^{2+} flux (see reviews, [70,71]). It is involved in synthesis, folding, trafficking and post-translational modifications of proteins. This includes proteins that reside in the ER as well as those destined for other organelles such as the Golgi and lysosomes, the plasma membrane and the extracellular space [70]. Indeed, control mechanisms exist in the ER to ensure high quality protein folding, which is essential for cell survival, function and homeostasis. Exposure to adverse environmental stimuli can disturb ER homeostasis, which leads to accumulation of misfolded and unfolded proteins in the ER lumen by a phenomenon known as ER stress [70]. Conditions that cause ER stress include exposure to environmental toxins, energy or nutrient deprivation, disturbance of calcium or redox homeostasis, hypoxia, inflammation, increased protein synthesis, impaired protein degradation and defective autophagy [15,18,70]. The induction of ER stress activates a survival response known as the Unfolded Protein Response (UPR), which helps cells to

adapt to ER stress and restore homeostasis [18,70]. UPR activation can promote cell survival or cell death, depending on the severity of ER stress.

The UPR involves three signalling mechanisms: i) transient inhibition of translation, thereby decreasing influx of new proteins into the ER; ii) up-regulation of genes encoding for chaperones and antioxidants, in order to increase folding, and for proteins involved in ER-associated protein degradation (ERAD); and iii) activation of ERAD, which allows translocation of unfolded or aggregated proteins from the ER to the cytosol where they are ubiquinated and degraded by the proteasome [15,18,70,71]. These mechanisms are controlled mainly by three sensors of the ER stress response: protein kinase RNA (PKR)-like ER kinase (PERK), inositol-requiring protein-1α (IRE-1α) and activating transcription factor-6α (ATF-6α) [72,73]. Under normal conditions, these three proteins are sequestrated in the ER lumen by interactions with the chaperone binding immunoglobulin protein (BiP)/glucose-regulated protein 78 (GRP78), which keeps them inactive (Fig. 4A) [15,70]. However, during ER stress, BiP binds preferentially to unfolded proteins that accumulate in the ER. This process releases the three ER stress sensors, which then become activated [15,70]. BiP also directs misfolded proteins for degradation by the ERAD system.

To transiently attenuate global protein synthesis, activated PERK phosphorylates the eukaryotic initiation factor 2α (eIF2α), which takes part in general inhibition of the initiation of mRNA translation

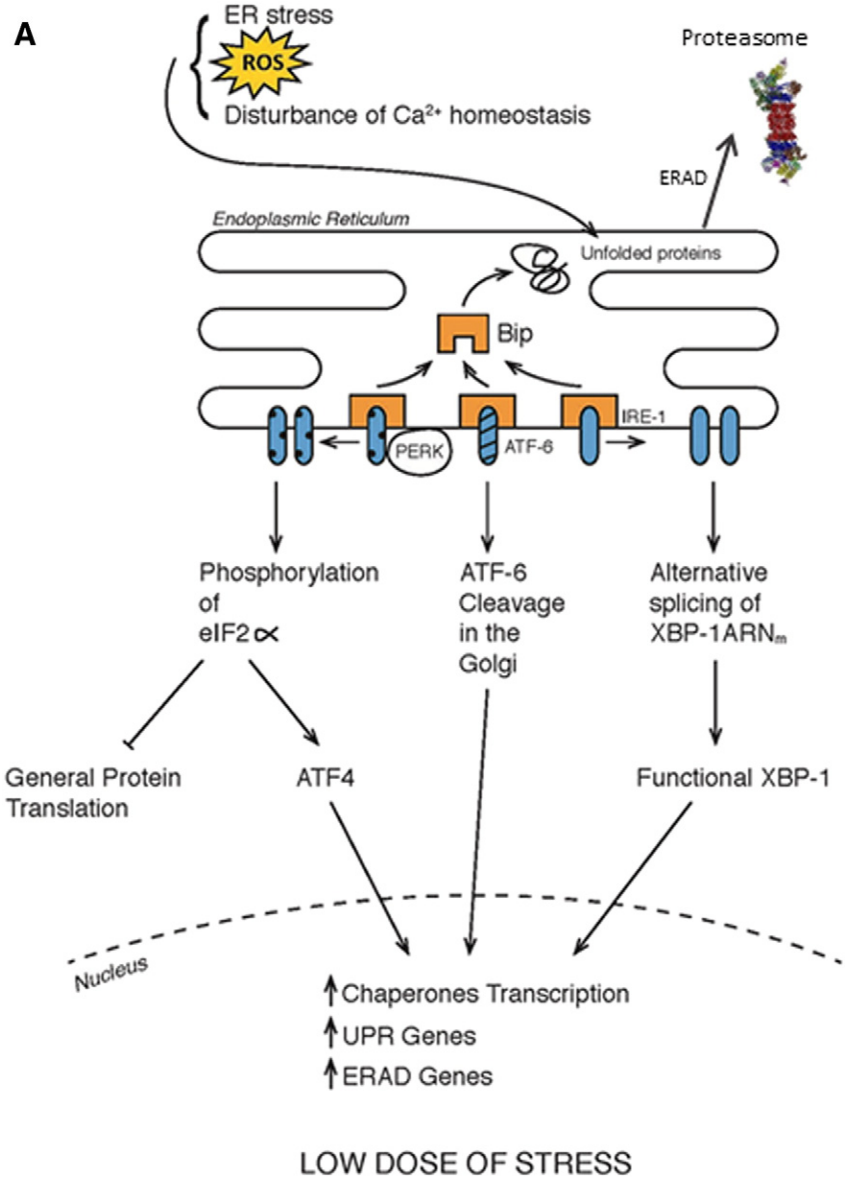

Fig. 4. (A) ROS activate the ER stress response and (B) cause apoptosis through the ER. A. Low dose stress stimulates activation of three ER stress sensors, PERK, ATF-6α and IRE1α that are involved in the unfolded protein response (UPR). Bip is released from these sensors and binds to unfolded proteins in the ER lumen. Together, the three ER stress sensors inhibit protein translation, increase chaperone expression and enhance ER-associated protein degradative (ERAD) pathways. Activation of PERK phosphorylates eIF2α, which inhibits general protein translation. This allows selective translation of ATF-4, which activates transcription of chaperones such as BiP, and upregulates genes for the UPR and ERAD. ATF-6α undergoes proteolytic cleavage in the Golgi apparatus. IRE1α activation causes alternative splicing of XBP1 mRNA, leading to expression of the active XBP1 transcription factor. IRE-1α and ATF-6α both activate transcription pathways that increase the UPR and ERAD. B. Higher dose ER stress triggers apoptosis through several pathways. Caspases-9 and -3 can be activated by mitochondrial-dependent and independent pathways. Activated IRE1α recruits TRAF2 and ASK1, causing activation of JNK and mitochondrial apoptosis. ER caspase-4/-12 is activated by calpain, and possibly caspase-7, leading to activation of caspase-9 and caspase-3. Cleavage of BAP31 at the ER membrane by caspase-8 generates a p20 fragment, which leads to activation of mitochondrial apoptosis. Ca^{2+} released from the ER is taken up by mitochondria, leading to depolarization of the inner mitochondrial membrane (IMM). Activation of PERK and ATF-6α induces CHOP, which appears to activate apoptosis by up-regulating expression of pro-apoptotic genes such as Bax and Bim and/or by inhibiting expression of the Bcl-2 gene.

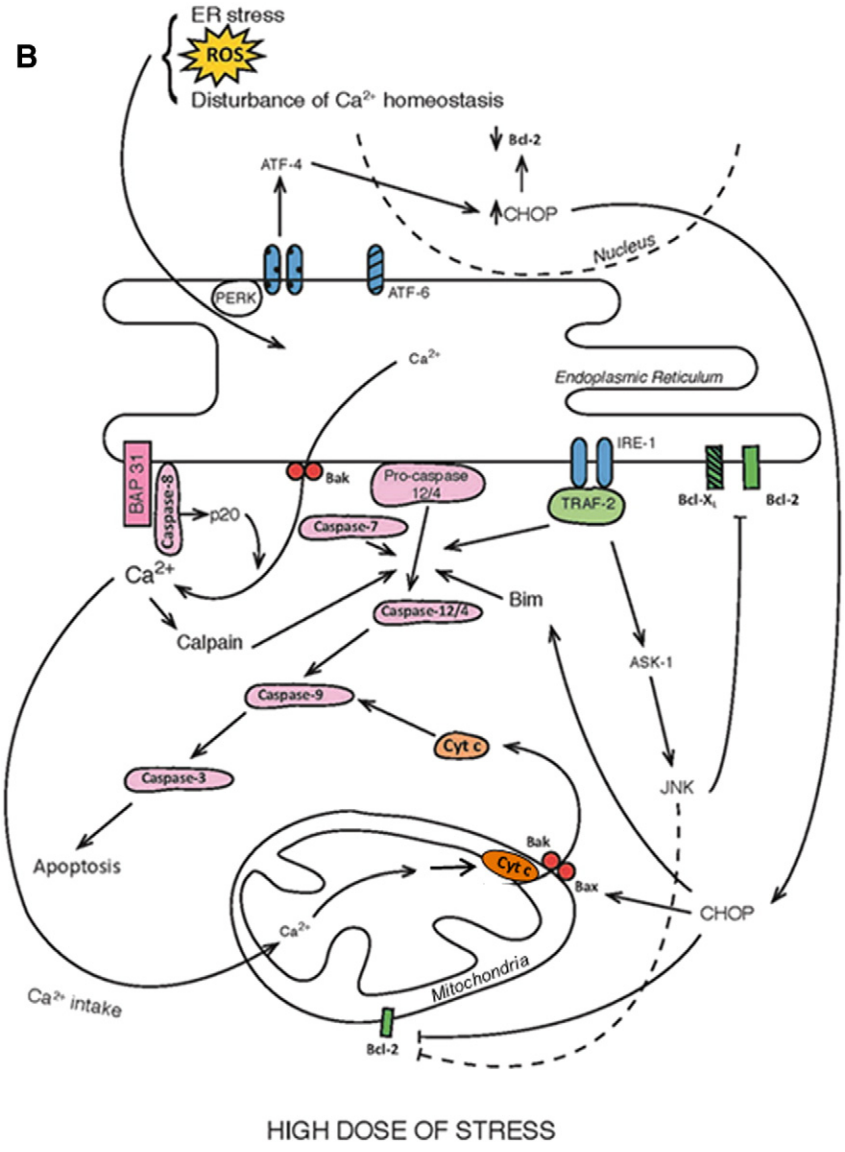

Fig. 4 (*continued*).

