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Redox Regulation of Apoptosis before and after Cytochrome C Release

Quan Chen[1]*, Meredith Crosby[2] and Alex Almasan[3]**

Department of Cancer Biology, Lerner Research Institute and [3]Department of Radiation, Oncology, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA;
[1]The National Key Laboratory of Biomembrane and Membrane Biotechnology, Chinese Academy of Sciences, Beijing 100080, People's Republic of China;
[2]Department of Environmental Health Sciences, Case Western Reserve University, School of Medicine, Cleveland, OH, 44106, USA

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Programmed cell death, or apoptosis, is one of the most studied areas of modern biology. Apoptosis is a genetically regulated process, which plays an essential role in the development and homeostasis of higher organisms. Mitochondria, known to play a central role in regulating cellular metabolism, was found to be critical for regulating apoptosis induced under both physiological and pathological conditions. Mitochondria are a major source of reactive oxygen species (ROS) but they can also serve as its target during the apoptosis process. Release of apoptogenic factors from mitochondria, the best known of which is cytochrome c, leads to assembly of a large apoptosis-inducing complex called the apoptosome. Cysteine proteases (called caspases) are recruited to this complex and, following their activation by proteolytic cleavage, activate other caspases, which in turn target for specific cleavage a large number of cellular proteins. The redox regulation of apoptosis during and after cytochrome c release is an area of intense investigation. This review summarizes what is known about the biological role of ROS and its targets in apoptosis with an emphasis on its intricate connections to mitochondria and the basic components of cell death.

Programmed cell death (or apoptosis) is a genetically regulated process, which plays an essential role in the development and homeostasis of higher organisms. Abnormal apoptosis is one of the primary causes of various diseases such as cancer, degenerative diseases, and autoimmune diseases (Bauer et al., 1995; Fadeel et al., 1999b; Martin and Green, 1994; Orrenius, 1995; Reec, 1999; Tatton and Chalmers-Redman, 1998; Thompson, 1995). Cell death may be triggered by a wide variety of death signals. These consist of death ligands and their interaction with their receptors, perturbations of redox and energy metabolism, ceramide generation, Ca^{2+} mobilization, or activation of Bcl-2 family proteins (Ashkenazi, 2002; Chen et al., 2001; Cory and Adams, 2002 Kroemer et al., 1997; Macho et al., 1997; Vander Heiden and Thompson, 1999). Mitochondria function as sentinels that receive these cell death signals and commit cells to apoptosis by releasing death factors into the cytosol, such as cytochrome c (cyt c) (Liu et al., 1996), Smac/DIABALO (Du et al., 2000; Wu et al., 2000) AIF (Joza et al., 2001), or Endonuclease G (Li et al., 2001; Swerdlow et al., 1996). The best-characterized factor is cyt c, which plays a dual role in triggering the apoptotic process, and in maintaining mitochondrial oxidative phosphorylation. Cyt c is a soluble protein located outside of the inner mitochondrial membrane. Under normal physiological conditions, it functions as an electron carrier in the respiratory chain between complexes III and IV. Once released into the cytosol during apoptosis, cyt c binds to Apaf-1 (a human homologue of the *C. elegans* Ced-4), thus forming a complex called the apoptosome, which recruits and activates pro-caspase-9 (Li et al., 1997; Liu et al., 1996). Thereafter, activated caspase-9 initiates the activation of downstream caspases, which cleave cellular substrates at specific tetra-peptide sequences on the carboxyl termini of aspartate residues.

