Journal of Bioenergetics and Biomembranes, Vol. 31, No. 4, 1999

Mitochondria at the Crossroad of Apoptotic Cell Death

Kenneth Thress,[1] Sally Kornbluth,[1,2] and Jesse J. Smith[1]

In the past few years, it has become widely appreciated that apoptotic cell death generally involves activation of a family of proteases, the caspases, which undermine the integrity of the cell by cleavage of critical intracellular substrates. Caspases, which are synthesized as inactive zymogens, are themselves caspase substrates and this cleavage leads to their activation. Hence, the potential exists for cascades of caspases leading to cell death. However, it has been recently recognized that another, perhaps more prominent route to caspase activation, involves the mitochondria. Upon receipt of apoptotic stimuli, either externally or internally generated, cells initiate signaling pathways which converge upon the mitochondria to promote release of cytochrome C to the cytoplasm; cytochrome *c*, thus released, acts as a potent cofactor in caspase activation. Even cell surface "death receptors" such as Fas, which can trigger direct caspase activation (and potentially a caspase cascade), appear to utilize mitochondria as part of an amplification mechanism; it has been recently demonstrated that activated caspases can cleave key substrates to trigger mitochondrial release of cytochrome *c*, thereby inducing further caspase activation and amplifying the apoptotic signal. Therefore, mitochondria play a central role in apoptotic cell death, serving as a repository for cytochrome *c*.

KEY WORDS: Mitochondria; apoptosis; caspases; cytochrome *c*; Fas; bcl-2.

INTRODUCTION

Apoptosis is a program of cellular suicide critical for the development and homeostasis of multicellular organisms. Whereas necrotic cell death elicits an inflammatory response that injures neighboring tissue, the apoptotic process is used by multicellular organisms to rid themselves of harmful or superfluous cells without damaging adjacent, healthy cells. Apoptotic cell death can be initiated by a variety of stimuli acting through a myriad of signaling pathways. However, data accumulated over the past 5 years suggests that many of these pathways converge upon the mitochondria. These organelles, responsible for the bulk of the cell's energy production, have a "dark side;" through several distinct pathways, they participate in apoptotic cell killing.

THE MITOCHONDRIA IN NONAPOPTOTIC CELL DEATH

Because mitochondria are the primary agents of ATP production in the cell, any event that grossly disrupts mitochondrial function will, almost inevitably, lead to cell death. Such events, most commonly a result of accidental cellular damage, lead to electron transport dysfunction, ATP depletion, and necrotic cell death. In contrast, most apoptotic pathways do not invoke an initial decrease in ATP levels. In fact, it has been shown in certain systems that ATP is required for propagation of an apoptotic death signal (Eguchi *et al.*, 1997).

Mitochondria of all aerobic cells produce superoxide anions and other reactive oxygen species as normal byproducts of electron transfer (Fridovich, 1978). Excessive ROS production has long been known to induce necrotic cell death by causing massive oxidative

[1] Department of Pharmacology and Cancer Biology, Duke University Medical Center, C366 LSRC, Research Drive, Durham, NC 27710.
[2] Author to whom all correspondence should be sent (email: komb001@mc.duke.edu).

0145-479X/99/0800-0321$16.00/0

damage to cellular components (Mignotte and Vayssiere, 1998). Consequently, cells employ antioxidants and other safeguards to keep these potentially dangerous molecules in check (Halliwell and Gutteridge, 1990). Recently, data has accrued to suggest that ROS may play a role in apoptotic, as well as necrotic cell death. In particular, it was shown that apoptosis can be inhibited or delayed by antioxidants; further, the levels of ROS increase during some types of apoptotic cell death (Bredesen, 1995; Greenlund *et al.*, 1995; Quillet-Mary *et al.*, 1997). Conversely, it has been reported that some apoptotic stimuli can function in the near absence of oxygen where there would be almost no ROS (Jacobson and Raff, 1995; Shimizu *et al.*, 1995). Even in instances where a rise in ROS does accompany apoptosis, however, this may be a late event in the apoptotic process (Kroemer *et al.*, 1995), reflecting the final stages of apoptotic cellular degeneration.

