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Research Overview

Molecular Machinery and Signaling Events in Apoptosis

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ABSTRACT Apoptosis is a process of major biomedical interest, since its ineffectiveness or inappropriate activation appears to be involved in the pathogenesis of a broad variety of human diseases (neoplasia, autoimmune disorders, viral and neurodegenerative diseases, to name a few). On this topic, extensive experimental work has allowed in the past years the clarification of the complex biochemical machinery that commits a cell to apoptosis and executes the death program. As to the signaling mechanisms, it is now evident that apoptosis can be initiated by different stimuli and/or genetic programs that are differentially decoded inside the cell. While the past years have witnessed a major advancement on this topic, much still needs to be learned of the cross-talk between the various signaling pathways involved in decoding the apoptotic stimuli, as well as the activation of other cell functions. In this review we first describe the properties and activation mechanisms of the caspases, the effector proteases of apoptosis. In the second part we discuss the current evidence for the involvement of calcium, the ubiquitous second-messenger decoding a wide variety of physiological stimuli, and highlight the potential targets of the apoptotic calcium signal. Drug Dev. Res. 52:558–570, 2001.

Key words: molecular machinery; signaling events; apoptosis; caspases

BIOCHEMISTRY OF APOPTOSIS: CASPASES AND REGULATORY MECHANISM

Apoptosis is a genetically regulated (programmed cell death) and finely tuned process of cell elimination essential for the embryogenesis, development, and tissue homeostasis of multicellular organisms [Kerr et al., 1972; Raff et al., 1993]. Apoptosis takes part in the normal development and functions of organisms as different as nematodes [Ellis et al., 1991; Hengartner and Horvitz, 1994; Vaux and Korsmeyer, 1999], insects, or humans. Dysregulation or impairment of apoptosis can therefore have deleterious consequences. In humans, important pathological conditions such as neurodegenerative and autoimmune diseases, cancer, or AIDS [Thompson, 1995; Fisher et al., 1995; Drappa et al., 1996; Uren and Vaux, 1996; Hetts, 1998] have defective apoptosis as the main cause. Cell death by apoptosis is accompanied by a stereotyped and interconnected series of events among which cell collapse, formation of membrane blebs, chromatin condensation, and DNA degradation are well recognized. Selective degradation of intracellular substrates during apoptosis also occurs and it is mainly due to the activity of a recently identified family of highly conserved cysteine proteases, named caspases (for cysteinyl aspartate-specific proteinases) [Alnemri et al., 1996; Nicholson and Thornberry, 1997; Nagata, 1997; Thornberry and Lazebnik, 1998]. The importance of caspases in the execution of the apoptotic program is emphasized by the

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fact that inhibition of their activity reduces apoptosis, suggesting that at least part of the substrates they cleave are indispensable for cell life. Caspases selectively cleave a set of about 100 targets, although the estimated number could be around 200 [Nicholson, 1999]. An essential requirement for their proteolytic activity is an aspartate residue in the P_1 position of the substrate. Cleavage by caspases usually results in the degradation and inactivation of their substrates, e.g., the enzymes involved in DNA cleavage and repair (DNA-PK, PARP, topoisomerase-I, MCM3, DFF, CAD) with the consequent impairment of DNA repairing machinery [Kaufmann et al., 1993; Lazebnik et al., 1994]. Proteolytic cleavage by caspases can in some cases activate their substrates, e.g., by removal of regulatory domains or inactivation of regulatory subunits (SREBP, PKCs, $cPLA_2$, PAK2, Bid). Structural components of the cytoskeleton (gelsolin, fodrin, catenins) [Kothakota et al., 1997] and nuclear membrane (lamin A, B1) are also cleaved by caspases during apoptosis [Oberhammer et al., 1994; Rao et al., 1996]. Proteins involved in signal transduction (Raf-1, protein kinases, protein phosphatases) or in the processing of cytokine precursors (proIL-1β, proIL-16, proIL-18) have also been identified as caspase substrates. Inhibition of cytokine secretion can have important consequences for viral infection. Viruses have evolved proteins capable of inhibiting caspases involved in the immune response and cell death, thus preventing production of inflammatory cytokines and apoptosis through death receptor activation, an outcome that would eliminate infected cells. A nice example of this defensive mechanism is that adopted by a cowpox virus that produces a caspase inhibitor named CrmA (cytokine response modifier A), which blocks casp-1 and casp-8 [Ray et al., 1992; McFadden et al., 1995; Komiyama et al., 1994]. Another strategy adopted by viruses to inhibit the apoptotic response of the infected cell is the expression of FLICE-inhibitory proteins (vFLIPs), interfering with the signaling of the death receptors [Thome et al., 1997; Glykofrydes et al., 2000]. Inhibitors of apoptosis (IAPs) have also been found in organisms as different as yeasts, nematodes, insects, and mammals, where they seem to play multiple roles [Fraser et al., 1999].

CASPASES (FIG. 1)

Fourteen mammalian members have been identified so far. They represent a multigene family that can be divided into two major subfamilies comprising enzymes either related to caspase-1/ICE or to the mammalian counterparts of CED-3 [Nicholson, 1999]. They can also be grouped on the basis of the substrate preference, the length of their prodomain, or by participation in apoptosis or inflammation. Two major gene clusters have been identified: one maps to chromosome 11q22.2-q22.3 and comprises genes encoding caspases-1, -4, -5; while the second is located on chromosome 2q33-q34 and encodes caspases-8 and -10 [Nasir et al., 1997; Rasper et al., 1998].

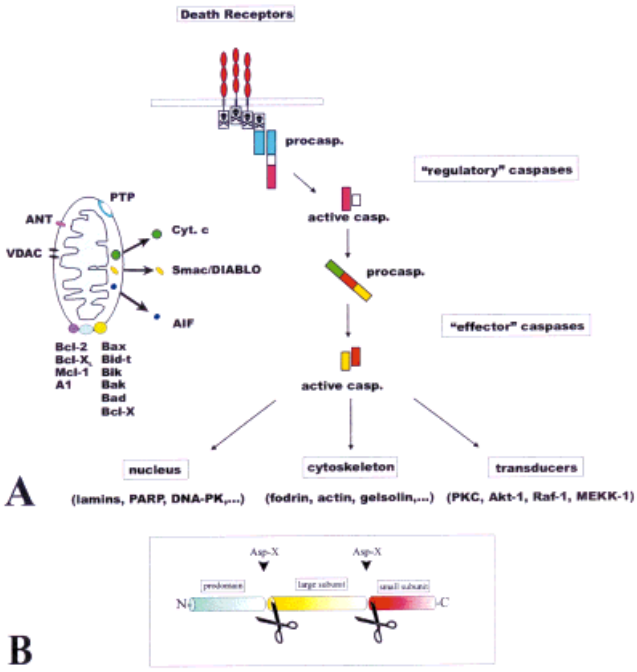

Fig. 1. **A**: Intracellular events triggered by death receptors activation. **B**: Schematic representation of procaspase cleavage.

Caspases are synthesized as enzymatically inert zymogens (procaspases) consisting of three main parts: a prodomain and a large and a small subunit that are split by cleavage at two aspartate sites [Asp (P_1)-X(P_1')]. The generated fragments (large and small subunits) associate to form dimers or tetramers: the active caspase [Earnshaw et al., 1999]. The large subunit contributes to the active site with a Cys and a His residue, whereas both subunits contribute to form the S_1 subsite that recognizes the crucial aspartic acid of the substrate [Wilson et al., 1994; Rano et al., 1997; Rotonda et al., 1996].

With respect to the prodomains, caspases can be distinguished into the so-called Class I or "regulatory caspases" or caspases with long prodomains, and Class II or "effector caspases," with short prodomains. Casp-1, -2, -4, -5, -8, -9, -10, -11, -12, and -13, belong to the first group and are thought to play a function in the recruitment-activation steps, while casp-3, -6, -7, and -14 can be ascribed to the second group and are thought to be downstream in the activation cascade.

Caspases can be activated in different ways, depending on the length and sequence of their prodomains. Caspases with long prodomains can be activated at the death domains (DD) of cytotoxic receptors (CD95/Fas/APO-1, TNFR1, etc.) [Tartaglia et al., 1993] through

homophilic interactions with adaptor molecules coupling caspases to the receptors [Chinnaiyan et al., 1995; Boldin et al., 1995, 1996; Muzio et al., 1996]. Recruitment between CARD-domains such as that involved in apoptosis-activating factor 1 (Apaf-1) -mediated activation of procasp-9, or RAIDD-mediated activation of casp-2 has also been shown.