[15,70]. However, phosphorylated eIF2α specifically upregulates translation of the ATF-4 mRNA. The transcription factor ATF-4 then translocates to the nucleus where it activates UPR genes that encode for proteins such as the chaperone BiP, as well as those involved in amino acid biosynthesis and transport, and the antioxidant response (Fig. 4A) [70]. ATF-4 also activates the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP) (or growth arrest and DNA damage-inducible gene 153 (GADD153)).

IRE-1α and ATF-6α both activate transcription pathways that increase protein folding, transport and degradation (see review [70]). Once activated, IRE-1α catalyses the alternative splicing of the X-Box binding protein (XBP-1) mRNA, which leads to expression of a functional transcription factor XBP-1s (Fig. 4A). XBP-1s translocates to the nucleus where it activates transcription of genes encoding for chaperones located in the ER as well as for degradation pathways, thus increasing the capacity of the ER to deal with damaged and unfolded proteins. Furthermore, IRE-1α activation can promote mRNA decay to reduce the protein folding load in the ER. This process is known as regulated IRE-dependent decay (RIDD) [70]. Finally, when ATF-6α is released from BiP, it translocates to the Golgi where it undergoes cleavage and activation (Fig. 4A). The cytosolic cleavage fragment of ATF-6α is then targeted to the nucleus where it activates genes that encode for proteins involved in the UPR. These proteins include chaperones such as BiP, ER protein 57 (ERp57), GRP94 and proteins involved in ERAD [70].

The UPR thus constitutes an adaptive, anti-apoptotic survival response during exposure to ER stress [70]. However, when the UPR fails to restore homeostasis in the ER under conditions of severe or prolonged ER stress, cells undergo ER stress-mediated apoptosis [15,18,70,74].

2.2.3.2. ER stress and apoptosis. There are several different mechanisms by which cells can undergo apoptosis following unresolved ER stress [18,71,72,74]. These mechanisms involve several of the factors involved in the UPR, and can be dependent on, or independent of mitochondria.

In addition to its role in selective splicing of XBP-1 mRNA during the ER stress response, IRE-1α appears to play a central role in the switch from a survival response to cell death [18,70,72]. During prolonged conditions of ER stress, IRE-1α can directly bind to unfolded proteins [75]. IRE-1α can also interact with Traf-2, which then recruits and activates apoptosis signalling kinase-1 (ASK-1) [76]. This causes phosphorylation and activation of c-Jun N-terminal kinase (JNK) and p38 MAPK, which can promote apoptosis (Fig. 4B) [70,71,76]. Indeed, activated JNK

translocates to the mitochondrial membrane, where it blocks Bcl-2 and allows activation of pro-apoptotic members of the Bcl-2 family that are critical for cytochrome *c* release from mitochondria and induction of apoptosis [15].

Although Bcl-2 family proteins are best characterised at the mitochondria level, they are also localised at the ER and nuclear membranes [77,78]. IRE-1α-mediated JNK activation causes phosphorylation and inactivation of Bcl-2 located at the ER membrane [76]. Phosphorylation by JNK inhibits anti-apoptotic Bcl-2 [71], which leads to its proteasomal degradation [79]. ER-localised Bcl-2 has been shown to inhibit apoptosis and to regulate ER Ca^{2+} homeostasis, whereas pro-apoptotic members of the Bcl-2 family such as Bax and Bak are able to promote Ca^{2+} release from the ER, in a similar manner to their action at the mitochondrial membrane [71,76,80]. Consequently, when inactivated by JNK, Bcl-2 is no longer able to inhibit pro-apoptotic members of the Bcl-2 family and unable to regulate Ca^{2+} efflux from the ER [81]. This leads to massive and/or prolonged influx of Ca^{2+} into mitochondria, which can activate the MPTP complex and trigger mitochondrial swelling and disruption of the OMM, resulting in release of pro-apoptotic factors into the cytosol and apoptosis [36]. Activated JNK can also phosphorylate and activate the ER pro-apoptotic Bcl-2 protein Bim [76]. Under physiological conditions, pro-apoptotic activity of Bim is inhibited by binding to dynein motor complexes [82]. Phosphorylation by JNK releases Bim from these complexes and allows activation of Bax-mediated apoptosis [83].

Under conditions of severe ER stress, the PERK-eIF2α-ATF-4 pathway also activates the transcription factor CHOP, which in turn upregulates expression of pro-apoptotic proteins such as DR5, Bim and PUMA, and downregulates expression of Bcl-2 (see reviews [71,76]). Among other things, CHOP downregulates Bcl-2 by repressing its promoter, and promotes activation and translocation of Bax to mitochondria (Fig. 4B) [76]. Another manner in which CHOP can cause apoptosis is through activation of growth arrest and DNA damage-inducible 34 (GADD34), which stimulates dephosphorylation of eIF2α and reverses the translational block [76]. This increases protein synthesis and contributes to additional accumulation of unfolded proteins in the ER, ATP depletion, oxidative stress and apoptosis. In addition, this allows the translation of mRNAs encoding for pro-apoptotic proteins. In addition, CHOP can be regulated post-translationally by p38 MAPK phosphorylation, which increases its transcriptional activity [76]. p38 is a substrate of ASK-1, which is recruited to the IRE1α–TRAF2 complex during ER stress [76].

Another mechanism of ER-mediated apoptosis involves the caspase-12/caspase-4 pathway [15,18]. Caspase-12/-4 can be activated by several different mechanisms. Procaspase-12 in mice is bound to the cytosolic face of the ER membrane and can be activated by cleavage involving the Ca^{2+}-dependent cysteine protease m-calpain [18,24,84]. Cytosolic caspase-7 translocates to the ER membrane and can also cleave and activate caspase-12 in response to excess ER stress [15,24]. Under normal conditions, Traf-2 forms a complex with procaspase-12. However, during excess ER stress, Traf-2 also takes part in caspase-12 processing by releasing it, thus allowing its activation. This appears to provide a link between the IRE-1α-Traf-2 complex and ER stress–induced apoptosis [18]. During ER stress, pro-apoptotic Bcl-2 family member Bim is recruited to the ER membrane, where it can intervene in caspase-12 processing [85]. On the other hand, anti-apoptotic proteins such as Bcl-X_L can dimerise with Bim, inhibiting its translocation to the ER membrane [85]. Once activated, caspase-12 can activate caspase-9 that in turn processes caspase-3, in a cytochrome *c*-independent pathway [15,18]. However, the role of caspase-12 as an initiating caspase in ER stress-induced apoptosis is unclear. In humans, functional caspase-12 is lacking in most cells because the caspase-12 gene contains several inactivating mutations [86]. Instead, caspase-4 appears to carry out the equivalent function in ER stress-induced apoptosis [25]. Caspase-2 has also been implicated in initiating ER stress-mediated apoptosis [71].

ER stress induces the formation of a complex between procaspase-8 and Bap31, an integral membrane protein of the ER [71,76]. This results in cleavage of the cytosolic tail of Bap31 by caspase-8, leading to production of a pro-apoptotic p20Bap31 cleavage fragment (Fig. 4B). The p20 fragment can then direct pro-apoptotic signals between the ER and mitochondria [76]. p20 triggers Ca^{2+} efflux from the ER where it is rapidly taken up mitochondria, and mitochondrial recruitment of dynamin-related protein 1 (Drp1). Once Ca^{2+} levels in the mitochondrial matrix reach a critical threshold, the MPTP becomes activated, resulting in increased MOMP. This culminates in pro-apoptotic cytochrome *c* release from mitochondria and cytochrome *c*-dependent cell death [76].

ER-localized Bap31 can interact with the outer membrane mitochondrial fission protein Fission 1 homolog (Fis1) [87]. Fis1 is the receptor for cytosolic Drp1 and appears to be involved in its recruitment to mitochondria [88]. Drp1 mediates scission of the OMM, which leads to dramatic fragmentation and scission of the mitochondrial network. Concurrent with cytochrome *c* release, the mitochondrial network undergoes fission.

The Fis1–Bap31 complex at the ER-mitochondria interface serves as a platform, known as the ARCosome, which recruits and activates procaspase-8, thereby linking two major organelles that are involved in intrinsic apoptotic signalling [39]. It was proposed that Ca^{2+} release from the ER could be a mechanism that allows the cell to achieve significant amplification by engaging mitochondria in apoptosis.