Cyt c release from mitochondria was thought to be an irreversible step for the apoptotic process. The release could be the result of outer mitochondrial membrane breakdown or an opening of the permeability transition

To whom correspondence should be addressed.
*Tel: 86-10-6252-9232, Fax: +6256-5689
E-mail: chenq@panda.ioz.ac.cn
**Tel: 216-444-9970, Fax: 216-445-6292
E-mail: almasaa@ccf.org

pore (PTP). Bax/Bak oligomerization (Antonsson et al., 2000; Hsu and Youle, 1997; Wei et al., 2000; Wei et al., 2001; Wolter et al., 1997) or the Ca^{2+} sensitive voltage-dependent anion channel (VDAC) (Shimizu et al., 1999; Tsujimoto and Shimizu, 2002) were proposed to form a megachannel which mediates cyt c release. However, the molecular mechanism of cyt c release from the mitochondria into the cytosol remains unclear. Emerging evidence suggests that the apoptotic execution process is highly regulated even after cyt c is released. While there are a large number of review articles in the literature, which summarize the fast-advancing field of apoptosis (Cory and Adams, 2002; Green and Evan, 2002; Harris and Thompson, 2000; Vander Heiden and Thompson, 1999; Wang, 2001), we will mainly focus on the redox regulation of apoptosis during and after cyt c release.

Mitochondria and Free Radical Generation During Apoptosis

Reactive oxygen species (ROS) are generated during normal processes of mitochondrial oxidative phosphorylation. Under physiological conditions, electrons carried by the electron transport chain can leak out of the pathway and pass directly to oxygen, generating superoxide anion, $O_2^{\cdot-}$. Approximately 0.4-4% of the oxygen consumed within the cell is reduced incompletely to generate $O_2^{\cdot-}$ (Boveris and Chance, 1973). Other sources of $O_2^{\cdot-}$ include enzymes such as cytochrome P450 in the endoplasmic reticulum (ER), lipoxygenases, cyclooxgenases, xanthine oxidase and NADPH oxidase in the cytosol. The dismutation of $O_2^{\cdot-}$ by superoxide dismutase (SOD) results in the generation of H_2O_2. Under normal physiological conditions, hydrogen peroxide (H_2O_2) is converted into H_2O by mitochondrial glutathione (GSH) peroxidase at the expense of GSH. However, this reaction is dependent upon the ratio of GSH:GSSG(oxidized form). If there is not enough reduced substrate (2 GSH→GSSG) available, H_2O_2 can react with Fe^{2+} to form hydroxyl radicals (OH) via the Fenton reaction. Additionally, OH can also be generated via the metal catalyzed Haber-Weiss reaction (Cadenas and Davies, 2000; Carmody and Cotter, 2001; Slater et al., 1995).

It has been suggested that mitochondrial ROS play a critical role in cellular cytotoxicity in TNF, Fas, p53, myc, and virus-induced apoptosis (Carmody and Cotter, 2001; Hildeman et al., 1999; Matsumura et al., 2000; Sidoti-de Fraisse et al., 1998; Slater et al., 1995; Vafa et al., 2002; Weis et al., 1995). Expression of a typical anti-oxidant gene, the mitochondrial matrix MnSOD, was able to protect these cells from TNF-induced apoptosis (Bruce-Keller et al., 1999; Kokoszka et al., 2001; Sugawara et al., 2002). ROS as an oxidant could passively attack cellular components to elicit cumulative oxidative damage and apoptosis or necrosis, depending on the severity of the damage. This view was recently redefined because signaling by ROS does not seem to be random (Carmody and Cotter, 2001; Curtin et al., 2002).