Transactivation of caspases is also possible. Due to the Asp-X site, procaspases can be cleaved by other caspases (casp-9 activates procasp-3, casp-3 activates procasp-6, etc.). Granzyme B released by T lymphocytes can also activate caspases [Darmon et al., 1995; Andrade et al., 1998] and a recent report showed that at least procasp-3 can be activated by binding to RGD peptides [Buckley et al., 1999].

Caspase Members

Casp-1 is synthesized as a 45-KDa zymogen (p45) which originates two subunits (p10 and p20) that associate to form the active caspase [Thornberry et al., 1992]. Casp-1 is mainly localized in the cytoplasm and translocates to the nucleus during apoptosis. Casp-1 has been also found on the external surface of the plasma membrane, where it can cleave secreted proIL-1β. This caspase has been recognized as the enzyme responsible for the processing of proIL-1β and proIL-18 in their mature biologically active forms [Ceretti et al., 1992; Thornberry et al., 1992; Gu et al., 1997; Dinarello, 1998]. Casp-1 can be recruited at the DD of death receptors [Miura et al., 1993]. Inhibition of its activity slows the progression of Huntington's disease in mice [Ona et al., 1999]. Monocytes from casp-1 knockout mice (casp-$1^{-/-}$) do not secrete IL-1β upon treatment with LPS or nigericin [Kuida et al.,1995; Li et al., 1995]; furthermore, and quite intriguingly, production of IL-1α is also impaired, suggesting that casp-1 activity is required for IL-1α secretion.

Casp-2 (ICH-1/Nedd2) is present in two splicing variants of 435 and 312 amino acids, respectively, named casp-2_L/ICH-1_L (proapoptotic) and casp-2_S/ICH-1_S (antiapoptotic) [Wang et al., 1994]. Procasp-2 can bind the adaptor molecule RAIDD (RIP-associated Ich-1/CED-3 homolog protein with a DD). Casp-2 is found in the mitochondrial matrix and translocates to the cytoplasm during apoptosis [Susin et al., 1999a]. Casp-2 has also been found in the nucleus [Colussi et al., 1998]. Newborn casp-$2^{-/-}$ mice show a reduced number of facial motoneurons. Deficiency of casp-2_L in ovary tissue leads to an increase in the number of oocytes that are also refractory to some apoptotic inducers.

For its central role in apoptosis, casp-3 (YAMA/apopain/CPP32) is one of the most thoroughly investigated caspases. Procasp-3 has a molecular mass of 32 kDa and upon cleavage generates large and small subunits of 17 kDa or 20 kDa, and 12 kDa, respectively. Casp-3 translocates from mitochondria to the cytosol and from cytosol to the nucleus. It is highly expressed in lymphoid cell lines and can be activated in vitro by granzyme B. Casp-3 is responsible for the cleavage of a large number of substrates. The DNA-repairing enzyme poly(ADP ribose) polymerase (PARP) is a target of casp-3 and undergoes inactivation upon cleavage. Casp-3 can also mediate processing and secretion of IL-16 [Zhang et al., 1998]. Cleavage of huntingtin, a protein involved in Huntington's disease by casp-3, has also been demonstrated [Goldberg et al., 1996]. Casp-$3^{-/-}$ mice show hyperplasia in the brain, accompanied by skull defects. An excessive number of neurons is found, particularly in cortical areas, as a consequence of decreased apoptosis during embryonic development [Kuida et al., 1996].

Casp-4 (ICE_{rel}II/TX/ICH-2) and casp-5 (ICE_{rel}III/TY) show high homology [Munday et al., 1995]. Casp-6 (Mch2) translocates from the cytoplasm to the nucleus during apoptosis. Casp-$6^{-/-}$ mice seem to have a normal development [Zheng et al., 1999]. Casp-7 (Mch3/CMH-1/ICE-LAP3) translocates from the cytosol to mitochondria and the microsomal fraction; activation and translocation of casp-7 correlates with the cleavage of a marker of the microsomal compartment, SREB-1 [Chandler et al., 1998]. Casp-$7^{-/-}$ mice die at the embryonic stage.

Casp-8 (FLICE/Mch5/MACH) is recruited at the CD95 and TNFR1 through interaction of the death effector domains (DEDs) present on both FADD (Fas-associated protein with death domain) and TRADD proteins. A role for casp-8 in the regulation of muscle development and erythropoiesis has been hypothesized. Casp-$8^{-/-}$ mice die in utero and show impaired heart formation and abundant hemorrhage in the liver and abdomen. Defects in these systems could be due to impaired signaling of death receptors such as CD95, DR3, or DR5, as similar abnormalities were found in FADD-deficient mice [Yeh et al., 1998].

Casp-9 (Mch6/ICE-LAP6) is localized in the mitochondrial matrix, but it translocates to the cytosol upon activation [Susin et al., 1999a]. Phosphatidylserine (PS) flipping is impaired in casp-$9^{-/-}$ thymocytes induced to undergo apoptosis with dexamethasone, suggesting that this caspase or another casp-9-activated caspase could be responsible for PS exposure. As for casp-$3^{-/-}$ mice, casp-$9^{-/-}$ knockouts show increased neuronal number in the brain. Casp-10 (Mch4/FLICE-2) has two DED sequences and like casp-8 is thought to interact with FADD. Mutations in the casp-10 gene are responsible for defective apoptosis of lymphocytes and dendritic cells in autoimmune lymphoproliferative syndrome type II [Wang et al., 1999a]. Casp-11 is involved in inflammation and is activated by cathepsin B [Schotte et al., 1998]. Murine casp-11 interacts with and activates casp-1; casp-$11^{-/-}$ mice are resistant to lipopolysaccharide-induced endotoxic shock [Wang et al., 1998]. Casp-

12 is localized in the endoplasmic reticulum (ER) [Nakagawa et al., 2000] and is activated by ER stress. Casp-13 (ERICE) [Humke et al., 1998] shows a high homology with casp-1. Its overexpression induces apoptosis in different cell lines and it is activated by casp-8. It has been hypothesized that casp-13 could have a role in receptor-stimulated cell death.

Casp-14 (MICE) has a very short prodomain and interacts preferentially with caspases bearing long prodomains (casp-1, casp-2, casp-4, casp-8, casp-10) [Hu et al., 1998]. In mice, expression of casp-14 is high in embryonic tissues, but absent in the adult.

MITOCHONDRIA RELEASE APOPTOTIC FACTORS

Mitochondria play an important role in the amplification of the apoptotic process by releasing proapoptotic factors such as cytochrome c (cyt c), apoptosis-inducing factor (AIF), and the recently identified Smac/DIABLO. For a long time the only known role of cyt c was that of catalyzing the transfer of electrons between Complex III and IV in the mitochondrial respiratory chain. More recently, an additional surprising function has been discovered for cyt c in apoptosis: during the apoptotic process this protein translocates from the mitochondrial intermembrane space to the cytoplasm, forming a complex with Apaf-1, a mammalian homolog of Ced-4, and procasp-9. This caspase is then activated and can in turn activate procasp-3 [Li et al., 1997; Zou et al., 1997]. Addition of cyt c to cytosolic extracts activates procaspases. Microinjection of cyt c is known to induce activation of caspases and apoptosis in a Bcl-2-inhibitable manner [Zhivotovsky et al., 1998; Brustugun et al., 1998]. The flavoprotein AIF is an oxidoreductase synthesized in the cytosol and imported into the mitochondrial intermembrane space. Given that in cell-free systems AIF induces chromatin condensation and large-scale DNA fragmentation [Susin et al., 1999b; Lorenzo et al., 1999; Daugas et al., 2000], it has been suggested that AIF is released from mitochondria during apoptosis and concurs to nuclear modifications. The proapoptotic factor Smac/DIABLO [Du et al., 2000; Verhagen et al., 2000] is also located in the mitochondrial intermembrane space and is released upon induction of apoptosis. Smac binds to and inactivates IAPs, allowing aggregation of Apaf-1 and casp-9 and the consequent formation of the apoptosome [Srinivasula et al., 2000].