3. ROS and Apoptosis

At lower doses, ROS such as H_2O_2 have been linked to induction of cell survival responses, whereas higher doses activate death processes such as apoptosis [6]. At lower doses, ROS activate the tumour suppressor protein p53 [6]. p53 plays a key role in the control of cellular stress responses, inducing either cell cycle arrest in order to promote DNA repair and survival, or cell death by apoptosis, depending on the context [89]. Under normal conditions, p53 has a short life-time and is maintained at low levels through ubiquitination by Mdm2, leading to its proteasomal degradation. When the cell faces stress conditions or DNA damage, p53 is released from Mdm2 and stabilised by post-translational modifications, thus avoiding proteasomal degradation, before associating with DNA [89]. If the stress is low, p53 induces cell cycle arrest, DNA repair and senescence. However if damage is too severe, p53 can regulate apoptosis transcriptionally by down-regulating pro-survival proteins such as Bcl-2, Bcl-X_L, IAPs and survivin, and upregulating pro-apoptotic members [89]. p53 activates the transcription of pro-apoptotic genes that are crucial for inducing the intrinsic pathway of apoptosis, such as Bax, Bid, Puma, Noxa and Apaf-1, but also extrinsic pro-apoptotic factors such as Fas, FasL, DR-4 and DR-5 [89]. Moreover, cytosolic p53 can translocate to mitochondria where it can interact directly with anti-apoptotic proteins such as Bcl-2, Bcl-X_L and Mcl-1 and the pro-apoptotic proteins Bax and Bak, which allows MOMP, release of pro-apoptotic factors and apoptosis [45]. In addition, cytosolic p53 can activate Bax directly by causing its structural rearrangement [45]. p53 thus enhances mitochondrial membrane permeabilisation and subsequent release of pro-apoptotic factors from mitochondria [90].

Apoptosis induced by H_2O_2 has been associated with increased protein expression of p53, Puma, Noxa and Bax, and p53 phosphorylation at Ser15 and Ser46, in several cell types including rat neural AF5, glioma, colon cancer and human cervical carcinoma HeLa cells [91–94]. During H_2O_2-induced apoptosis in HeLa cells, p53 was an upstream factor for the upregulation of Puma and translocation of Bax to mitochondria [92].

The redox regulation of caspase activity appears to involve post-translational modifications of their catalytic site cysteine residues [56,95]. The catalytic site cysteines of most caspases are susceptible to oxidation, whereas procaspase-9, procaspase-3 and caspase-3 are susceptible to S-glutathiolation [56,95]. While caspases can be activated by oxidants such as H_2O_2 [65,92], oxidants can also irreversibly inactivate their enzymatic activity [95]. This is the case for caspases-3, -8 and -9.

Moreover, cellular GSH can also regulate caspase-3 activity, mediated by S-glutathiolation, which decreases accessibility for proteolytic cleavage, resulting in resistance to apoptosis. However, the overall control of this process and its consequences for apoptosis induction are still not elucidated [56]. For example, are there other apoptotic components upstream of caspase-3 that are targets for S-glutathiolation and which specific cysteines are susceptible?

3.1. ROS and the Mitochondrial Pathway

The mitochondrial pathway of apoptosis is activated in response to a variety of cellular stresses, including mitochondrial DNA (mtDNA) damage, growth factor deprivation, heat shock, hypoxia, ER stress and developmental cues [15,36,56,95]. ROS have been tightly linked to activation of the mitochondrial pathway [36]. Indeed, mitochondria are the location where most intracellular ROS are produced, as a result of leakage from the respiratory electron transport chain [10]. Mitochondria-derived ROS are then able to target nearby structures such as mtDNA, which is susceptible to oxidative damage [36]. mtDNA damage would impair mtRNA transcription of proteins involved in the electron transport chain, causing disruption of respiratory chain function, further increases in ROS generation, leading to loss of mitochondrial membrane potential and impaired ATP synthesis [36]. These events culminate in apoptosis through the mitochondrial pathway.

ROS such as H_2O_2 and superoxide can cause cytochrome *c* release from mitochondria and induction of apoptosis through the mitochondrial pathway [56,65,96,97]. Protein components of the MPTP such as VDAC, ANT and cyclophilin D are targets of ROS and undergo oxidative modifications, which will stimulate MPTP opening [56]. H_2O_2 caused initial mitochondrial membrane hyperpolarisation that led to collapse of mitochondrial membrane potential ($\Delta\Psi_m$), mitochondrial translocation of Bax and Bad, and cytochrome *c* release [95]. Significant loss of cytochrome *c* from mitochondria will further increase ROS generation due to disruption of the electron transport chain.

In HeLa cells, H_2O_2-induced apoptosis through the mitochondrial pathway was mediated by p53 as an upstream factor [92]. H_2O_2 caused both caspase-dependent and caspase-independent apoptosis. Caspase-dependent apoptosis involved loss of mitochondrial membrane permeability, cytochrome *c* release into the cytosol, and caspase-9-mediated activation of caspase-3. Caspase-independent apoptosis involved release of AIF from mitochondria and its translocation to the nucleus [65]. Furthermore, JNK activation by oxidative stress could phosphorylate and inactivate anti-apoptotic factors such as Bcl-2 and Bcl-X_L, while phosphorylating and activating pro-apoptotic members of this family [12].

ROS can oxidise other targets such as the phospholipid cardiolipin, which binds cytochrome *c* to the outer leaflet of the IMM [95]. Under normal conditions, cytochrome *c* shuttles electrons between complexes III and IV of the mitochondrial electron transport chain. Cardiolipin oxidation by ROS decreases cytochrome *c* binding and increases the level of free cytochrome *c* potentially released through the OMM in the cytosol, where it initiates the apoptotic cascade [95].

Oxidative stress and mitochondrial Ca^{2+} accumulation can both trigger opening of the pore in the IMM, by a process known as mitochondrial permeability transition (MPT) [36]. Although Ca^{2+} is a key factor in pore opening, other factors such as ATP depletion, oxidative stress, low pH and high inorganic phosphate can also facilitate pore opening [98]. This leads to osmotic swelling and OMM rupture, followed by release of mitochondrial proteins such as cytochrome *c*. The consequence of pore opening appears to depend on the level of Ca^{2+}; higher concentrations favour necrosis while lower levels promote apoptosis. Furthermore, ROS generation can significantly increase Ca^{2+}-induced cell deterioration [36]. In fact, ROS decrease the level of Ca^{2+} required to trigger MPT and subsequent cell damage.

Disruption of cellular GSH redox status due to GSH oxidation or GSH efflux can contribute to oxidant-mediated apoptosis. GSH appears to play a central role in protecting cells against activation of apoptosis by different stimuli. This topic is discussed in detail in the following review [56].

3.2. ROS and the death receptor pathway

Links between ROS and extrinsic-induced apoptosis also exist. The cytokine TNF-α is an important regulator of the complex signalling networks that promote either cell survival, or cell death by apoptosis [52]. TNF-α can also cause caspase-independent cell death by necrosis (necroptosis), which involves ROS generated from either mitochondrial or non-mitochondrial sources [99].

ROS and TNF-α influence each other in a positive feedback loop [57]. At low doses, ROS play an important role in regulating the TNF-R1 signalling pathway, whereas at higher doses, this leads to DNA damage and cell death. There is considerable debate as to whether mitochondrial ROS can activate or suppress the transcription factor NF-κB. It is clear that ROS can mediate activation of NF-κB through TNF-R1 signalling, which leads to upregulation of antioxidants such as catalase and MnSOD [57]. These antioxidants can offset TNF-induced apoptosis by neutralising mitochondrial-derived ROS. In addition, NF-κB stimulates the transcription of anti-apoptotic genes such as IAP and Bcl-X_L, which can prevent induction of TNF-R1-mediated apoptosis by blocking caspase-8 activation [12]. However, it is generally accepted that TNF-derived ROS can inhibit NF-κB activation, decreasing NF-κB-mediated survival signalling, thus promoting apoptosis [100]. On the other hand, mitochondria-derived ROS appear to promote, rather than inhibit, TNF-mediated NF-κB activation [100]. It appears that mitochondrial ROS inactivate the phosphatases that regulate activity of the kinases controlling NF-κB signalling. Consequently, ROS-mediated phosphatase inhibition would cause enhanced phosphorylation of the inhibitor IκB, triggering its degradation and allowing NF-κB activation.

The mechanisms by which TNF stimulation leads to increased mitochondrial ROS generation in cells are not understood. Once activated by TNF-R1, caspase-8 can bind to ROS modulator-1 (ROMO-1) that is located in the OMM [57]. ROMO1 then sequesters Bcl-X_L, which triggers loss of mitochondrial membrane potential and increases generation of ROS. ROS in turn trigger activation of ASK-1. Under normal conditions, ASK-1 remains inactive by binding to reduced thioredoxin [95]. However, ROS oxidise thioredoxin, which releases ASK-1, allowing it to activate its downstream targets JNK and p38 MAPK, leading to TNF-induced apoptosis. ASK-1 can also be inactivated by protein phosphatase-5, which is regulated by ROS [57]. ROS can also inactivate JNK-inactivating phosphatases, which leads to JNK activation, followed by cytochrome *c* release, caspase-3 activation and apoptosis.

Fas-mediated apoptosis can also be activated by ROS such as H_2O_2 [65]. In HeLa cells, H_2O_2 caused up-regulation of FasL, FADD translocation to the plasma membrane and caspase-8 activation. Once activated, caspase-8 processed caspase-2. Activated caspase-8 and caspase-2 both caused cleavage of Bid to t-Bid, thus enhancing mitochondrial-induced apoptosis [92]. Furthermore, p53 was an upstream initiating factor in the activation of Fas-mediated apoptosis by oxidative stress [65]. H_2O_2 also induced death receptor-mediated apoptosis in other cell types. H_2O_2 increased mRNA levels for FasL and Fas in murine intestinal epithelial cells [101] and up-regulation of Fas in HUVECs [102]. In HL-60 cells, H_2O_2 caused activation of caspase-8 and caspase-3 [103].

During stress conditions, death-associated protein 6 (Daxx) can interact with Fas through its DD, leading to activation of ASK-1, which in turn activates JNK [104] (Fig. 3). JNK triggers Bcl-2 phosphorylation, thus favouring apoptosis through the mitochondrial pathway.