ROS were shown to activate the mitochondrial uncoupling protein-2 (UCP-2), which is located at the inner membrane of mitochondria. UCP-2 is a homolog of the ATP/ADP translocator (ANT), which is one of the components of the PTP complex. UCP-2 has been implicated in proton transport across the mitochondrial inner membrane in the presence of fatty acids and plays an important role in the maintenance of mitochondrial membrane potential. Interestingly, UCP-2 has been shown to be induced by genotoxic stress and to be associated with ROS production in the mitochondria (Arsenijevic et al., 2000; Casteilla et al., 2001; Echtay et al., 2002a; Echtay et al., 2002b; Voehringer et al., 2000). The role of UCPs in ROS production remains to be determined. Also, recent evidence indicates that $O_2^{\cdot-}$, but not H_2O_2, directly induces PTP opening by targeting VDAC, another PTP complex protein located on the outer mitochondrial membrane (Madesh and Hajnoczky, 2001). $O_2^{\cdot-}$ generated by Xanthine/Xanthine oxidase induce PTP opening and cyt c release. These effects were inhibited by the PTP inhibitors Cyclosporin A (CsA) and Bcl-xL (Chen et al., 2003). The specific targeting of $O_2^{\cdot-}$ to VDAC was demonstrated by using VDAC antibodies, which specifically block VDAC channel activities (Shimizu et al., 2001), thereby b ocking $O_2^{\cdot-}$ induced PTP opening and cyt c release. These data could explain, at least in part, how ROS could induce cyt c release and apoptosis.

Other types of free radical species, such as reactive nitrogen species (RNS), also play important roles in oxidative stress and apoptosis. A large number of cells release nitric oxide (NO), which is formed either during the NOS-catalyzed conversion of arginine to citrulline, or by mitochondria. NO reacts with $O_2^{\cdot-}$ and $ONOO^-$ to form inert nitrite, or with GSH to form the NO donor, GSNO. High levels of $ONOO^-$ have significant cell damaging effects. Under physiological conditions, cellular NO is a ubiquitous signaling molecule that activates soluble guanylate cyclase. It could also act as a signaling molecule to induce mitochondrial biogenesis (Nisoli et al., 2003). In addition, NO inhibits the mitochondrial cyt c oxidase in competition with oxygen at high efficiency. This interaction may play a significant role in regulating mitochondrial respiration (Beltran et al., 2000; Brown, 2001). Depending on the systems used, NO may have either a pro-apoptotic or an anti-apoptotic role (Almeida et al., 2001). Recently, it was suggested that during early apoptosis, NO plays a protective role by inhibiting cyt c oxidase activity and inducing hyper-polarization of the mitochondrial membrane. However, prolonged exposure to NO could lead to oxidative stress by inhibiting cyt c oxidase and S-nitrosylation of mitochondrial enzymes, thus, promoting apoptosis (Almeida et al., 2001; Beltran et al., 2000; Beltran et al., 2002; Brown and Borutaite, 1999; Brown and Borutaite, 2001).

Feedback Amplification of Cyt C Release and ROS Production by Caspases

Despite rapid progress in understanding apoptosis, the regulation of ROS production during cell death and its relationship to other apoptotic events are still not clear. There is evidence suggesting that inhibition of caspases can effectively prevent ROS production (Green and Kroemer, 1998; Luetjens et al., 2000; Marzo et al., 1998b). We recently demonstrated that there are distinct phases of ROS production from mitochondria and that there is feedback amplification of ROS production from mitochondria by caspases (Chen et al., 2003). The late phase of ROS production and other associated redox events such as glutathione depletion were inhibited by z-VAD-fmk, a pancaspase inhibitor and by ectopic expression of a dominant negative caspase-9. Interestingly, the late increase of ROS production appears to be associated with depletion of cyt c from mitochondria. In line with these observations, we also observed that there were distinct phases of cyt c release following genotoxic stress (Chen et al., 2000), indicating that that cyt c release from mitochondria was a two step process (Chen et al., 2003; Chen et al., 2000; Ott et al., 2002). Furthermore, we showed that there was a feedback amplification of cyt c release and PTP opening by caspases during genotoxic stress-induced apoptosis. Thus, during the early stage of apoptosis, there was a small amount of cyt c release, which was sufficient enough to activate caspases. Once activated, caspases could induce a feedback amplification of cyt c release and mitochondrial dysfunction. These PTP-inducing effects could be mediated by cytosolic factors, such as truncated Bid (Bossy-Wetzel and Green, 1999; Slee et al., 2000) or by caspases that directly target mitochondrial proteins (Chen et al., 2003; Chen et al., 2000; Ricci et al., 2003). Our most recent results show that recombinant Caspase-3 directly induces mitochondrial PTP opening in isolated mitochondria in vitro, arguing that caspases could independently induce mitochondrial apoptotic events (Xia et al., 2002).