Moreover, several different pro- (Bad, Bak, Bax, Bik, Bid, Bcl-X_S) or antiapoptotic (Bcl-2, Bcl-X_L, Mcl-1, A1) proteins belonging to the Bcl-2 family [Adams and Cory, 1998] reside in or translocate to the mitochondria. Since some of these proteins (Bcl-2, Bax, Bcl- X_L, Bid) can form pores in lipid membranes [Schendel et al., 1997; Minn et al., 1997], it was hypothesized that this property could allow them to collaborate in the formation of multimeric complexes in the mitochondrial membrane. Bcl-2 is also known to be localized on the outer surface of the nuclear envelope and of the ER membrane. This oncogene inhibits release of cyt c during apoptosis [Kluck et al., 1997], but it has also been hypothesized that Bcl-2 could prevent apoptosis by acting on the ER Ca^{2+} pool, although no general consensus on the mechanism involved has been reached so far [He et al., 1997; Kuo et al., 1998; Pinton et al., 2000; Foyouzi-Youssefi et al., 2000]. Inhibition of the import of the apoptogenic protein p53 to the nucleus by Bcl-2 has been also shown [Beham et al., 1997]. The proapoptotic Bcl-2 family member Bid is normally located in the cytoplasm. It can be processed by caspases, as it has a casp-8 cleavage site. Caspase cleavage of inactive p22 BID gives a major p15 and minor p13 and p11 fragments. The p15 portion (tBid) can translocate to the mitochondria and induce release of cyt c [Luo et al., 1998; Kim et al., 2000]. Bid can also induce oligomerization and insertion of Bax into the outer mitochondrial membrane [Eskes et al., 2000]. Mice deficient for Bid injected with an anti-CD95 antibody are resistant to CD95-induced hepatocellular apoptosis and show no activation of casp-3 and casp-7 and no cyt c release. Bad is phosphorylated by calcineurin and translocates to the mitochondrial outer membrane forming heterodimers with Bcl-X_L and promoting apoptosis [Wang et al., 1999b]. An increase in the mitochondrial volume in apoptotic cells has also been shown [Vander Heiden et al., 1997], thus leading some authors to hypothesize that release of cyt c could be the consequence of the rupture of the outer mitochondrial membrane, although release of cyt c can also occur independently of this phenomenon [Doran and Halestrap, 2000; Kim et al., 2000]. According to some authors, the apoptotic process is preceded by collapse of the mitochondrial potential, opening of a multiprotein structure named the permeability transition pore (PTP) [see Bernardi, 1999b, for a detailed review]. The PTP assembles at sites of contacts between the inner and outer mitochondrial membranes, swelling the matrix and rupturing the outer membrane with the ensuing change in the permeability of the outer mitochondrial membrane. Release of apoptogenic factors from mitochondria follows [Petronilli et al., 1994; Skulachev, 1996; Bernardi et al., 1998; Petit et al., 1998]. According to other authors, the release of cyt c and the activation of caspases occur before and independently of the loss of mitochondrial membrane potential [Bossy-Wetzel et al., 1998; Finucane et al., 1999; Li et al., 2000a].

INVOLVEMENT OF CALCIUM (FIG. 2)

Calcium Signaling Alterations in Apoptosis

When the systems responsible for the regulation of cellular Ca^{2+} homeostasis are irreversibly compromised,

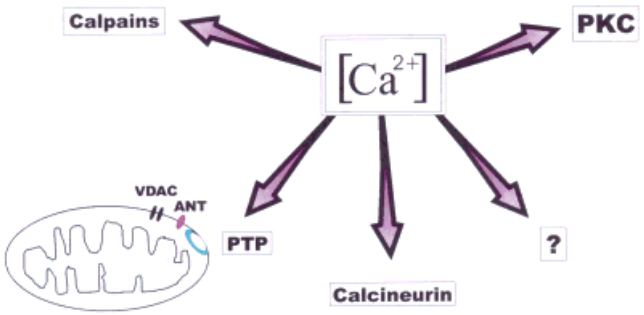

Fig. 2. Putative Ca^{2+}-activated effectors during apoptosis.

a cell is condemned to die. Cell death can occur either in a disordered manner, by necrosis (i.e., through activation of Ca^{2+}-activated hydrolyzing enzymes), or in a more controlled way, by apoptosis.

The possibility that apoptosis is also regulated by more subtle alterations in intracellular Ca^{2+} homeostasis is now supported by a variety of experimental evidence. In neurons and other cell types, for example, an uncontrolled increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) such as that induced by alterations of ER Ca^{2+} pool is sufficient to induce apoptosis [Nicotera and Orrenius, 1992]. Indeed, pharmacological agents such as thapsigargin and cyclopiazonic acid, which induce large increases in the cytosolic Ca^{2+} concentration, by emptying the intracellular Ca^{2+} stores, have been shown to induce apoptosis in a murine lymphoma cell line [Bian et al., 1997].

Indirect support for this hypothesis comes from the demonstration that overexpression of cytosolic Ca^{2+} buffering proteins such as calbindin-D28K protects neurons from apoptosis. Along the same lines, overexpression of calbindin-D28K [Dowd et al., 1992] was shown to block the proapoptotic actions of mutant presenilin 1 [Guo et al., 1998], reducing oxidative stress and preserving mitochondrial functions. The action of Ca^{2+} on neuronal survival is, however, complex. Excess Ca^{2+} promotes apoptosis, but controlled, small increases in cytosolic $[Ca^{2+}]$ have beneficial effects. Thus, inhibition of calcium signaling through glutamatergic Ca^{2+} channels, for example, by ethanol (which blocks NMDA receptor-dependent Ca^{2+} signaling), causes massive neuronal apoptosis during brain development [Ikonomidou et al., 1999, 2000]. Similarly, a modest increase of $[Ca^{2+}]_i$ promotes the survival of neurons in culture [Gallo et al., 1987; Koike et al., 1989].

In addition to the numerous studies that, especially in neuronal cells, demonstrate that a dysregulation of Ca^{2+} homeostasis promotes cell death, a few additional pieces of evidence support the notion that Ca^{2+} plays a key role in cell death. For example, in familial Alzheimer's disease (FAD) (i.e., cases exhibiting a clear Mendelian (autosomal dominant) transmission), there are mutations in two genes encoding closely related (~70% identical) proteins, the presenilins (PS1 and PS2). While these mutations appear causally linked to the production of β-amyloid and neuronal degeneration, their pathogenic mechanism is still undefined. Recent evidence indicates that their primary role could be that of modulating Ca^{2+} signaling in neuronal cells by controlling Ca^{2+} release from intracellular stores. Moreover, presenilins have been shown to interact with antiapoptotic members of the Bcl-2 family and mutations of these genes result in an increased susceptibility to apoptotic stimuli [Mattson et al., 2000]. A second line of evidence pointing to a key role for Ca^{2+} in apoptotic cell death comes from the demonstration that oncogenes that protect against cell death perturb intracellular Ca^{2+} homeostasis. The first example was provided by the study of the prototype of this class of oncogenes, *bcl*-2. The *bcl*-2 product shows a unique intracellular distribution, as it is localized in organelles (i.e., ER and mitochondria) having an important role in controlling Ca^{2+} signaling and homeostasis. It has been demonstrated that Bcl-2 can induce a decrease in the Ca^{2+} concentration of the ER by increasing the leak of the cation from this organelle [Pinton et al., 2000; Foyouzi-Youssefi et al., 2000]. These data are in agreement with the observation that Bcl-2 can form pores in lipid membranes [Minn et al., 1997], thus allowing efflux of the cation from the organelle lumen. Altered Ca^{2+} handling in Bcl-2 overexpressing cells could thus account, at least partially, for its antiapoptotic function.

Another interesting example along this line is represented by an oncogene recently described in a human hepatocarcinoma. This oncogene is generated by the integration of the hepatitis B virus genome in the gene encoding the protein SERCA1. Viral activation was shown to cis-activate SERCA1 chimeric transcripts with splicing of exon 4 and/or exon 11. Splicing of exon 11 creates a frameshift and a premature stop codon in exon 12. The encoded protein lacks most of the cytosolic N and P domains and critical Ca^{2+}-binding regions of the transmembrane region. This protein is incapable of active Ca^{2+} pumping [Chami et al., 2000], but is causally involved in the neoplastic phenotype. Although the molecular mechanism of this oncogene has not been explained yet, it may be speculated that the mutated SERCA could either interfere with the activity of endogenous pumps and/or it could act as a Ca^{2+} leak pathway from the ER. These data are consistent with the recent observations that overexpression of SERCA in HeLa cells increases the susceptibility of cells to apoptotic agents [Ma et al., 1999] (Pinton et al., unpublished observations).

Intracellular Targets of a Ca^{2+}-Mediated Apoptotic Factor

The evidence, presented in the previous section, supporting a role for Ca^{2+} as a messenger of apoptosis,

may appear puzzling, given that the same transduction mechanism is used for regulating a variety of cell functions (ranging from the activation of cells to contract, secrete, move, and even divide). Indeed, widely diverse physiological stimuli (e.g., neurotransmitters, hormones, growth factors, etc.) induce (by opening Ca^{2+} channels located in the plasma membrane or in intracellular stores) increases in cytosolic Ca^{2+} concentration. These increases, which exhibit a high degree of spatiotemporal complexity (i.e., they may occur in propagating waves, localized rises, or repetitive spikes) are specifically decoded into defined intracellular actions. In this scenario, cell death could be one of the consequences of a Ca^{2+} signal that follows the recruitment of specific effectors and/or the cooperation of other intracellular events. While a complete understanding of this complex interaction appears a challenging, but still distant goal, we discuss here some of the potential transducers of the Ca^{2+}-mediated death signal, reviewing the evidence that well-known mediators of Ca^{2+} action could be involved in apoptosis.