Members of the Nox family are an important source of ROS ($O_2^{\bullet -}$) generation in cells [105]. Noxs are transmembrane proteins that are specifically located in subcellular compartments such as lipid rafts, caveolae, endosomes and the nucleus [106]. For Fas and TNF-α, death ligand-receptor binding caused lipid raft formation, recruitment and activation of Nox, and ROS generation. These processes constituted lipid

raft-derived redox signalling platforms, which promoted death receptor activation and induction of apoptosis [107]. The physiological significance of ROS-dependent receptor-mediated apoptosis compared to the classical ligand/receptor-induced activation of apoptotic signalling is not completely understood [95].

ROS have been implicated in the regulation of TRAIL-mediated apoptosis [106], although the mechanisms involved are not clear. In particular, ROS can upregulate gene expression for DRs such as TRAIL, which is abrogated by antioxidants such as catalase and *N*-acetylcysteine. In addition, ROS-mediated upregulation of TRAIL appears to be mediated by factors such as CHOP and p53. Furthermore, substances that increase ROS generation appear to sensitize cells to TRAIL-mediated apoptosis [106].

3.3. ROS and the ER Pathway

In the ER lumen, the redox status is very important because protein folding and disulfide bond formation require an oxidant environment. The oxidizing environment of the ER lumen has a high ratio of oxidized to reduced glutathione (GSSG/GSH) compared to the reducing environment of the cytosol [108]. Disulfide bond formation is under the control of chaperone proteins such as the protein disulfide isomerase (PDI) [109]. PDI is the most abundant chaperone in the ER. During formation of disulfide bonds in nascent proteins, PDI catalyses the oxidation of two cysteine residues between polypeptide substrates, while itself undergoing two electron reduction at its active site. The ER membrane-associated protein, endoplasmic reticulum oxidoreductin-1 (ERO1), is capable of restoring PDI to its oxidized state, but this leads to ROS generation [109]. Electrons are transferred from protein thiol groups of PDI to O_2 and ERO1, through a flavin adenine dinucleotide (FAD)-dependent reaction. FAD acts as an electron acceptor and its reduced form ($FADH_2$) is reoxidised by a reaction with O_2, which results in generation of H_2O_2. It is not clear whether the ER-derived H_2O_2 produced during oxidative folding is toxic or acts as a signalling molecule [110]. Furthermore, increased levels of GSH in the ER can lead to excessive generation of H_2O_2 through ERO1. Glutathione (GSH) is a substrate for PDI-catalysed oxidation, which leads to increased levels of GSSG when ERO1 is active. The increased levels of H_2O_2 and GSSG cause a disturbance in the redox equilibrium of the ER lumen and lead to ER stress. Moreover, the activities of both ERO1 and PDI are redox regulated [110].

The impairment of ER proteostasis is another condition that would increase the need for ERO1-derived disulfides [110]. Misfolded proteins in the ER are thought to be subjected to fruitless cycles of aberrant disulfide bond formation and reduction. This would increase production of ERO1-derived H_2O_2, which would in turn cause ER stress and activate UPR signalling. However, the source and nature of ER stress-derived ROS, and whether they are ERO1-derived, is not entirely clear.

Furthermore, ROS levels may increase when GSH levels are depleted due to reduction of improperly-paired disulfide bonds in misfolded proteins. It was estimated that about 25% of cellular ROS may be generated from the formation of disulfide bonds in the ER during oxidative protein folding [111]. Proteins with many disulfide bonds would play a larger role in ROS generation in the ER compared to those with fewer disulfide bonds. The generation of ROS as by-products of protein oxidation in the ER could then cause ER stress-mediated cell death. Other antioxidants found in the ER include glutathione peroxidases 7 and 8, and peroxiredoxin 4, which all detoxify H_2O_2 [110]. The ER-antioxidant system is thus essential for maintaining ER homeostasis because changes in the redox status result in oxidative modification and/or misfolding of newly-synthesised proteins, which triggers ER stress.

ROS generation could increase during protein misfolding through changes in oxidative phosphorylation, which would arise from Ca^{2+} release from the ER or energy depletion. However, excess ROS are able to trigger protein misfolding, leading to UPR activation and induction of CHOP, which can be prevented by the antioxidant butylated hydroxyanisol (BHA) [112]. Furthermore, excess ROS production could also interfere with protein folding by inactivating the PDI/ERO1 thiol-disulfide exchange and/or by causing incorrect disulfide bond formation.

Activation of the UPR by mild oxidative stress appears to be an adaptive response to maintain cell survival and function, whereas more severe or prolonged oxidative stress and protein misfolding can initiate apoptotic signalling pathways [76]. During persistent ER stress, activation of IRE1α leads to ASK and p38 MAPK activation [18], which further activate CHOP and contribute to generation of ROS [109]. Among other things, CHOP activates ERO1 transcription [76], which could lead to acute ROS production [109], favouring an over-oxidizing environment. This would exacerbate PDI activity, causing extra disulfide bond formation and re-oxidation of PDI in a vicious loop, as well as accumulation of misfolded proteins [74]. ERO1 can also activate the inositol triphosphate receptor (IP_3R), which stimulates massive Ca^{2+} transport from the ER to mitochondria, thereby triggering mitochondrial-mediated cell death [76]. Furthermore, ER-stress-mediated apoptosis is under the control of JNK, which is regulated by ASK-1, whose function is directly controlled by oxidative status. Indeed, thioredoxin-mediated ASK-1 oxidation is necessary for ASK-1 conjugation with Traf-2 and subsequent activation of JNK [95].

In addition to eIF2α, PERK can phosphorylate nuclear erythroid 2 p45-related factor 2 (Nrf2) [113]. This leads to dissociation of the Nrf2-Keap1 complex and promotes the expression of genes containing antioxidant response elements (ARE), thereby preventing oxidative stress by induction of antioxidant genes such as heme oxygenase 1 (HO-1) [113]. Nrf2 also increases expression of glutamate cysteine ligase (GCL), the main enzyme involved in synthesis of the antioxidant GSH.

During ER stress, ROS can stimulate Ca^{2+} release from the ER lumen [109]. There are tight interactions between the ER and mitochondria through specialised ER compartments such as mitochondria-associated membranes (MAM), which control the transport of small molecules and ions between these organelles [39]. Due to proximity of mitochondria to the ER, high levels of Ca^{2+} released from the ER are then taken up by mitochondria [36]. The increased levels of Ca^{2+} in mitochondria stimulate the Ca^{2+}-sensitive matrix dehydrogenases, which increase metabolic activities by providing NADH for mitochondrial respiration and ATP production [36]. Increased mitochondrial respiration results in an increase in mitochondrial ROS generation through complexes I and III. Furthermore, mitochondrial Ca^{2+} overload stimulates opening of the MPTP, leading to release of ATP, GSH and cytochrome *c* to further increase ROS levels [110].

Interestingly, ROS can induce expression of the pro-apoptotic BH3-only Bcl-2 protein Bim [114]. In addition, JNK phosphorylates Bim, which releases it from the inhibitory dynein complexes, thus allowing activation of Bax-Bak [83]. Ca^{2+} release from the ER through Bax-Bak channels allows signal transmission from the ER to mitochondria and Bax-dependent apoptosis.

There appears to be a functional connection between ER stress and mitochondrial ROS production. It should be noted that ERO1 is a transcriptional target of the ATF-4/CHOP pro-apoptotic pathway, which stimulates mitochondrial ROS production [70,110]. Mitochondrial ROS production was dependent on levels of ERO1, which is concentrated in MAMs. ERO1 also promotes IP_3R-mediated Ca^{2+} efflux from the ER that is taken up by mitochondria through the MAM [76]. Therefore, ER stress induces oxidative stress and impairs mitochondrial function, which leads to cell death in a CHOP-dependent manner.

The pro-oxidant H_2O_2 activated the UPR in several different cell types. A relatively short exposure (15 min) of HeLa cells to H_2O_2 (15–50 μM) activated the UPR: the expression of the ER stress sensors p-PERK, p-eIF2α and p-IRE1α increased, while cleavage of ATF6 occurred [24]. H_2O_2 (50 to 500 μM, 24 h) caused induction of GRP78/Bip, p-PERK and p-eIF2α in human oral keratinocytes and oral cancer cells [115]. In mesenchymal stem cells, H_2O_2 (120 μM, 6 to 24 h) increased the expression of Bip [116]. On the other hand, longer exposure times

(1 − 3 h) to H_2O_2 induced ER-mediated apoptosis. In HeLa cells, expression of CHOP increased along with cleavage of the calpain inhibitor calpastatin, leading to increased enzymatic activity of the ER proteases calpain, caspase-4, caspase-12 and caspase-7 [24]. The activation of ER caspase-4 and caspase-12 was decreased by the calcium chelator BAPTA-AM, and by inhibitors of calpain and caspase-7, confirming the roles of calcium, calpain and caspase-7 in the activation of ER-mediated apoptosis by H_2O_2. Further downstream in the signalling cascade, caspase-4 and caspase-12 caused activation of caspase-9 and caspase-3, followed by ER-dependent DNA fragmentation and apoptosis [24]. Caspase-4 cleavage and up-regulation of CHOP were induced by peroxide (200 to 500 μM, 24 h) in human oral keratinocytes and oral cancer cells [115]. The exposure of mesenchymal stem cells to H_2O_2 (120 μM) for times ranging from 6 to 24 h caused cleavage of procaspase-12 [116]. In rat hepatocytes, the pro-oxidant tert-butylhydroperoxide (tBOOH) caused calpain-mediated cleavage of procaspase-12 at the ER and its translocation to the nucleus, where increased caspase-12 activity was found [117]. Therefore, the adaptive survival response (UPR) dominated during milder conditions of pro-oxidant stress, whereas ER-mediated apoptosis occurred under more severe conditions [24].