A recent report suggests that cyt c release from mitochondria is an "all or nothing" event (Goldstein et al., 2000; Martinou et al., 2000; Waterhouse et al., 2002). Our previous results implicating a feedback amplification of cyt c release by caspases are not contradictory, but rather, are complementary to this observation. It is possible that only a small proportion of mitochondria within any individual cell open the PT pore following irradiation, resulting in release of cyt c from the affected mitochondria. It remains to be determined whether the limited cyt c release is from a small number of individual mitochondria that lost all their cyt c, or alternatively, all mitochondria lost a small proportion of their cyt c. Moreover, the degree to which the loss of cyt c in an individual mitochondrion could result in the catastrophic outcome for ROS production is unclear. Since cyt c is an abundant protein in the mitochondria, it is possible that a small amount of cyt c release will not impair mitochondrial function. Furthermore, cyt c release is a reversible process (Chen et al., 1998; Martinou et al., 1999; Waterhouse et al., 2001a). Our results indicate that cells maintain their ability to survive even when cyt c is partially released and mitochondrial membrane potential is reduced. Indeed, exogenous addition of cyt c to mitochondria maintains the mitochondrial membrane potential and the mitochondrial ATP generation (Waterhouse et al., 2001b).

Caspases are able to cleave a number of proteins that have an essential role in various cellular functions. Such targets include Bcl-2 and Bcl-xL, which both guard the mitochonhdrial integrity and prevent cyt c release (Chen et al., 2000; Cheng et al., 1997; Kirsch et al., 1999). Truncated Bcl-xL could form a mitochondrial pore, which would be big enough to allow cyt c release (Basanez et al., 2001). This proteolytic cleavage converts these anti-apoptotic molecules (Yan molecules) to pro-apoptotic molecules (Yin molecules). Given the fact that ROS is able to induce cyt c release, we postulate that there might be a self-amplifying process for cyt c release, caspase activation, and ROS production. It is possible that the positive feedback amplification of cyt c release and the consequential mitochondrial dysfunction represent the point of no-return for these cells.

Cytochrome C as an Antioxidant

Cyt c could also function as a potent anti-oxidant, which is a role beyond its utility as an electron carrier and cofactor required for activating the apoptosome in the presence of ATP (Korshunov et al., 1999; Skulachev, 1998). Under normal physiological conditions, cyt c is attached to the outside of the inner mitochondrial membrane to function as an electron carrier. With a rise of mitochondrial ROS levels, cyt c detaches from the inner mitochondrial membrane and is capable of oxidizing $O_2^{\cdot-}$ to form molecular oxygen, a reaction supported by the cyt c oxidase. This could significantly buffer the oxidizing power of the $O_2^{\cdot-}$ and diminish the damaging effects of superoxide radicals during mitochondrial respiration. If the buffering capacity is overridden by the increasing levels of ROS, cyt c is released from mitochondria through the PTP or, alternatively, through the oligomerized Bax/Bak super-channels. This model is supported by evidence that cyt c release is associated with decreased levels of intracellular GSH. GSH itself is not the direct stimulus for cyt c release, but its depletion enhances cyt c release and the damaging effects of multiple death stimuli. This may help to explain how GSH depletion could overcome the Bcl-2 suppression of cyt c release and apoptosis.