MITOCHONDRIA

Mitochondria are the paradigm of the double-edged sword effect of Ca^{2+} on cell life and death. On the one hand, work by our and other groups has shown that, despite the low affinity of mitochondrial Ca^{2+} transporters, large Ca^{2+} fluxes occur across the mitochondrial membranes when a physiological stimulus elicits a $[Ca^{2+}]_i$ rise, because these organelles are not exposed to the (lower) bulk $[Ca^{2+}]_i$ increase, but to microdomains generated in the proximity of the open Ca^{2+} channels. In other words, the strategic location of mitochondria close to the source of the $[Ca^{2+}]_i$ rise (the ER and/or the plasma membrane) allows them to be exposed to $[Ca^{2+}]$ that meet the affinity of their transporters and allows the rapid and large accumulation of the cation in the matrix. In turn, this accumulation has an important physiological role because, by stimulating intramitochondrial effectors (such as the Ca^{2+}-dependent dehydrogenases of the Krebs cycle), it allows the prompt tuning of organelle metabolism (and hence ATP production) to the increased needs of an activated cell [see Duchen, 1999; Rizzuto et al., 2000, for review].

On the other hand, mitochondria, as discussed earlier, are important checkpoints of the apoptotic process, as they may release caspase co-factors [Berridge et al., 1998; Mignotte and Vayssiere, 1998; Green and Reed, 1998; Bernardi, 1999a; Kroemer and Reed, 2000; Desagher and Martinou, 2000; Duchen, 2000; Crompton, 2000]. Among the signals that promote this decisive event, Ca^{2+} may have a central role. Indeed, in the as-yet unsettled identification of the molecular mechanism of this release, one of the favorite hypotheses involves a large conductance nonspecific channel of the inner mitochondrial membrane, commonly referred to as the PTP or mitochondrial megachannel.

Mitochondrial permeability transition became the focus of intense research after the discovery that cyt c is required for apoptosis in a cell-free system [Liu et al., 1996; Zou et al., 1997]. PTP may participate in the regulation of matrix Ca^{2+}, pH, transmembrane potential, and volume. PTP is a Ca^{2+}-, voltage-, pH-, and redox-gated channel that shows several subconductance levels and little, if any, ionic selectivity [Bernardi 1999b; Crompton, 1999].

The PTP appears to operate at the crossroads of two distinct physiological pathways, i.e., the Ca^{2+} signaling network during the life of the cell, and the effector phase of the apoptotic cascade during Ca^{2+}-dependent cell death. Accordingly, two different conformations of the PTP have been suggested to exist: 1) a low-conductance state that allows the diffusion of small ions like Ca^{2+}, is pH-operated, and undergoes spontaneous closures; and 2) a high-conductance state that allows the nonselective diffusion of large molecules and disrupts mitochondrial structure and (indirectly) causes the release of proapoptotic factors.

Ichas and Mazat proposed that the switching from low- to high-conductance state is an irreversible process that is strictly dependent on the saturation of the internal Ca^{2+}-binding sites of the PTP [Ichas and Mazat, 1998]. Mitochondria that undergo the mitochondrial permeability transition may cause cyt c release and thus initiate apoptosis.

Transient pore opening might allow Ca^{2+} release following matrix Ca^{2+} overload [Bernardi, 1999b] as a protective mechanism against mitochondrial Ca^{2+} overload.

Different reports have shown that increased mitochondrial Ca^{2+} accumulation is a trigger for the release of cyt c from the mitochondrial intermembrane space into the cytosol. For example, Jurgensmeier et al. [1998] observed that Ca^{2+} and the proapoptotic protein Bax induce cyt c release from mitochondria via different mechanisms, with the former involving organellar swelling and outer membrane rupture and the latter occurring through a swelling-independent mechanism. Krajewski et al. [1999] showed that Ca^{2+} and Bax induced the release of both cyt c and procaspase-9 (caspase-9 is critical for cyt c-dependent apoptosis) into the supernatants of isolated mitochondria and Ca^{2+}-induced release was completely suppressed by cyclosporin A (an inhibitor of PTP). Szalai et al. [1999] reported the cooperative action of physiological stimuli causing mitochondrial Ca^{2+} accumulation and apoptotic agents in inducing the opening of the PTP, with ensuing cyt c release. In agreement with these results, we have observed that Bcl-2 causes a decrease in agonist-dependent mitochondrial Ca^{2+} increases [Pinton et al., 2000].

A role for mitochondrial Ca^{2+} homeostasis in the

control of apoptosis is also proposed by Zhu et al. [2000a]. In this study, however, the triggering mechanism for the activation of caspases is suggested to be a marked reduction in global calcium signaling obtained by incubating the cells at low extracellular [Ca^{2+}].

CYTOPLASMIC EFFECTORS

Calcineurin

Calcineurin is a calcium- and calmodulin-dependent serine-threonine protein phosphatase. [see Rusnak and Mertz, 2000, for review]. As a serine-threonine phosphatase, calcineurin participates in a number of calcium-dependent signal transduction pathways. Calcineurin is the only known serine-threonine phosphatase whose activity is stimulated by Ca^{2+} [Liu et al., 1991]. Several pieces of evidence indicate that calcineurin plays an important role in some types of apoptosis. For example: 1) prolonged increases in cytosolic [Ca^{2+}] lead to activation of calcineurin, with ensuing cell death by apoptosis [Rao et al., 1997], through a process blocked by Bcl-2 [Shibasaki and McKeon, 1995]; 2) calcineurin activity is essential in the apoptotic death of murine T cell hybridomas [Fruman et al., 1992]; 3) calcineurin can dephosphorylate BAD (a proapoptotic member of the Bcl-2 family), thus enhancing BAD heterodimerization with Bcl-X_L and promoting apoptosis [Wang et al., 1999]. On the latter topic, it should be stressed that elevation of [Ca^{2+}] does not always turn on cell death, but can also have the opposite effect, i.e., protection from apoptosis. For example, Yano et al. [1998] showed that a [Ca^{2+}] increase, via activation of Ca^{2+}/calmodulin-dependent protein kinase kinase (CaM-KK), turns on protein kinase B, which phosphorylates BAD and protects cells from death.

PKC

Protein kinases have been implicated both in the upstream induction phase of apoptosis and in the downstream execution stage, as direct targets of caspases. In this section we focus on the role of PKC, given that its role in programmed cell death has been intensively investigated in the last few years. Other serine/threonine protein kinases play a role in apoptosis, e.g., the mitogen-activated protein kinase (MAPK) family, cyclic AMP-dependent protein kinase (PKA), and protein kinase B (PKB), but their involvement has not been completely characterized and the reader is referred to recent reviews on this topic for more details [Cross et al., 2000].

The term "protein kinase C" (PKC) identifies a family of phospholipid-dependent serine/threonine kinases that are activated by diverse intracellular factors, including diacylglycerol and Ca^{2+}. The various PKC isoforms have been subdivided into three classes: the classical, or conventional, PKCs (α, βI, βII, and γ) that are activated by Ca^{2+} and diacylglycerol; the novel PKCs (δ, ε, η, and θ) that are activated by diacylglycerol, but are Ca^{2+}-independent; the atypical PKCs (λ and ζ) which are insensitive to both Ca^{2+} and diacylglycerol [for review, see Cross et al., 2000; Musashi et al., 2000; Dempsey et al., 2000].

As in the case of Ca^{2+}, PKCs can have a dual role in apoptosis, i.e., activation of specific PKC isoforms may protect or induce cell death [Lavin et al., 1996]. Among the classical PKCs, TPA (12-0-tetradecanoylphorbol-13-acetate) induces cyt c release (and cell death) in U-937 leukemia cells by a PKC β-dependent mechanism [Pandey et al., 2000]. In gastric cancer cells, indomethacin-induced apoptosis is in part mediated by differential regulation of PKC isoform expression and enhanced expression of exogenous PKC β protects against indomethacin-induced apoptosis [Zhu et al., 2000].

Powell et al. [1996] demonstrated that in a cell line derived from a human prostate cancer the presence on the cell membrane of PKC α correlates with spontaneous apoptosis, while its absence is associated with resistance to TPA-induced apoptosis.

Among the novel isoforms, most evidence supports a key role of PKC δ. For example, Lynch et al. [2000] showed that basic fibroblast growth factor inhibits apoptosis of spontaneously immortalized granulosa cells by reducing [Ca^{2+}] changes through a PKC δ-dependent pathway.