4. Other Regulated Cell Death Pathways

Since the discovery of apoptosis, several other regulated pathways of cell death have been recognised [105]. These include autophagy, necroptosis and anoikis. These modalities will not be described in detail in this review and the reader is referred to recent reviews on these subjects.

4.1. Autophagy

Autophagy (or macroautophagy) is a regulated cellular catabolic process that eliminates damaged or superfluous cytoplasmic contents and organelles (e.g. mitochondria, ER) (see review, [118]). It is a normal process that is involved in important functions such as cell growth, development, aging and immunity [15]. Deregulation of autophagy has been implicated in several human diseases such as cancer, cardiac disease, and neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases [15,105]. Different conditions of stress (e.g. hypoxia, nutrient starvation, hormonal imbalance, oxidative stress, protein aggregates) can enhance autophagy. Autophagy is generally a stress adaptation pathway that promotes cell survival during starvation or acts as a defence mechanism against different environmental stresses. During autophagy, the cytoplasmic contents of a cell are sequestered within double membrane vacuoles (autophagosomes) [118]. The outer membrane of the autophagosome fuses with the membrane of lysosomes to form an autolysosome, in which cytoplasmic contents are degraded by lysosomal enzymes such as cathepsins B, D and L [15]. Under conditions of nutrient deprivation, degradation of cytoplasmic material during autophagy generates amino acids and energy to maintain cell survival [118]. Autophagy is regulated by a full set of autophagy-related genes (*Atg*) and by the mammalian target of rapamycin (mTOR) kinase signalling pathway [15,105,118]. mTOR kinase is the major inhibitory signal that turns off autophagy when sufficient quantities of nutrients and growth factors are available.

Although autophagy is generally recognised as a cell survival process, it can also act as a caspase-independent cell death pathway. In terms of morphology, autophagic cell death is characterised by dying cells containing abundant autophagosomes and the lack of phagocyte participation in cell death [118]. Beyond the morphology, the process by which autophagic cells subsequently die is not clear and has been the subject of controversy. Is this cell death by autophagy or cell death with autophagy? Much less is known about the mechanisms that regulate autophagic cell death and how this may relate to diseases such as cancer [118]. More research is required to clearly delineate how autophagy triggers cell death.

4.2. Necroptosis

Necrosis was originally classified as an accidental form of cell death, where there was explosive release of cellular contents following rupture of the cytoplasmic membrane [14]. Necroptosis eventually emerged as a highly regulated process that can be activated by TNF-α and a pan-caspase inhibitor (z-VAD-FMK) [119]. Similar to necrosis, morphological features of necroptosis include cellular rounding, swelling, cytoplasmic granulation and plasma membrane rupture. This leads to release of cellular contents into the surrounding tissue, thus provoking an inflammatory response [51]. Necroptosis is a regulated pathway of cell death that contributes to certain diseases, notably those associated with inflammation and cancer (see reviews [51,120]). It is thought to be a form of cell death that compensates for the inhibition of apoptosis due to certain cellular contexts or caused by viruses, cancer, etc.

Necroptosis is activated in response to death receptors (e.g. TNF-R1, Fas), Toll-receptors, viruses and DNA damage [51,120]. In the presence of TNF-α, complex 1 consisting of Rip1, TRADD, cIAP1/2 and Traf forms on the cytoplasmic side of TNF-R1 [121]. Rip1 is involved in this signalling pathway and its polyubiquitination by cIAP1/2 leads to activation of the transcription factor NFκB. When caspase-8 is inactivated, deubiquitination of Rip1 leads to its association with Rip3. When Rip1 and Rip3 become phosphorylated, they interact to form the necrosome (complex 2). In the absence of Rip3, Rip1 can mediate apoptosis. Rip3 appears to facilitate a molecular switch from apoptosis to necroptosis.

Mixed lineage kinase domain-like (MLKL) protein and PGAM5L, a mitochondrial protein, both interact with Rip3 and are involved in the necroptosis signalling cascade [121]. MLKL is phosphorylated by Rip3 and acts downstream of Rip3 as an adaptor protein that is essential for necroptosis induction. PGAM5L is a protein phosphatase that is involved in the Rip1 and Rip3 containing necrosome. PGAM5L recruits Drp1 to mitochondria, which expedites mitochondrial fission and necroptotic cell death. Interestingly, PGAM5L is a substrate for Keap1, which is an inhibitor of Nrf2. The transcription factor Nrf2 activates the antioxidant response and thus contributes to detoxification of intracellular ROS [113]. This provides a molecular link between ROS and the necroptotic signalling pathway. In addition, ROS have been implicated in the induction and execution of cell death by necroptosis [122].

The understanding of mechanisms of necroptosis is not very advanced and future mechanistic studies are required. These mechanisms are likely to be dependent on cell type and tissue. Once these mechanisms are more clearly delineated, necroptosis is likely to play an important role in the development of novel cancer treatment strategies. In addition, improved understanding of necroptosis should advance treatments for multiple inflammation-based diseases. There are other emerging forms of regulated necrosis such as pyroptosis and ferroptosis [120], whose mechanisms will be surely uncovered during the coming years.

4.3. Anoikis

Anoikis was first characterised by Frisch and Francis in 1994 [123]. It is a cell death program where a cell dies when it becomes detached from the extracellular matrix (ECM) (see reviews [121,124]). For certain cell types such as epithelial cells, attachment to the ECM is essential for their ability to carry out vital cellular functions such as metabolism, proliferation and survival. Detachment from the ECM can trigger a wide variety of cellular changes. A major role of anoikis is to limit the progression of cancer. For cancer cells to undergo metastasis, they need to inhibit anoikis [121]. Hence, resistance to anoikis facilitates the progression of tumour cells and enables metastasis.

Anoikis is initiated through adhesion molecules that are involved in cell-cell contacts such as E-cadherin and integrins that are involved in

cell-ECM [124]. E-cadherin is a transmembrane protein whose cytoplasmic extremity is bound to β-catenin, which is in turn bound to α-catenin. This complex anchors E-cadherin to the actin cytoskeleton, allowing it to regulate cell adhesion. E-cadherin appears to regulate anoikis through the RAF/ERK and PI3K/Akt pathways. The ligated conformation of integrins can activate signalling pathways that promote cell survival such as the PI3K/Akt pathway, whereas their unligated conformation can stimulate apoptosis mediated by caspase-8.

ECM-detached cells have several metabolic defects including increased levels of ATP and ROS, which can lead to non-apoptotic cell death (see review [121]). These regulated death pathways include entosis, autophagy and necroptosis. Entotic cells are internalised by host cells through activation of Rho and ROCK, before undergoing non-autophagic degradation involving lysosomes. Some entotic cells can exit the host and return to their normal functions. ECM-detached cells can undergo autophagy to prolong cell survival until nutrient rescue mechanisms are activated. On the other hand, autophagy can be activated as a death mechanism in cells under duress due to ECM-detachment. The loss of β1 integrin engagement can promote autophagy. Necroptosis can be linked to ECM-detachment through its regulation of ROS, mediated by Rip3. Anoikis can also lead to cell death mediated by both intrinsic and extrinsic pathways of apoptosis [124]. The intrinsic pathway is mediated by MOMP and caspase-9 activation whereas the extrinsic pathway involves the TNF-R death receptor and caspase-8 activation. Further advances are required to fully understand the mechanisms involved in the molecular regulation of anoikis as well as the different modes by which these cells undergo cell death.

5. Concluding Remarks

Three different types of programmed cell death have been identified: apoptosis, autophagy and necroptosis. An important concept is that these cell death pathways each act as backup in the event that the other pathways are inhibited. These cell death pathways modulate each other by mutual inhibitory processes and are controlled by multiple feedback loops [51]. During recent years, our understanding of the mechanisms involved in regulation of the signalling pathways of apoptosis has evolved significantly. However, many important questions remain unanswered. These include further clarification of the molecular links that trigger the transition between cell survival and cell death for each of the death receptor and ER signalling pathways. In addition, apoptosis through the ER pathway appears to involve several distinct signalling mechanisms whose respective roles require clarification. Moreover, the complex interplay between apoptosis and other signalling pathways involved in cell survival and cell death such as autophagy and necroptosis requires further mechanistic insights. Autophagy has been mainly characterised as a cell survival phenomenon. However, it appears to play a role in cell death by mechanisms that have not been entirely clarified. Further confounding factors are the influence of different cell types and the responses to different environmental factors. The recent interest in the involvement of miRNAs in cell survival and death signalling pathways is likely to open up a multitude of new avenues of research in this field.

ROS play a central role in cell signalling and in the regulation of the main pathways of apoptosis mediated by mitochondria, death receptors and the ER. ROS are also involved in other regulated pathways of cell survival and cell death such as autophagy and necroptosis. In particular, the role of ROS in the complex interplay and crosstalk between these different signalling pathways remains to be further unravelled during the coming years. The deregulation of these different cell survival and cell death pathways is likely to have important pathological consequences for oxidative stress-associated diseases such as cancer, neurodegenerative diseases, ischemia-reperfusion injury and diabetes. Advances in our understanding of how different stress sensors such as ROS enable a switch from survival to death should lead to new strategies to prevent or treat some of these diseases.