Another level of cyt c regulated activation of caspases could be based on the redox state of cytoplasmic cyt c (Hampton et al., 1998). It has been suggested that cyt c

will only induce programmed cell death if it is present in the cytoplasm in the oxidized state. The presence of high levels of cytoplasmic GSH maintain cyt c in an inactive (reduced) state (Ueda et al., 1998). Therefore, when cyt c is released by mitochondria and programmed cell death is not the required outcome, a fail-safe mechanism for cellular integrity remains. If the redox status of the cell is disturbed, however, perhaps in the presence of ROS, GSH concentrations will drop and cyt c will shift towards the oxidized state, allowing programmed cell death to proceed. This explains how redox status and GSH is regulating caspase activation even after cyt c is released (Hancock et al., 2001). Indeed, the redox status of cyt c, and thus its structure, can be altered by the presence of ROS and reduced glutathione (GSH).

It appears that the relationship between ROS and cyt c release is quite complex. On one hand, release of cyt c could disrupt the mitochondrial respiratory chain and lead to an increase in ROS production (Cai et al., 1998; Kirkland and Franklin, 2001). Considering that cyt c is one of the most abundant proteins localized outside of the inner mitochondrial membrane, it remains to be determined to what extent cyt c release is hampering ATP production, thus resulting in elevated ROS production. On the other hand, ROS could directly attack mitochondria or VDAC, leading to cyt c release. In addition, cyt c functions as a potent anti-oxidant that scavenges ROS in the mitochondria (see above). Thus, in the healthy living cells, the production of ROS in mitochondria is finely tuned by the regulation of electron flow of the respiratory chain, by the mitochondrial matrix scavenging enzyme systems, and by anti-oxidant scavengers including cyt c. However, once the system is perturbed, hyper-production of ROS has a catastrophic effect by targeting key components of mitochondria to induce cyt c release.

Redox Regulation of Caspase Activity

Considerable evidence supports the role of redox molecules in signaling toward apoptosis (Hirota et al., 1997; Hu et al., 2001; Liu et al., 2000; Powis et al., 1998; Saitoh et al., 1998). It has been shown that GSH and thioredoxin are critical components of the TNF and Fas-activated apoptotic pathways (Armstrong and Jones, 2002; Armstrong et al., 2002; Ueda et al., 2002). Recent evidence further indicates that S-nitrosylation and oxidation events at critical thiol residues represent the major mechanism for regulating caspase activities. Not surprisingly, there are conflicting reports in support of NO having both pro- and anti-apoptosis properties depending on the biological system investigated. It is reported that NO serves as an anti-apoptotic regulator of caspase activitity via S-nitrosylation of the Cys-163 residue of caspase-3 (Rossig et al., 1999). In contrast, denitrosylation of caspase-3 during Fas activated apoptosis was associated with an increase in intracellular caspase activity (Mannick et al., 1999). Examining the ability of recombinant human thioredoxin to activate caspase-3 indicated that caspase activation was correlated with the number of reduced cysteine residues in thioredoxin. Both reduced insulin and BSA were as effective as thioredoxin in activating caspase-3. These results suggest that the redox state plays a direct regulatory role in caspase activation (Baker et al., 1997; Baker et al., 2000; Ueda et al., 2002).

HSP Regulation of Cyt C and Caspases: Relevant to its Reducing Ability

Heat shock proteins (HSPs) function collectively to protect cells from the potentially fatal consequences of adverse environmental, physical, or chemical stresses through their ability to prevent protein aggregation and to promote the refolding of denatured proteins (Parsell and Lindquist, 1993). It is not surprising that small HSPs protect against apoptosis mediated by different agents, including staurosporine, etoposide, and Fas ligand. The protective function of the HSPs may be extended to include an antiapoptotic role for several members of the HSP family, including HSP90, HSP70, and HSP27 (Beere and Green, 2001; Beere et al., 2000). It has been reported that HSP70 and HSP90 directly bind to caspase-3 or Apaf-1 to negatively regulate its activation (Concannon et al., 2001; Jaattela et al., 1998; Li et al., 2000; Saleh et al., 2000). Furthermore, a small HSP known as HSP27, inhibits cyt-c-mediated activation of caspases by directly binding to the cytochrome c released from the mitochondria (Garrido et al., 1999; Garrido et al., 2001). This event thus prevents the cyt-c-mediated interaction of Apaf-1 with procaspase-9. These findings are not surprising since HSPs are very sticky proteins and may interact with a number of other proteins in response to various cellular stresses.