PKC δ appears to have a role also in the release of cyt c from mitochondria. Indeed, TPA induces translocation of PKC δ from the cytoplasm to mitochondria and translocation of PKC δ results in release of cyt c and the activation of caspase-3 [Majumder et al., 2000]. Mitochondrial localization is not unique to the PKC δ isoform, since PKC α has been shown to be localized, in some cell types, in mitochondria where it may cause Bcl-2 phosphorylation and suppression of apoptosis [Ruvolo et al., 1998].

Most of the examples described above concern apoptosis induced in vitro, under selected experimental conditions. A particularly interesting phenomenon, of major physiological relevance (that involves both Ca^{2+} and PKC), is that related to the termination of the immune response. To maintain T cell homeostasis, once the antigen has been cleared activated lymphocytes are removed by apoptosis [Russell et al., 1995]. This form of apoptosis involves the TCR-induced expression of the CD95 ligand (CD95L) on the surface of T cells [Brunner et al., 1995; Nagata, 1997]. Once CD95L is expressed on the T cell surface, it induces T cell apoptosis through activation of CD95 [Ashkenazi and Dixit, 1998]. It has been shown that PKC θ in cooperation with calcineurin plays an essential role in regulating CD95 expression and activation-induced cell death [Villalba et al., 1999].

A role in apoptosis appears to also be played by the atypical isoforms. Ceramide, a lipid mediator of apoptosis, increase PKC ζ phosphorylation and activity and the re-

cent demonstration that PKC ζ moves to the nucleus upon ceramide production suggests that it may regulate the transcriptional apparatus during ceramide signaling [Bertolaso et al., 1998].

Finally, direct support for a role of PKC in apoptosis comes from the demonstration that the antibiotic calphostin C (a potent PKC inhibitor) induces rapid apoptosis in human acute lymphoblastic leukemia (ALL) by two additive effects: the inhibition of PKC and the modulation of a cytosolic Ca^{2+} rise. Indeed, calphostin C induces rapid Ca^{2+} mobilization from intracellular stores of ALL cell lines, and its cytotoxicity for ALL cell lines well correlated with the magnitude of this Ca^{2+} signal. In fact, calphostin C-induced death is suppressed by loading the cells with the Ca^{2+} chelator BAPTA [Zhu et al., 1998, 1999].

Calpains

As mentioned above, many biochemical and genetic studies on apoptosis have revealed that intracellular proteases are key players in this process. In particular, early studies have pointed to the primacy of caspase proteases as mediators of the execution phase. More recently, however, there is evidence that proteases other than caspases, in particular, calpains, may also participate in apoptosis. Calpains are intracellular Ca^{2+}-dependent proteases that have been found in virtually all mammalian cells [see Suzuki et al., 1995; Lane et al., 1992; Croall et al., 1991; Carafoli et al., 1998, for reviews]. Calpain substrates include cytoskeletal and associated proteins, membrane receptors and transporters, steroid receptors, alpha- and beta-fodrin, calmodulin-dependent protein kinases, ADP-ribosyltransferase (ADPRT/PARP), and the tau protein [Chan et al. 1999; Wang, 2000].

A direct involvement of calpains in some apoptotic programs has been recently demonstrated. In the neuronal line P19, activation of calpains was shown to be an early event that preceded the appearance of the typical morphological hallmarks of apoptosis and caused degradation of cytoskeletal proteins in the cell death program activated by hydrogen peroxide. In particular, it was demonstrated that calpains were responsible for the degradation of cytoskeletal proteins and preceded the appearance of the typical morphological hallmarks of apoptosis [Ishihara et al., 2000]. In the pancreatic beta-cell line MIN6, excess NO production was proposed to induce apoptosis by causing an increase in intracellular $[Ca^{2+}]$ and activation of calpains [Nakata et al., 1999]. A similar effect was observed in PC12 cells, suggesting a general role of calpains in neuronal (or neuroendocrine) apoptosis under oxidative stress and Ca^{2+} influx [Ray et al., 2000]. Whether the process involving calpains is an alternative to that of caspases has not yet been determined. It should be mentioned, however, that recent results obtained in the U937 cell line suggest that cadmium may induce apoptosis by two independent pathways, the Ca^{2+}-calpains-dependent and the caspase-mitochondria-dependent pathways [Li et al., 2000b].

CONCLUSIONS

The past years have witnessed an explosive expansion in our knowledge of the molecular mechanisms that commit a cell to apoptosis and carry out the complex series of biochemical reactions involved in this process. Numerous and interacting triggers (genetic programs, plasma membrane receptor activation, etc.) and regulatory mechanisms (effector systems, co-factors, inhibitors) have been identified. The cross-talk between molecular actors and signaling mechanisms of apoptosis is today a major research topic, since, on the one hand, defects in apoptosis allow neoplastic and virally infected cells to escape elimination by the immune system, and on the other inappropriate triggering of apoptosis is a cause of serious neurodegenerative diseases (e.g., Alzheimer's and Parkinson's disease). Thus, only complete understanding of how apoptotic signals are conveyed and decoded by different cell types may allow researchers to directly and safely develop a tailored pharmacology of apoptosis and to identify the molecular targets of innovative drugs. In this context, wide interest has been generated by converging evidence demonstrating that the best-characterized intracellular second-messenger, Ca^{2+}, can act as a mediator of apoptosis. This is an important concept, because it implies that the same messenger can decode extracellular stimuli not only into the most diverse patterns of cell response (secretion, contraction, motility, and so on) but also in the major choice between life and death: a Ca^{2+} signal is elicited by both growth factor stimulation (with ensuing cell proliferation) and apoptotic agents (causing cell death). The obvious consequence is that the specificity of the different stimuli must rest either in the complex spatiotemporal mode of Ca^{2+} signaling and/or in the interaction with other transduction pathways. In this review, we have summarized, in the context of the biochemical mechanisms of apoptosis, the increasing evidence supporting a role for Ca^{2+} as a crucial regulator of biochemical pathways of apoptosis and we have highlighted the intracellular effector systems. Although current work by many groups will certainly shed light on these topics and soon make this review obsolete, we hope that this brief summary may prove useful in identifying and testing mechanisms and pharmacological tools.

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REFERENCES

Adams JM, Cory S. 1998. The Bcl-2 protein family: arbiters of cell survival. Science 281:1322–1326.

Alberici A, Moratto D, Benussi L, Gasparini L, Ghidoni R, Gatta LB, Finazzi D, Frisoni GB, Trabucchi M, Growdon JH, Nitsch RM, Binetti G. 1999. Presenilin 1 protein directly interacts with Bcl-2. J Biol Chem 274:30764–30769.

Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. 1996. Human ICE/CED-3 protease nomenclature. Cell 87:171.

Andrade F, Roy S, Nicholson D, Thornberry N, Rosen A, Casciola-Rosen L. 1998. Granzyme B directly and efficiently cleaves several downstream caspase substrates: implications for CTL-induced apoptosis. Immunity 8:451–460.

Ashkenazi A, Dixit VM. 1998. Death receptors: signaling and modulation. Science 281:1305–1308.

Bian X, Hughes FM Jr, Huang Y, Cidlowski JA, Putney JW Jr. 1997. Roles of cytoplasmic Ca2+ and intracellular Ca2+ stores in induction and suppression of apoptosis in S49 cells. Am J Physiol 272:C1241–1249

Beham A, Marin MC, Fernandez A, Herrmann J, Brisbay S, Tari AM, Lopez-Berestein G, Lozano G, Sarkiss M, McDonnell TJ. 1997. Bcl-2 inhibits p53 nuclear import following DNA damage. Oncogene 15:2767–2772.

Bernardi P. 1999a. Mitochondria in muscle cell death. Ital J Neurol Sci 20:395–400.

Bernardi P. 1999b. Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol Rev 79:1127–1155.

Bernardi P, Colonna R, Costantini P, Eriksson O, Fontaine E, Ichas F, Massari S, Nicolli A, Petronilli V, Scorrano L. 1998. The mitochondrial permeability transition. Biofactors 8:273–281.

Berridge MJ, Bootman MD, Lipp P. 1998. Calcium: a life and death signal. Nature 395:645–648.

Bertolaso L, Gibellini D, Secchiero P, Previati M, Falgione D, Visani G, Rizzoli R, Capitani S, Zauli G.1998. Accumulation of catalytically active PKC-zeta into the nucleus of HL-60 cell line plays a key role in the induction of granulocytic differentiation mediated by all-trans retinoic acid. Br J Haematol 100:541–549.

Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. 1995. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. J Biol Chem 270:7795–7798.

Boldin MP, Goncharov TM, Goltsev YV, Wallach D. 1996. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85:803–815.

Bossy-Wetzel E, Newmeyer DD, Green DR. 1998. Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization. EMBO J 17:37–49.