Abbreviations

AIF	Apoptosis inducing factor
ALS	Amyotrophic lateral sclerosis
AMPK	AMP-activated protein kinase
Apaf-1	Apoptotic peptidase activating factor 1
ASK-1	Apoptosis signalling kinase 1
ATF-6α	Activating transcription factor-6α
Atg	Autophagy-related gene
ATM	Ataxia-telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3 related
Baf	Bafilomycin A1
Bcl-2	B-cell-lymphoma protein 2
BH	Bcl-2 homology
BiP	Binding immunoglobulin protein
Ca^{2+}	Calcium (II)
Chk1	Checkpoint kinase 1
CHOP	CCAAT-enhancer-binding protein homologous protein
Daxx	Death-associated protein 6
DD	Death domain
DED	Death-effector domain
DISC	Death inducing signalling complex
DR	Death receptor
DRP	Dynamin-related protein 1
ECM	Extracellular matrix
eIF2α	Eukaryotic initiation factor 2α
Endo G	Endonuclease G
ER	Endoplasmic reticulum
ERAD	ER-associated protein degradation
ERK	Extracellular regulated kinase
ERO1	Endoplasmic reticulum oxidoreductin-1
ERp57	ER protein 57
FAD	Flavin adenine dinucleotide
FADD	Fas-associated death domain
FasL	Fas ligand
Fis1	Fission 1 homolog
FLIP	FLICE inhibitory protein
GADD153	Growth arrest and DNA damage-inducible gene 153
GCL	Glutamate cysteine ligase
GRP94	Glucose-regulated protein 94
GSH	Glutathione
H_2O_2	Hydrogen peroxide
$OH^{\bullet}$	Hydroxyl radical
HSP	Heat shock protein
IAP	Inhibitor of apoptosis protein
ICAD	Inhibitor of caspase activated DNase
IL	Interleukin
IMM	Inner mitochondrial membrane
IP_3R	Inositol triphosphate receptor
IRE-1α	Serine/threonine-protein kinase/endoribonuclease-1α
JNK	c-Jun N-terminal kinase
Keap1	Kelch like-ECH-associated protein 1
LDH	Lactate dehydrogenase
3-MA	3-Methyladenine
MAM	Mitochondria-associated membranes
MAPK	Mitogen-activated protein kinase
Mdm2	Mouse double minute 2 homolog
MLKL	Mixed lineage kinase domain-like
MOMP	Mitochondrial outer membrane permeability
MPT	Mitochondrial permeability transition
MPTP	Membrane permeability transition pore
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
NF-κB	Nuclear factor kappa-B
NLS	Nuclear localisation signal
Nox	NADPH oxidase

Nrf2 Nuclear factor (erythroid-derived 2)-like 2
$O_2^{\cdot-}$ Superoxide anion
OMM Outer mitochondrial membrane
PAK2 p21-activated kinase 2
PARP Poly-(ADP-ribose) polymerase
PERK Protein kinase RNA-like ER kinase
PI3K Phosphatidylinositol 3-kinase
PIDD p53-inducible death domain-containing protein
PKC Protein kinase C
RAIDD RIP-associated ICH-1 homologous protein with a death domain
RIDD Regulated IRE-dependent decay
RIP Receptor-interacting protein
RNS Reactive nitrogen species
ROCK1 or 2 Rho-associated kinase 1 or 2
ROMO1 ROS modulator-1
ROS Reactive oxygen species
Smac/Diablo Second mitochondria-derived activator of caspases/direct inhibitor of apoptosis (IAP)-binding protein with low pI
t-Bid truncated Bid
TNF-R Tumour necrosis factor receptor
TNFRSF25 TNF receptor superfamily 25
TRADD TNF-R-associated adaptor protein with death domain
Traf-2 TNF-associated factor-2
TRAIL TNF-related apoptosis-inducing ligand
TSC2 Tuberous sclerosis complex 2
UPR Unfolded protein response
XIAP X-linked inhibitor of apoptosis
XBP1 X-Box binding protein

Transparency Document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

The authors wish to thank the Natural Sciences and Engineering Council of Canada (#36725-11) for financial support (DAB), the J.A. DeSève Foundation for graduate scholarship support (MRD), Olivier Mornet for preparation of the figures, and Dr. Mélanie Grondin for assistance with text revisions.

References

[1] B. Halliwell, Free radicals and antioxidants - quo vadis? Trends Pharmacol. Sci. 32 (2011) 125–130.
[2] C.C. Winterbourn, Are free radicals involved in thiol-based redox signaling? Free Radic. Biol. Med. 80 (2015) 164–170.
[3] L. Covarrubias, D. Hernandez-Garcia, D. Schnabel, E. Salas-Vidal, S. Castro-Obregon, Function of reactive oxygen species during animal development: passive or active? Dev. Biol. 320 (2008) 1–11.
[4] J. Zhang, X. Wang, V. Vikash, Q. Ye, D. Wu, Y. Liu, W. Dong, ROS and ROS-mediated cellular signaling, Oxidative Med. Cell. Longev. 2016 (2016) 4350965.
[5] Y.S. Bae, H. Oh, S.G. Rhee, Y.D. Yoo, Regulation of reactive oxygen species generation in cell signaling, Mol. Cells 32 (2011) 491–509.
[6] V.O. Kaminskyy, B. Zhivotovsky, Free radicals in cross talk between autophagy and apoptosis, Antioxid. Redox Signal. 21 (2014) 86–102.
[7] K. Brieger, S. Schiavone, F.J. Miller Jr., K.H. Krause, Reactive oxygen species: from health to disease, Swiss Med. Wkly. 142 (2012) 13659–13664.
[8] J.P. Kehrer, L.O. Klotz, Free radicals and related reactive species as mediators of tissue injury and disease: implications for health, Crit. Rev. Toxicol. 45 (2015) 765–798.
[9] P. Ghezzi, V. Jaquet, F. Marcucci, H.H. Schmidt, The oxidative stress theory of disease: levels of evidence and epistemological aspects, Br. J. Pharmacol. (2016).
[10] B.C. Dickinson, C.J. Chang, Chemistry and biology of reactive oxygen species in signaling or stress responses, Nat. Chem. Biol. 7 (2011) 504–511.
[11] B. Halliwell, Free radicals and antioxidants: updating a personal view, Nutr. Rev. 70 (2012) 257–265.
[12] J.D. West, L.J. Marnett, Endogenous reactive intermediates as modulators of cell signaling and cell death, Chem. Res. Toxicol. 19 (2006) 173–194.
[13] R. Franco, R. Sanchez-Olea, E.M. Reyes-Reyes, M.I. Panayiotidis, Environmental toxicity, oxidative stress and apoptosis: menage a trois, Mutat. Res. 674 (2009) 3–22.
[14] S. Orrenius, P. Nicotera, B. Zhivotovsky, Cell death mechanisms and their implications in toxicology, Toxicol. Sci. 119 (2011) 3–19.
[15] P. Pallepati, D.A. Averill-Bates, Reactive oxygen species, cell death signaling and apoptosis, in: K. Pantopoulos, H. Schipper (Eds.), Princ Free Rad Biomed, Nova Science Publishers, Inc., Place Published 2012, pp. 513–546.
[16] S.B. Gibson, Investigating the role of reactive oxygen species in regulating autophagy, Methods Enzymol. 528 (2013) 217–235.
[17] J.F. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[18] S. Fulda, A.M. Gorman, O. Hori, A. Samali, Cellular stress responses: cell survival and cell death, Int. J. Cell Biol. 10 (2010) 1–23.
[19] D.A. Flusberg, P.K. Sorger, Surviving apoptosis: life-death signaling in single cells, Trends Cell Biol. 25 (2015) 446–458.
[20] S. Shalini, L. Dorstyn, S. Dawar, S. Kumar, Old, new and emerging functions of caspases, Cell Death Differ. 22 (2015) 526–539.
[21] H. Vakifahmetoglu-Norberg, E. Norberg, A.B. Perdomo, M. Olsson, F. Ciccosanti, S. Orrenius, G.M. Fimia, M. Piacentini, B. Zhivotovsky, Caspase-2 promotes cytoskeleton protein degradation during apoptotic cell death, Cell Death Dis. 4 (2013), e94.
[22] L.L. Fava, F.J. Bock, S. Geley, A. Villunger, Caspase-2 at a glance, J. Cell Sci. 125 (2012) 5911–5915.
[23] S. Garcia de la Cadena, L. Massieu, Caspases and their role in inflammation and ischemic neuronal death. Focus on caspase-12, Apoptosis 21 (2016) 763–777.
[24] P. Pallepati, D.A. Averill-Bates, Activation of ER stress and apoptosis by hydrogen peroxide in HeLa cells: protective role of mild heat preconditioning at 40 degrees C, Biochim. Biophys. Acta 1813 (2011) 1987–1999.
[25] A. Yamamuro, T. Kishino, Y. Ohshima, Y. Yoshioka, T. Kimura, A. Kasai, S. Maeda, Caspase-4 directly activates caspase-9 in endoplasmic reticulum stress-induced apoptosis in SH-SY5Y cells, J. Pharmacol. Sci. 115 (2011) 239–243.
[26] M. Leist, M. Jaattela, Four deaths and a funeral: from caspases to alternative mechanisms, Nat. Rev. Mol. Cell Biol. 2 (2001) 589–598.
[27] M. Olsson, B. Zhivotovsky, Caspases and cancer, Cell Death Differ. 18 (2011) 1441–1449.
[28] E.D. Crawford, J.A. Wells, Caspase substrates and cellular remodeling, Annu. Rev. Biochem. 80 (2011) 1055–1087.
[29] J.E. Ricci, C. Munoz-Pinedo, P. Fitzgerald, B. Bailly-Maitre, G.A. Perkins, N. Yadava, I.E. Scheffler, M.H. Ellisman, D.R. Green, Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain, Cell 117 (2004) 773–786.
[30] D.R. Green, F. Llambi, Cell death signaling, Cold Spring Harb. Perspect. Biol. 7 (2015) a006080.
[31] N. Kayagaki, S. Warming, M. Lamkanfi, L. Vande Walle, S. Louie, J. Dong, K. Newton, Y. Qu, J. Liu, S. Heldens, J. Zhang, W.P. Lee, M. Roose-Girma, V.M. Dixit, Non-canonical inflammasome activation targets caspase-11, Nature 479 (2011) 117–121.
[32] K.F. Ferri, G. Kroemer, Organelle-specific initiation of cell death pathways, Nat. Cell Biol. 3 (2001) 255–263.
[33] Z. Jin, W.S. El-Deiry, Overview of cell death signaling pathways, Cancer Biol. Ther. 4 (2005) 139–163.
[34] R.J. Youle, A.M. van der Bliek, Mitochondrial fission, fusion, and stress, Science 337 (2012) 1062–1065.
[35] T. Landes, J.C. Martinou, Mitochondrial outer membrane permeabilization during apoptosis: the role of mitochondrial fission, Biochim. Biophys. Acta 1813 (2011) 540–545.
[36] S. Orrenius, V. Gogvadze, B. Zhivotovsky, Calcium and mitochondria in the regulation of cell death, Biochem. Biophys. Res. Commun. 460 (2015) 72–81.
[37] P. Bernardi, The mitochondrial permeability transition pore: a mystery solved? Front. Physiol. 4 (2013) 3377–3389.
[38] A.P. Halestrap, What is the mitochondrial permeability transition pore? J. Mol. Cell. Cardiol. 46 (2009) 821–831.
[39] S. Grimm, The ER-mitochondria interface: the social network of cell death, Biochim. Biophys. Acta 1823 (2012) 327–334.
[40] M.P. Luna-Vargas, J.E. Chipuk, Physiological and pharmacological control of BAK, BAX, and beyond, Trends Cell Biol. (2016).
[41] B. Leibowitz, J. Yu, Mitochondrial signaling in cell death via the Bcl-2 family, Cancer Biol. Ther. 9 (2010) 417–422.
[42] T. Kaufmann, A. Strasser, P.J. Jost, Fas death receptor signalling: roles of bid and XIAP, Cell Death Differ. 19 (2012) 42–50.
[43] A. Glory, A. Bettaieb, D.A. Averill-Bates, Mild thermotolerance induced at 40 degrees C protects cells against hyperthermia-induced pro-apoptotic changes in Bcl-2 family proteins, Int. J. Hyperth. 30 (2014) 502–512.
[44] S.W. Tait, D.R. Green, Mitochondria and cell death: outer membrane permeabilization and beyond, Nat. Rev. Mol. Cell Biol. 11 (2010) 621–632.
[45] M.P. Luna-Vargas, J.E. Chipuk, The deadly landscape of pro-apoptotic BCL-2 proteins in the outer mitochondrial membrane, FEBS J. 283 (2015) 2676–2689.
[46] L. Happo, A. Strasser, S. Cory, BH3-only proteins in apoptosis at a glance, J. Cell Sci. 125 (2012) 1081–1087.
[47] L.N. Zhang, J.Y. Li, W. Xu, A review of the role of Puma, Noxa and Bim in the tumorigenesis, therapy and drug resistance of chronic lymphocytic leukemia, Cancer Gene Ther. 20 (2013) 1–7.
[48] J.C. Martinou, R.J. Youle, Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics, Dev. Cell 21 (2011) 92–101.
[49] M. Bonora, A. Bononi, E. De Marchi, C. Giorgi, M. Lebiedzinska, S. Marchi, S. Patergnani, A. Rimessi, J.M. Suski, A. Wojtala, M.R. Wieckowski, G. Kroemer, L. Galluzzi, P. Pinton, Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition, Cell Cycle 12 (2013) 674–683.