In addition to the direct interaction with caspases, HSPs could act to regulate the intracellular redox state and programmed cell death. Over-expression of small HSPs could increase the intracellular GSH levels and maintain the cells in a reduced state, a phenomenon that may be linked to the ability of these proteins to decrease the intracellular level of ROS in a GSH-dependent manner. Reactive cysteines of the 90-kDa heat shock protein HSP90, as well as two HSP90 peptides containing Cys-521 and Cys-589/590 are able to reduce cytochrome c (Nardai et al., 2000). HSP90 neither reduces the disulfide bonds of insulin nor possesses a NADPH: quinone oxidoreductase activity. The high and specific reactivity of the HSP90 cysteine groups toward cyt c may indicate a role of this chaperone in modulating the redox status of the cytosol in resting and apoptotic cells. HSP70 has also the ability to reduce cyt c (Simpkins et al., 1993). Interestingly, both molecules are able to negatively regulate the apoptosome activities after cyt c is released (see above).

Caspase Independent Cell Death: a Radical Way Toward Cell Death?

Recent reports indicate that cells treated with caspase inhibitors, or Apaf1-deficient cells unable to activate caspases, die of a necrosis-like cell death called apo-necrosis (Formigli et al., 2000). Moreover, inhibition of caspases by oncogenic Bcl-2/Bcl-xL, Ras, Raf, and mitogen-activated kinases delay, but do not prevent cell death (Lockshin and Zakeri, 2002). These cells show slower cell death without caspase activation and loss of clonogenicity. This seems to be a distinct form of cell death with intermediary characteristics between apoptosis and necrosis, without any detectable caspase activation, nuclear condensation, or DNA fragmentation, the hallmarks of apoptosis. However, mitochondria were not only morphologically abnormal, but functionally affected, as the mitochondrial transmembrane potential was lost in cells with intact plasma membrane integrity. These findings point to an intrinsic caspase-independent cell death pathway. This pathway could be evolutionarily preceding the caspase-dependent apoptotic pathway. Genome-wide analysis indicates that yeast and plant cells do not have any obvious homologues of the metazoan apoptosis regulators, such as those of the Bcl-2 gene family, caspases, Apaf-1, and p53. These cells may die by a caspase-independent pathway (Fleury et al., 2002). However, recent evidence suggest that yeast has a caspase-like protease, which may execute apoptosis (Madeo et al., 2002a; Madeo et al., 2002b).

The signals for this intrinsic apo-necrosis pathway remain obscure. It is known that caspase and Bax expression in yeast induce an apoptotic-like cell death (Gross et al., 2000; Matsuyama et al., 1999). Recent evidence suggests that oxidative signals may be responsible for yeast apoptosis (Frohlich and Madeo, 2000; Madeo et al., 1999). The depletion of GSH from yeast culture medium or addition of exogenous ROS also induces yeast cell death (Greenhalf et al., 1996; Madeo et al., 1999). Similarly, in mammalian cells, ROS could also mediate cell death both in a caspase-dependent and caspase-independent manner. Another possibility is that mitochondria are also the executioners for caspases-independent apoptosis, although this possibility remains to be proven. Nevertheless, mitochondria release endonuclease G and AIF to execute cell death without any help from caspases or even ROS (Li et al., 2001; Widlak et al., 2001). As these various caspase-dependent and caspase-independent cell death pathways are becoming better characterized, we may learn to differentiate them, fill in the many gaps in our understanding, and perhaps exploit the knowledge acquired for clinical benefit.