Brunner T, Mogil RJ, LaFace D, Yoo NJ, Machboubi A, Echeverri F, Martin SJ, Force WR, Lynch DH, Ware CF, Green DR. 1995. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature 373:441–444.

Brustugun OT, Fladmark KE, Doskeland SO, Orrenius S, Zhivotovsky B. 1998. Apoptosis induced by microinjection of cytochrome c is caspase-dependent and is inhibited by Bcl-2. Cell Death Differ 5:660–668.

Buckley CD, Pilling D, Henriquez NV, Parsonage G, Threlfall K, Scheel-Toellner D, Simmons DL, Akbar AN, Lord JM, Salmon M. 1999. RGD peptides induce apoptosis by direct caspase-3 activation. Nature 397:534–539.

Carafoli E, Molinari M.1998. Calpain: a protease in search of a function? Biochem Biophys Res Commun 247:193–203.

Ceretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, March CJ, Kronheim SR, et al. 1992. Molecular cloning of the interleukin-1 beta converting enzyme. Science 256:97–100.

Chami M, Gozuacik D, Saigo K, Capiod T, Falson P, Lecoeur H, Urashima T, Beckmann J, Gougeon ML, Claret M, le Maire M, Brechot C, Paterlini-Brechot P. 2000. Hepatitis B virus-related insertional mutagenesis implicates SERCA1 gene in the control of apoptosis. Oncogene 19:2877–2886.

Chan SL, Mattson MP. 1999. Caspase and calpain substrates: roles in synaptic plasticity and cell death. J Neurosci Res 58:167–190.

Chandler JM, Cohen GM, MacFarlane M. 1998. Different subcellular distribution of caspase-3 and caspase-7 following Fas-induced apoptosis in mouse liver. J Biol Chem 273:10815–10818.

Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 81:505–512.

Colussi PA, Harvey NL, Kumar S. 1998. Prodomain-dependent nuclear localization of the caspase-2 (Nedd2) precursor. A novel function for a caspase prodomain. J Biol Chem 273:24535–24542.

Croall DE, DeMartino GN. 1991. Calcium-activated neutral protease (calpain) system: structure, function, and regulation. Physiol Rev 71:813–847.

Crompton M. 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem J 341:233–249.

Crompton M. 2000. Mitochondrial intermembrane junctional complexes and their role in cell death. J Physiol 529:11–21.

Cross TG, Scheel-Toellner D, Henriquez NV, Deacon E, Salmon M, Lord JM. 2000. Serine/threonine protein kinases and apoptosis. Exp Cell Res 256:34–41.

Darmon AJ, Nicholson DW, Bleackley RC. 1995. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature 377:446–448.

Daugas E, Nochy D, Ravagnan L, Loeffler M, Susin SA, Zamzami N, Kroemer G. 2000. Apoptosis-inducing factor (AIF): a ubiquitous mitochondrial oxidoreductase involved in apoptosis. FEBS Lett 476:118–123.

Dempsey EC, Newton AC, Mochly-Rosen D, Fields AP, Reyland ME, Insel PA, Messing RO. 2000. Protein kinase C isozymes and the regulation of diverse cell responses. Am J Physiol Lung Cell Mol Physiol 279:L429–L438.

Desagher S, Martinou JC. 2000. Mitochondria as the central control point of apoptosis. Trends Cell Biol 10:369–377

Dinarello CA. 1998. Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. Ann NY Acad Sci 856:1–11.

Doran E, Halestrap AP. 2000. Cytochrome c release from isolated rat liver mitochondria can occur independently of outer-membrane rupture: possible role of contact sites. Biochem J 348:343–350.

Dowd DR, MacDonald PN, Komm BS, Haussler MR, Miesfeld RL. 1992. Stable expression of the calbindin-D28K complementary DNA interferes with the apoptotic pathway in lymphocytes. Mol Endocrinol 6:1843–1848.

Drappa J, Vaishnaw AK, Sullivan KE, Chu JL, Elkon KB. 1996. Fas gene mutations in the Canale-Smith syndrome, an inherited

lymphoproliferative disorder associated with autoimmunity. N Engl J Med 335:1643–1649.

Du C, Fang M, Li Y, Li L, Wang X. 2000. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 102:33–42.

Duchen MR. 1999. Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. J Physiol 1999 516:1–17.

Duchen MR. 2000. Mitochondria and calcium: from cell signalling to cell death. J Physiol 529:57–68.

Earnshaw WC, Martins LM, Kaufmann SH. 1999. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 68:383–424.

Ellis RE, Yuan JY, Horvitz HR. 1991. Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663–698.

Eskes R, Desagher S, Antonsson B, Martinou JC. 2000. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol Cell Biol 20:929–935.

Foyouzi-Youssefi R, Arnaudeau S, Borner C, Kelley WL, Tschopp J, Lew DP, Demaurex N, Krause KH. 2000. Bcl-2 decreases the free Ca2+ concentration within the endoplasmic reticulum. Proc Natl Acad Sci USA 97:5723–5728

Finucane DM, Waterhouse NJ, Amarante-Mendes GP, Cotter TG, Green DR. 1999. Collapse of the inner mitochondrial transmembrane potential is not required for apoptosis of HL60 cells. Exp Cell Res 251:166–174.

Fisher GH, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, Strober W, Lenardo MJ, Puck JM. 1995. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell 81:935–946.

Fraser AG, James C, Evan GI, Hengartner MO. 1999. Caenorhabditis elegans inhibitor of apoptosis protein (IAP) homologue BIR-1 plays a conserved role in cytokinesis. Curr Biol 9:292–301.

Fruman DA, Mather PE, Burakoff SJ, Bierer BE. 1992. Correlation of calcineurin phosphatase activity and programmed cell death in murine T cell hybridomas. Eur J Immunol 22:2513–2517.

Gallo V, Kingsbury A, Balazs R, Jorgensen OS. 1987. The role of depolarization in the survival and differentiation of cerebellar granule cells in culture. J Neurosci 7:2203–2213.

Glykofrydes D, Niphuis H, Kuhn EM, Rosenwirth B, Heeney JL, Bruder J, Niedobitek G, Muller-Fleckenstein I, Fleckenstein B, Ensser A. 2000. Herpesvirus Saimiri vFLIP provides an antiapoptotic function but is not essential for viral replication, transformation, or pathogenicity. J Virol 74:11919–11927.

Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK, Bromm M, Kazemi-Esfarjani P, Thornberry NA, Vaillancourt JP, Hayden MR. 1996. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. Nat Genet 13:442–449.

Green DR, Reed JC. 1998. Mitochondria and apoptosis. Science 281:1309–1312.

Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming MA, Hayashi N, Higashino K, Okamura H, Nakanishi K, Kurimoto M, Tanimoto T, Flavell RA, Sato V, Harding MW, Livingston DJ, Su MS. 1997. Activation of interferon-gamma-inducing factor mediated by interleukin-1 beta converting enzyme. Science 275:206–209.

Guo Q, Christakos S, Robinson N, Mattson MP. 1998. Calbindin D28k blocks the proapoptotic actions of mutant presenilin 1: reduced oxidative stress and preserved mitochondrial function. Proc Natl Acad Sci USA 95:3227–3232.

He H, Lam M, McCormick TS, Distelhorst CW. 1997. Maintenance of calcium homeostasis in the endoplasmic reticulum by Bcl-2. J Cell Biol 138:1219–1228.

Hengartner MO, Horvitz HR. 1994. Programmed cell death in Caenorhabditis elegans. Curr Opin Genet Dev 4:581–586.

Hetts SW. 1998. To die or not to die: an overview of apoptosis and its role in disease. JAMA 279:300–307.

Hu S, Snipas SJ, Vincenz C, Salvesen G, Dixit VM. 1998. Caspase-14 is a novel developmentally regulated protease. J Biol Chem 273:29648–29453.

Humke EW, Ni J, Dixit VM. 1998. ERICE, a novel FLICE-activatable caspase. J Biol Chem 273:15702–15707.

Ichas F, Mazat JP. 1998. From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. Biochim Biophys Acta 1366:33–50.

Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW. 1999. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 283:70–74.

Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Horster F, Tenkova T, Dikranian K, Olney JW. 2000. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science 287:1056–1060.

Ishihara I, Minami Y, Nishizaki T, Matsuoka T, Yamamura H. 2000. Activation of calpain precedes morphological alterations during hydrogen peroxide-induced apoptosis in neuronally differentiated mouse embryonal carcinoma P19 cell line. Neurosci Lett 279:97–100.

Jurgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC. 1998. Bax directly induces release of cytochrome c from isolated mitochondria. Proc Natl Acad Sci USA 95:4997–5002.

Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. 1993. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res 53:3976–3985.

Kerr JF, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–57.