[50] H.G. Wang, N. Pathan, I.M. Ethell, S. Krajewski, Y. Yamaguchi, F. Shibasaki, F. McKeon, T. Bobo, T.F. Franke, J.C. Reed, Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD, Science 284 (1999) 339–343.
[51] T. Vanden Berghe, W.J. Kaiser, M.J. Bertrand, P. Vandenabeele, Molecular crosstalk between apoptosis, necroptosis, and survival signaling, Mol. Cell. Oncol. 2 (2015), e975093.
[52] Z. Mahmood, Y. Shukla, Death receptors: targets for cancer therapy, Exp. Cell Res. 316 (2010) 887–899.
[53] C. Kantari, H. Walczak, Caspase-8 and bid: caught in the act between death receptors and mitochondria, Biochim. Biophys. Acta 1813 (2011) 558–563.
[54] P.A. Holoch, T.S. Griffith, TNF-related apoptosis-inducing ligand (TRAIL): a new path to anti-cancer therapies, Eur. J. Pharmacol. 625 (2009) 63–72.
[55] B. Pennarun, A. Meijer, E.G. de Vries, J.H. Kleibeuker, F. Kruyt, S. de Jong, Playing the DISC: turning on TRAIL death receptor-mediated apoptosis in cancer, Biochim. Biophys. Acta 1805 (2010) 123–140.
[56] M.L. Circu, T.Y. Aw, Glutathione and modulation of cell apoptosis, Biochim. Biophys. Acta 1823 (2012) 1767–1777.
[57] H. Blaser, C. Dostert, T.W. Mak, D. Brenner, TNF and ROS crosstalk in inflammation, Trends Cell Biol. 26 (2016) 249–261.
[58] M. Russo, A. Mupo, C. Spagnuolo, G.L. Russo, Exploring death receptor pathways as selective targets in cancer therapy, Biochem. Pharmacol. 80 (2010) 674–682.
[59] O. Micheau, S. Lens, O. Gaide, K. Alevizopoulos, J. Tschopp, NF-kappaB signals induce the expression of c-FLIP, Mol. Cell. Biol. 21 (2001) 5299–5305.
[60] J. Tschopp, M. Irmler, M. Thome, Inhibition of fas death signals by FLIPs, Curr. Opin. Immunol. 10 (1998) 552–558.
[61] A. Tinel, J. Tschopp, The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress, Science 304 (2004) 843–846.
[62] H. Vakifahmetoglu-Norberg, B. Zhivotovsky, The unpredictable caspase-2: what can it do? Trends Cell Biol. 20 (2010) 150–159.
[63] L. Bouchier-Hayes, D.R. Green, Caspase-2: the orphan caspase, Cell Death Differ. 19 (2012) 51–57.
[64] L. Dorstyn, J. Puccini, C.H. Wilson, S. Shalini, M. Nicola, S. Moore, S. Kumar, Caspase-2 deficiency promotes aberrant DNA-damage response and genetic instability, Cell Death Differ. 19 (2012) 1288–1298.
[65] P. Pallepati, D.A. Averill-Bates, Mild thermotolerance induced at 40 degrees C protects HeLa cells against activation of death receptor-mediated apoptosis by hydrogen peroxide, Free Radic. Biol. Med. 50 (2011) 667–679.
[66] M. Olsson, H. Vakifahmetoglu, P.M. Abruzzo, K. Hogstrand, A. Grandien, B. Zhivotovsky, DISC-mediated activation of caspase-2 in DNA damage-induced apoptosis, Oncogene 28 (2009) 1949–1959.
[67] G. Paroni, C. Henderson, C. Schneider, C. Brancolini, Caspase-2-induced apoptosis is dependent on caspase-9, but its processing during UV- or tumor necrosis factor-dependent cell death requires caspase-3, J. Biol. Chem. 276 (2001) 21907–21915.
[68] S. Sidi, T. Sanda, R.D. Kennedy, A.T. Hagen, C.A. Jette, R. Hoffmans, J. Pascual, S. Imamura, S. Kishi, J.F. Amatruda, J.P. Kanki, D.R. Green, A.A. D'Andrea, A.T. Look, Chk1 suppresses a caspase-2 apoptotic response to DNA damage that bypasses p53, Bcl-2, and caspase-3, Cell 133 (2008) 864–877.
[69] T.G. Oliver, E. Meylan, G.P. Chang, W. Xue, J.R. Burke, T.J. Humpton, D. Hubbard, A. Bhutkar, T. Jacks, Caspase-2-mediated cleavage of Mdm2 creates a p53-induced positive feedback loop, Mol. Cell 43 (2011) 57–71.
[70] M. Wang, R.J. Kaufman, Protein misfolding in the endoplasmic reticulum as a conduit to human disease, Nature 529 (2016) 326–335.
[71] G.C. Shore, F.R. Papa, S.A. Oakes, Signaling cell death from the endoplasmic reticulum stress response, Curr. Opin. Cell Biol. 23 (2011) 143–149.
[72] V.I. Rasheva, P.M. Domingos, Cellular responses to endoplasmic reticulum stress and apoptosis, Apoptosis 14 (2009) 996–1007.
[73] P. Walter, D. Ron, The unfolded protein response: from stress pathway to homeostatic regulation, Science 334 (2011) 1081–1086.
[74] I. Tabas, D. Ron, Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress, Nat. Cell Biol. 13 (2011) 184–190.
[75] B.M. Gardner, P. Walter, Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response, Science 333 (2011) 1891–1894.
[76] R. Sano, J.C. Reed, ER stress-induced cell death mechanisms, Biochim. Biophys. Acta 1833 (2013) 3460–3470.
[77] S. Zinkel, A. Gross, E. Yang, BCL2 family in DNA damage and cell cycle control, Cell Death Differ. 13 (2006) 1351–1359.
[78] A. Gross, BCL-2 family proteins as regulators of mitochondria metabolism, Biochim. Biophys. Acta 1857 (2016) 1243–1246.
[79] S.S. Lin, M.C. Bassik, H. Suh, M. Nishino, J.D. Arroyo, W.C. Hahn, S.J. Korsmeyer, T.M. Roberts, PP2A regulates BCL-2 phosphorylation and proteasome-mediated degradation at the endoplasmic reticulum, J. Biol. Chem. 281 (2006) 23003–23012.
[80] T. Vervliet, J.B. Parys, G. Bultynck, Bcl-2 proteins and calcium signaling: complexity beneath the surface, Oncogene (2016).
[81] D.G. Breckenridge, M. Germain, J.P. Mathai, M. Nguyen, G.C. Shore, Regulation of apoptosis by endoplasmic reticulum pathways, Oncogene 22 (2003) 8608–8618.
[82] S. Luo, D.C. Rubinsztein, BCL2L11/BIM: a novel molecular link between autophagy and apoptosis, Autophagy 9 (2013) 104–105.
[83] G.V. Putcha, S. Le, S. Frank, C.G. Besirli, K. Clark, B. Chu, S. Alix, R.J. Youle, A. LaMarche, A.C. Maroney, E.M. Johnson Jr., JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis, Neuron 38 (2003) 899–914.
[84] T. Nakagawa, J. Yuan, Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis, J. Cell Biol. 150 (2000) 887–894.
[85] N. Morishima, K. Nakanishi, K. Tsuchiya, T. Shibata, E. Seiwa, Translocation of Bim to the endoplasmic reticulum (ER) mediates ER stress signaling for activation of caspase-12 during ER stress-induced apoptosis, J. Biol. Chem. 279 (2004) 50375–50381.
[86] H. Fischer, U. Koenig, L. Eckhart, E. Tschachler, Human caspase 12 has acquired deleterious mutations, Biochem. Biophys. Res. Commun. 293 (2002) 722–726.
[87] R. Iwasawa, A.L. Mahul-Mellier, C. Datler, E. Pazarentzos, S. Grimm, Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction, EMBO J. 30 (2011) 556–568.
[88] C. Sheridan, S.J. Martin, Mitochondrial fission/fusion dynamics and apoptosis, Mitochondrion 10 (2010) 640–648.