Bcl-2 Regulation of Cyt C Release: An Anti-Oxidant Pathway to Inhibit Cyt c Release

Bcl-2 and related family proteins play an important role in regulating cyt c release, and caspase activation. The exact mechanisms of how Bcl-2 family proteins regulate these apoptosis associated events are not clear. At least two major functions are attributed to the protective role of Bcl-2 in apoptosis: its regulation of the cellular redox potential and cyt c release from mitochondria. Rather than simply acting as a regulator of apoptosis, it was suggested that Bcl-2 could be a general regulator of mitochondrial homeostasis (Vander Heiden et al., 1997). Alternatively, Bcl-2 could regulate the mitochondrial transition pore (MTP) opening by opposing the effect of Bax, a component of the MTP pore (Marzo et al., 1998a). Bcl-2 and Bcl-x/L regulate the homeostasis of mitochondria by blocking cyt c release from mitochondria, thereby preventing caspase activation. It is possible that Bcl-2, as a proto-oncoprotein, functions at multiple levels to prevent the morphological changes associated with apoptosis induced by diverse death stimuli. It has been suggested that Bcl-2 could prevent apoptosis by regulating the cellular redox potential. Bcl-2 could also act as an anti-oxidant by increasing the GSH pool and by redistributing of GSH to various cellular compartments, thus preventing ROS production, GSH depletion, and cellular damage caused by lipid peroxidation (Hockenbery et al., 1993; Voehringer and Meyn, 2000). Bcl-2 deficient mice show enhanced oxidative stress, perhaps due to their altered antioxidant pathways. However, other reports indicate that Bcl-2 could function as a pro-oxidant. The mechanism of how Bcl-2 regulates cellular redox potential and mitochondrial cyt c release are not clear. Bcl-2 itself is known to be able to increase the intracellular levels of GSH or its mobilization (Voehringer, 1999; Voehringer et al., 1998), although generalization of these findings may be difficult. However, the mechanism by which Bcl-2 acts to maintain cellular GSH levels is not clear. We recently documented that Bcl-2 prevented GSH depletion and increased ROS production during the late phase of apoptosis. Bcl-2 overexpression appears not to impact on the initial GSH loss immediately after ionizing radiation in our system, but rather prevents further loss of GSH at 24 h. Our results strongly indicate that Bcl-2 prevents GSH depletion by blocking cyt c depletion, which is mediated by caspases and the subsequent ROS increase. Our findings reconcile the dual role of Bcl-2 in regulating cyt c release and redox potential. Our data suggest that the functions of Bcl-2 in regulating the redox potential, ROS production, and cyt c release from mitochondria are interrelated, and the suppression of cyt c release could be the primary function of Bcl-2 (Chen et al., 2003). These results are consistent with a large body of evidence indicating that Bcl-2 prevents caspase activation, cyt c release, GSH depletion, and the associated mitochondrial dysfunction. Activated caspases could proteolytically cleave mitochondrial Bcl-2, converting this pro-apoptotic molecule into a Bax-like molecule that promotes apoptosis (Chen et al., 2000; Fadeel et al., 1999a; Kirsch et al.,

1999). Further understanding of the complex functional relationships between the biochemical and metabolic hallmarks of apoptosis, such as cyt c release, caspase activation, ROS production, PT, and GSH depletion, and their regulation by Bcl-2 are important for advancing our knowledge of apoptosis.

Concluding Remarks

Apoptosis or programmed cell death, is a highly regulated process before and after cyt c release, both at the level of mitochondria and the process of caspase activation. ROS could play a critical role in mediating caspase dependent of cell death by promoting cyt c release and independent pathways. Clearly, there seems to be multiple levels of fail-safe mechanisms in regulating cyt c-mediated activation of caspases. In accordance with this suggestion, our recent results prompt us to suggest that there are two distinct phases of cyt c release and ROS production and there is a self-amplifying process for ROS production, cyt c release and caspase activation during apoptosis. Further delineation of the apoptotic pathways holds promise for exploring novel pharmaceutical targets for drug discovery.

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