Kim TH, Zhao Y, Barber MJ, Kuharsky DK, Yin XM. 2000. Bid-induced cytochrome c release is mediated by a pathway independent of mitochondrial permeability transition pore and Bax. J Biol Chem 275:39474–39481.

Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. 1997. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science 275:1132–1136.

Koike T, Martin DP, Johnson EM Jr. 1989. Role of Ca2+ channels in the ability of membrane depolarization to prevent neuronal death induced by trophic-factor deprivation: evidence that levels of internal Ca2+ determine nerve growth factor dependence of sympathetic ganglion cells. Proc Natl Acad Sci USA 86:6421–6425.

Komiyama T, Ray CA, Pickup DJ, Howard AD, Thornberry NA, Peterson EP, Salvesen G. 1994. Inhibition of interleukin-1-beta converting enzyme by the cowpox virus serpin CrmA. An example of cross-class inhibition. J Biol Chem 269:19331–19337.

Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Koths K, Kwiatkowski DJ, Williams LT. 1997.

Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. Science 278:294–298.

Krajewski S, Krajewska M, Ellerby LM, Welsh K, Xie Z, Deveraux QL, Salvesen GS, Bredesen DE, Rosenthal RE, Fiskum G, Reed JC. 1999. Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia. Proc Natl Acad Sci USA 96:5752–5757.

Kroemer G, Reed JC. 2000. Mitochondrial control of cell death. Nat Med 6:513–519.

Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA. 1995. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. Science 267:2000–2003.

Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H, Rakic P, Flavell RA. 1996. Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. Nature 384:368–372.

Kuo TH, Kim HR, Zhu L, Yu Y, Lin HM, Tsang W. 1998. Modulation of endoplasmic reticulum calcium pump by Bcl-2. Oncogene 17:1903–1910.

Kusano H, Shimizu S, Koya RC, Fujita H, Kamada S, Kuzumaki N, Tsujimoto Y. 2000. Human gelsolin prevents apoptosis by inhibiting apoptotic mitochondrial changes via closing VDAC. Oncogene 19:4807–4814.

Lane RD, Allan DM, Mellgren RL. 1992. A comparison of the intracellular distribution of mu-calpain, m-calpain, and calpastatin in proliferating human A431 cells. Exp Cell Res 203:5–16.

Lavin MF, Watters D, Song Q. 1996. Role of protein kinase activity in apoptosis. Experientia 52:979–994.

Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG, Earnshaw WC. 1994. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature 371:346–347.

Li P, Allen H, Banerjee S, et al. 1995. Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. Cell 80:401–411.

Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiate an apoptotic protease cascade. Cell 91:479–489.

Li X, Du L, Darzynkiewicz Z. 2000a. During apoptosis of HL-60 and U-937 cells caspases are activated independently of dissipation of mitochondrial electrochemical potential. Exp Cell Res 257:290–297.

Li M, Kondo T, Zhao QL, Li FJ, Tanabe K, Arai Y, Zhou ZC, Kasuya M. 2000b. Apoptosis induced by cadmium in human lymphoma U937 cells through Ca2+-calpain and caspase- mitochondria-dependent pathways. J Biol Chem 275:39702–39709.

Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. 1991. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 66:807–815.

Liu X, Kim CN, Yang J, Jemmerson R, Wang X. 1996. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 86:147–157.

Lorenzo HK, Susin SA, Penninger J, Kroemer G. 1999. Apoptosis inducing factor (AIF): a phylogenetically oc release from mitochondria in response to activation of cell surface death receptors. Cell 94:481–490.

Lynch K, Fernandez G, Pappalardo A, Peluso JJ. 2000. Basic fibroblast growth factor inhibits apoptosis of spontaneously immortalized granulosa cells by regulating intracellular free calcium levels through a protein kinase Cdelta-dependent pathway. Endocrinology 141:4209–4217.

Ma TS, Mann DL, Lee JH, Gallinghouse GJ. 1999. SR compartment calcium and cell apoptosis in SERCA overexpression. Cell Calcium 26:25–36.

Majumder PK, Pandey P, Sun X, Cheng K, Datta R, Saxena S, Kharbanda S, Kufe D. 2000. Mitochondrial translocation of protein kinase C delta in phorbol ester-induced cytochrome c release and apoptosis. J Biol Chem 275:21793–21796.

Mattson MP, Zhu H, Yu J, Kindy MS. 2000. Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. J Neurosci 20:1358–1364

McFadden G, Graham K, Ellison K, Barry M, Macen J, Schreiber M, Mossman K, Nash P, Lalani A, Everett H. 1995. Interruption of cytokine networks by poxviruses: lessons from myxoma virus. J Leukoc Biol 57:731–738.

Mignotte B, Vayssiere JL. 1998. Mitochondria and apoptosis. Eur J Biochem 252:1–15.

Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB. 1997. Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature 385:353–357.

Mittl PR, Di Marco S, Krebs JF, Bai X, Karanewsky DS, Priestle JP, Tomaselli KJ, Grutter MG.1997. Structure of recombinant human CPP32 in complex with the tetrapeptide acetyl-Asp-Val-Ala-Asp fluoromethyl ketone. J Biol Chem 272:6539–6547.

Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J. 1993. Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the C. elegans cell death gene ced-3. Cell 75:653–660.

Munday NA, Vaillancourt JP, Ali A, Casano FJ, Miller DK, Molineaux SM, Yamin TT, Yu VL, Nicholson DW. 1995. Molecular cloning and pro-apoptotic activity of ICErelII and ICErelIII, members of the ICE/CED-3 family of cysteine proteases. J Biol Chem 270:15870–15876.

Musashi M, Ota S, Shiroshita N. 2000. The role of protein kinase C isoforms in cell proliferation and apoptosis. Int J Hematol 72:12–19.

Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, Dixit VM. 1996. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 85:817–827.

Nagata S. 1997. Apoptosis by death factor. Cell 88:355–365.

Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J. 2000. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 403:98–103.

Nakata M, Uto N, Maruyama I, Yada T. 1999. Nitric oxide induces apoptosis via Ca2+-dependent processes in the pancreatic beta-cell line MIN6. Cell Struct Funct 24:451–455.

Nasir J, Theilmann JL, Vaillancourt JP, Munday NA, Ali A, Scherer S, Beatty B, Nicholson DW, Hayden MR 1997. Interleukin-1 beta-converting enzyme (ICE) and related cell death genes ICErel-II and ICE-rel-III map to the same PAC clone at band 11q22.2-22.3. Mamm Genome 8:611–613.

Nicholson DW. 1999. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 6:1028–1042.

Nicholson DW, Thornberry NA. 1997. Caspases: killer proteases. Trends Biochem Sci 22:299–306.

Nicotera P, Orrenius S. 1992. Ca2+ and cell death. Ann NY Acad Sci 648:17–27.

Nicotera P, Leist M, Manzo L. 1999. Neuronal cell death: a demise with different shapes. Trends Pharmacol Sci 20:46–51.

Oberhammer FA, Hochegger K, Froschl G, Tiefenbacher R, Pavelka M. 1994. Chromatin condensation during apoptosis is accompanied by degradation of lamin A+B, without enhanced activation of cdc2 kinase. J Cell Biol 126:827–837.

Ona VO, Li M, Vonsattel JP, Andrews LJ, Khan SQ, Chung WM, Frey AS, Menon AS, Li XJ, Stieg PE, Yuan J, Penney JB, Young AB, Cha JH, Friedlander RM. 1999. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. Nature 399:263–267.

Pandey P, Nakazawa A, Ito Y, Datta R, Kharbanda S, Kufe D. 2000. Requirement for caspase activation in monocytic differentiation of myeloid leukemia cells. Oncogene 19:3941–3947.

Petit PX, Goubern M, Diolez P, Susin SA, Zamzami N, Kroemer G. 1998. Disruption of the outer mitochondrial membrane as a result of large amplitude swelling: the impact of irreversible permeability transition. FEBS Lett 426:111–116.

Petronilli V, Nicolli A, Costantini P, Colonna R, Bernardi P. 1994. Regulation of the permeability transition pore, a voltage-dependent mitochondrial channel inhibited by cyclosporin A. Biochim Biophys Acta 1187:255–259.

Pinton P, Ferrari D, Magalhaes P, Schulze-Osthoff K, Di Virgilio F, Pozzan T, Rizzuto R. 2000. Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx In bcl-2-overexpressing cells. J Cell Biol 148:857–862.

Powell CT, Brittis NJ, Stec D, Hug H, Heston WD, Fair WR. 1996. Persistent membrane translocation of protein kinase C alpha during 12-0-tetradecanoylphorbol-13-acetate-induced apoptosis of LNCaP human prostate cancer cells. Cell Growth Differ 7:419–428.

Raff MC, Barres BA, Burne JF, Coles HF, Ishizaki Y, Jacobson MD. 1993. Programmed cell death and the control of cell survival. Science 262:695–700.