[89] K. Yoshida, Y. Miki, The cell death machinery governed by the p53 tumor suppressor in response to DNA damage, Cancer Sci. 101 (2010) 831–835.
[90] N. Dashzeveg, K. Yoshida, Cell death decision by p53 via control of the mitochondrial membrane, Cancer Lett. 367 (2015) 108–112.
[91] K. Datta, P. Babbar, T. Srivastava, S. Sinha, P. Chattopadhyay, p53 dependent apoptosis in glioma cell lines in response to hydrogen peroxide induced oxidative stress, Int. J. Biochem. Cell Biol. 34 (2002) 148–157.
[92] P. Pallepati, D. Averill-Bates, Mild thermotolerance induced at 40 degrees C increases antioxidants and protects HeLa cells against mitochondrial apoptosis induced by hydrogen peroxide: role of p53, Arch. Biochem. Biophys. 495 (2010) 97–111.
[93] C. McNeill-Blue, B.A. Wetmore, J.F. Sanchez, W.J. Freed, B.A. Merrick, Apoptosis mediated by p53 in rat neural AF5 cells following treatment with hydrogen peroxide and staurosporine, Brain Res. 1112 (2006) 1–15.
[94] J. Yu, L. Zhang, PUMA, a potent killer with or without p53, Oncogene 27 (Suppl. 1) (2008) 71–83.
[95] M.L. Circu, T.Y. Aw, Reactive oxygen species, cellular redox systems, and apoptosis, Free Radic. Biol. Med. 48 (2010) 749–762.
[96] J. Chandra, A. Samali, S. Orrenius, Triggering and modulation of apoptosis by oxidative stress, Free Radic. Biol. Med. 29 (2000) 323–333.
[97] M. Madesh, G. Hajnoczky, VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome *c* release, J. Cell Biol. 155 (2001) 1003–1015.
[98] P. Pizzo, T. Pozzan, Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics, Trends Cell Biol. 17 (2007) 511–517.
[99] P. Vandenabeele, L. Galluzzi, T. Vanden Berghe, G. Kroemer, Molecular mechanisms of necroptosis: an ordered cellular explosion, Nat. Rev. Mol. Cell Biol. 11 (2010) 700–714.
[100] M.J. Morgan, Z.G. Liu, Crosstalk of reactive oxygen species and NF-kappaB signaling, Cell Res. 21 (2011) 103–115.
[101] T.L. Denning, H. Takaishi, S.E. Crowe, I. Boldogh, A. Jevnikar, P.B. Ernst, Oxidative stress induces the expression of Fas and Fas ligand and apoptosis in murine intestinal epithelial cells, Free Radic. Biol. Med. 33 (2002) 1641–1650.
[102] T. Suhara, K. Fukuo, T. Sugimoto, S. Morimoto, T. Nakahashi, S. Hata, M. Shimizu, T. Ogihara, Hydrogen peroxide induces up-regulation of Fas in human endothelial cells, J. Immunol. 160 (1998) 4042–4047.
[103] S. Zhuang, J.T. Demirs, I.E. Kochevar, p38 mitogen-activated protein kinase mediates bid cleavage, mitochondrial dysfunction, and caspase-3 activation during apoptosis induced by singlet oxygen but not by hydrogen peroxide, J. Biol. Chem. 275 (2000) 25939–25948.
[104] P. Salomoni, A.F. Khelifi, Daxx: death or survival protein? Trends Cell Biol. 16 (2006) 97–104.
[105] L. Portt, G. Norman, C. Clapp, M. Greenwood, M.T. Greenwood, Anti-apoptosis and cell survival: a review, Biochim. Biophys. Acta 1813 (2011) 238–259.
[106] G. Mellier, S. Pervaiz, The three Rs along the TRAIL: resistance, re-sensitization and reactive oxygen species (ROS), Free Radic. Res. 46 (2012) 996–1003.
[107] A.Y. Zhang, F. Yi, S. Jin, M. Xia, Q.Z. Chen, E. Gulbins, P.L. Li, Acid sphingomyelinase and its redox amplification in formation of lipid raft redox signaling platforms in endothelial cells, Antioxid. Redox Signal. 9 (2007) 817–828.
[108] D. Van der Vlies, M. Makkinje, A. Jansens, I. Braakman, A.J. Verkleij, K.W. Wirtz, J.A. Post, Oxidation of ER resident proteins upon oxidative stress: effects of altering cellular redox/antioxidant status and implications for protein maturation, Antioxid. Redox Signal. 5 (2003) 381–387.
[109] H.M. Zeeshan, G.H. Lee, H.R. Kim, H.J. Chae, Endoplasmic reticulum stress and associated ROS, Int. J. Mol. Sci. 17 (2016) 327–347.
[110] A. Delaunay-Moisan, C. Appenzeller-Herzog, The antioxidant machinery of the endoplasmic reticulum: protection and signaling, Free Radic. Biol. Med. 83 (2015) 341–351.
[111] B.P. Tu, J.S. Weissman, Oxidative protein folding in eukaryotes: mechanisms and consequences, J. Cell Biol. 164 (2004) 341–346.
[112] J.D. Malhotra, H. Miao, K. Zhang, A. Wolfson, S. Pennathur, S.W. Pipe, R.J. Kaufman, Antioxidants reduce endoplasmic reticulum stress and improve protein secretion, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 18525–18530.
[113] S.B. Cullinan, J.A. Diehl, PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress, J. Biol. Chem. 279 (2004) 20108–20117.
[114] H. Sade, A. Sarin, Reactive oxygen species regulate quiescent T-cell apoptosis via the BH3-only proapoptotic protein BIM, Cell Death Differ. 11 (2004) 416–423.
[115] S.K. Min, S.K. Lee, J.S. Park, J. Lee, J.Y. Paeng, S.I. Lee, H.J. Lee, Y. Kim, H.O. Pae, S.K. Lee, E.C. Kim, Endoplasmic reticulum stress is involved in hydrogen peroxide induced apoptosis in immortalized and malignant human oral keratinocytes, J. Oral Pathol. Med. 37 (2008) 490–498.
[116] H. Wei, Z. Li, S. Hu, X. Chen, X. Cong, Apoptosis of mesenchymal stem cells induced by hydrogen peroxide concerns both endoplasmic reticulum stress and mitochondrial death pathway through regulation of caspases, p38 and JNK, J. Cell. Biochem. 111 (2010) 967–978.
[117] K. Haidara, M. Marion, M. Gascon-Barre, F. Denizeau, D.A. Averill-Bates, Implication of caspases and subcellular compartments in tert-butylhydroperoxide induced apoptosis, Toxicol. Appl. Pharmacol. 229 (2008) 65–76.
[118] L. Lin, E.H. Baehrecke, Autophagy, cell death, and cancer, Mol. Cell. Oncol. 2 (2015), e985913.
[119] T. Hirsch, P. Marchetti, S.A. Susin, B. Dallaporta, N. Zamzami, I. Marzo, M. Geuskens, G. Kroemer, The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death, Oncogene 15 (1997) 1573–1581.
[120] B. Hanson, Necroptosis: a new way of dying? Cancer Biol. Ther. (2016) 1–12.

[121] C.L. Buchheit, R.R. Rayavarapu, Z.T. Schafer, The regulation of cancer cell death and metabolism by extracellular matrix attachment, Semin. Cell Dev. Biol. 23 (2012) 402–411.
[122] W. Wu, P. Liu, J. Li, Necroptosis: an emerging form of programmed cell death, Crit. Rev. Oncol. Hematol. 82 (2012) 249–258.
[123] S.M. Frisch, H. Francis, Disruption of epithelial cell-matrix interactions induces apoptosis, J. Cell Biol. 124 (1994) 619–626.
[124] S. Malagobadan, N.H. Nagoor, Evaluation of MicroRNAs regulating anoikis pathways and its therapeutic potential, Biomed. Res. Int. 2015 (2015) 716816.