Rano TA, Timkey T, Peterson EP, Rotonda J, Nicholson DW, Becker JW, Chapman KT, Thornberry NA.1997. A combinatorial approach for determining protease specificities: application to interleukin-1beta converting enzyme (ICE). Chem Biol 4:149–155.

Rao L, Perez D, White E. 1996. Lamin proteolysis facilitates nuclear events during apoptosis. J Cell Biol 135:1441–1455.

Rao A, Luo C, Hogan PG. 1997. Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol 15:707–747.

Rasper DM, Vaillancourt JP, Hadano S, Houtzager VM, Seiden I, Keen SL, Tawa P, Xanthoudakis S, Nasir J, Martindale D, Koop BF, Peterson EP, Thornberry NA, Huang J, MacPherson DP, Black SC, Hornung F, Lenardo MJ, Hayden MR, Roy S, Nicholson DW. 1998. Cell death attenuation by "Usurpin", a mammalian DED-caspase homologue that precludes caspase-8 recruitment and activation by the CD95 (Fas, APO-1) receptor complex. Cell Death Differ 5:271–288.

Ray CA, Pickup DJ. 1996. The mode of death of pig kidney cells infected with cowpox virus is governed by the expression of the crmA gene. Virology 217:384–391.

Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath PR, Salvesen GS, Pickup DJ. 1992. Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. Cell 69:597–604.

Ray SK, Fidan M, Nowak MW, Wilford GG, Hogan EL, Banik NL. 2000. Oxidative stress and Ca2+ influx upregulate calpain and induce apoptosis in PC12 cells. Brain Res 852:326–334.

Rizzuto R, Bernardi P, Pozzan T. 2000. Mitochondria as all-round players of the calcium game. J Physiol 529:37–47.

Rotonda J, Nicholson DW, Fazil KM, Gallant M, Gareau Y, Labelle M, Peterson EP, Rasper DM, Ruel R, Vaillancourt JP, Thornberry NA, Becker JW. 1996. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. Nat Struct Biol 3:619–625.

Rusnak F, Mertz P. 2000. Calcineurin: form and function. Physiol Rev 80:1483–1521.

Russell JH. 1995. Activation-induced death of mature T cells in the regulation of immune responses. Curr Opin Immunol 7:382–388.

Ruvolo PP, Deng X, Carr BK, May WS.1998. A functional role for mitochondrial protein kinase C alpha in Bcl2 phosphorylation and suppression of apoptosis. J Biol Chem 73:25436–25442.

Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC. 1997. Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci USA 94:5113–5118.

Schotte P, Van Criekinge W, Van de Craen M, Van Loo G, Desmedt M, Grooten J, Cornelissen M, De Ridder L, Vandekerckhove J, Fiers W, Vandenabeele P, Beyaert R. 1998. Cathepsin B-mediated activation of the proinflammatory caspase-11. Biochem Biophys Res Commun 251:379–387.

Shibasaki F, McKeon F. 1995. Calcineurin functions in Ca(2+)-activated cell death in mammalian cells. J Cell Biol 131:735–743.

Skulachev VP. 1996. Why are mitochondria involved in apoptosis? Permeability transition pores and apoptosis as selective mechanisms to eliminate superoxide-producing mitochondria and cell. FEBS Lett 397:7–10.

Srinivasula SM, Datta P, Fan XJ, Fernandes-Alnemri T, Huang Z, Alnemri ES. 2000. Molecular determinants of the caspase-promoting activity of Smac/DIABLO and its role in the death receptor pathway. J Biol Chem 275:36152–36157.

Susin SA, Lorenzo HK, Zamzami N, Marzo I, Brenner C, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G. 1999a. Mitochondrial release of caspase-2 and -9 during the apoptotic process. J Exp Med 189:381–394.

Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G. 1999b. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397:441–446.

Suzuki K, Sorimachi H, Yoshizawa T, Kinbara K, Ishiura S.1995. Calpain: novel family members, activation, and physiologic function. Biol Chem Hoppe Seyler 376:523–529.

Szalai G, Krishnamurthy R, Hajnoczky G. 1999. Apoptosis driven by IP(3)-linked mitochondrial calcium signals. EMBO J 18:6349–6361.

Tartaglia LA, Ayres TM, Wong GH, Goddel DV. 1993. A novel domain within the 55 kd TNF receptor signals cell death. Cell 74:845–853.

Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer JL, Schroter M, Scaffidi C, Krammer PH, Peter ME, Tschopp J. 1997. Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature 386:517–521.

Thompson CB. 1995. Apoptosis in the pathogenesis and treatment of disease. Science 267:1455–1462.

Thornberry NA, Bull HG, Calayacay JR, et al. 1992. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature 356:768–774.

Thornberry NA, Lazebnik Y. 1998. Caspases: enemies within. Science 28:1312–1316.

Uren AG, Vaux DL. 1996. Molecular and clinical aspects of apoptosis. Pharmacol Ther 72:37–50.

Vander Heiden MG, Chandel NS, Williamson EK, Schumacker PT,

Thompson CB. 1997. Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria. Cell 91:627–637.

Vaux DL, Korsmeyer SJ. 1999. Cell death in development. Cell 96: 245–254.

Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ, Vaux DL. 2000. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 102:43–53.

Villalba M, Kasibhatla S, Genestier L, Mahboubi A, Green DR, Altman A. 1999. Protein kinase c theta cooperates with calcineurin to induce Fas ligand expression during activation-induced T cell death. J Immunol 163:5813–5819.

Wang KK. 2000. Calpain and caspase: can you tell the difference? Trends Neurosci 23:20–26.

Wang L, Miura M, Bergeron L, Zhu H, Yuan J. 1994. *Ich-1*, and *Ice/ced-3*-related gene, encodes both positive and negative regulators of programmed cell death. Cell 78:739–750.

Wang S, Miura M, Jung YK, Zhu H, Li E, Yuan J. 1998. Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. Cell 92:501–509.

Wang J, Zheng L, Lobito A, Chan FK, Dale J, Sneller M, Yao X, Puck JM, Straus SE, Lenardo MJ. 1999a. Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell 98:47–58.

Wang HG, Pathan N, Ethell IM, Krajewski S, Yamaguchi Y, Shibasaki F, McKeon F, Bobo T, Franke TF, Reed JC. 1999b. Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. Science 284:339–343.

Wilson KP, Black JA, Thomson JA, et al. 1994. Structure and mechanism of interleukin-1 beta converting enzyme. Nature 370:270–275.

Yano S, Tokumitsu H, Soderling TR. 1998. Calcium promotes cell survival through CaM-K kinase activation of the protein-kinase-B pathway. Nature 396:584–587.

Yeh WC, Pompa JL, McCurrach ME, Shu HB, Elia AJ, Shahinian A, Ng M, Wakeham A, Khoo W, Mitchell K, El-Deiry WS, Lowe SW, Goeddel DV, Mak TW. 1998. FADD: essential role for embryo development and signalling from some, but not all, inducers of apoptosis. Science 279:1954–1958.

Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, Kornfeld H. 1998. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem 273:1144–1149.

Zheng TS, Hunot S, Kuida K, Flavell RA. 1999. Caspase knockouts: matters of life and death. Cell Death Differ 6:1043–1053.

Zhivotovsky B, Orrenius S, Brustugun OT, Doskeland SO. 1998. Injected cytochrome c induces apoptosis. Nature 391:449–450.

Zhou YP, Teng D, Dralyuk F, Ostrega D, Roe MW, Philipson L, Polonsky KS. 1998. Evidence for the role of intracellular Ca2+ stores and arachidonic acid metabolism. J Clin Invest 101:1623–1632.

Zhu DM, Narla RK, Fang WH, Chia NC, Uckun FM. 1998. Calphostin C triggers calcium-dependent apoptosis in human acute lymphoblastic leukemia cells. Clin Cancer Res 4:2967–2976.

Zhu DM, Fang WH, Narla RK, Uckun FM. 1999. A requirement for protein kinase C inhibition for calcium-triggered apoptosis in acute lymphoblastic leukemia cells. Clin Cancer Res 5:355–360.

Zhu LP, Yu XD, Ling S, Brown RA, Kuo TH. 2000a. Mitochondrial Ca(2+) homeostasis in the regulation of apoptotic and necrotic cell deaths. Cell Calcium 28:107–117.

Zhu GH, Wong BC, Slosberg ED, Eggo MC, Ching CK, Yuen ST, Lai KC, Soh JW, Weinstein IB, Lam SK. 2000b. Overexpression of protein kinase C-beta1 isoenzyme suppresses indomethacin-induced apoptosis in gastric epithelial cells. Gastroenterology 118:507–514.

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. 1997. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 90:405–413.