MITOCHONDRIAL RELEASE OF PROAPOPTOTIC REGULATORS

While the roles of ROS and electron transport disruption in apoptosis are still unclear, there is little question that mitochondria do participate actively in apoptotic cell death through the release of proapoptotic (apoptogenic) factors. By far, the most widely studied of these factors is cytochrome *c*, as evidenced by over 100 published reports of its apoptotic role in little over 2 years. Using cell-free extracts for the reconstitution of apoptotic processes, X. Wang and colleagues purified three apoptotic protease activating factors (Apaf 1–3), which were required for *in vitro* apoptosis (Li *et al.*, 1997; Liu *et al.*, 1996; Zou *et al.*, 1997). One of these factors (Apaf-2) was identified as cytochrome *c*. Subsequently, it was shown in a variety of different systems that cytochrome *c* is released from mitochondria following propagation of a "death" signal (e.g., see below and Jurgensmeier *et al.*, 1998; Kluck *et al.*, 1997a). Cytochrome *c* may not be the sole factor of importance released from the mitochondria in response to apoptotic stimuli. Indeed, a novel apoptotic protein, termed AIF (apoptosis initiating factor) was also found to translocate from mitochondria to the cytosol following proapoptotic signaling (Susin et al., 1996, 1997, 1999).

CONSEQUENCES OF CYTOCHROME *c* RELEASE

It is widely accepted that many, if not all, apoptotic cell deaths are ultimately achieved through activation of a family of proteases, known as caspases. Proteolytic cleavage of key caspase targets undermines the structural integrity of the cell, ultimately leading to cell death. For example, it has been shown that cleavage of nuclear lamins by caspases is critical for the apoptotic dismantling of the nucleus, since mutant variants of lamins, which are resistant to cleavage, prevent this process. It is likely that similar cleavage of other specific targets lead to all of the morphological manifestations of apoptosis.

Caspases are synthesized as inactive zymogens containing N-terminal-prodomains, which are proteolytically removed from the mature protease. Since removal of the prodomains renders the caspases active, prodomain cleavage is an important regulatory point in implementation of the apoptotic program. This activation can be achieved by two different mechanisms: (1) Cleavage in *trans* by another caspase molecule. The sequences at the junction of the prodomains and protease-encoding regions of the caspases are themselves caspase substrates. This allows the formation of "protease cascades," whereby one caspase activates another, and so on, leading to amplification of the apoptotic signal (reviewed in Martins and Earnshaw, 1997); (2) binding of "adaptor proteins" to the prodomains. This is believed to facilitate intramolecular cleavage and activation of the caspases. In several cases, it has been shown that overproduction of prodomain-interacting proteins can trigger apoptosis via binding and activation of their cognate caspases (Boldin *et al.*, 1995, 1996; Chinnaiyan *et al.*, 1995; Duan *et al.*, 1997; Muzio *et al.*, 1996).

Since the activity of AIF, described above, is inhibitable by ZVAD-fmk, a broad-spectrum caspase inhibitor, AIF may act directly on a caspase, or be a caspase itself. In the case of cytochrome *c*, however, it is now understood that cytochrome *c* released from mitochondria serves as a cofactor in cytoplasmic caspase activation. Specifically, cytochrome *c* can bind to the apoptogenic factor, Apaf-1, which, in turn, leads to processing and activation of one of the caspase family members, caspase 9 (Li *et al.*, 1997). This can trigger processing of other, effector caspases, ultimately leading to cell death (Fig. 1).

THE HIGH ROAD AND THE LOW ROAD: TWO ROUTES TO CASPASE ACTIVATION

Among the most potent and direct triggers of apoptotic cell death are extracellular ligands, which engage cell surface "death receptors." Death signaling

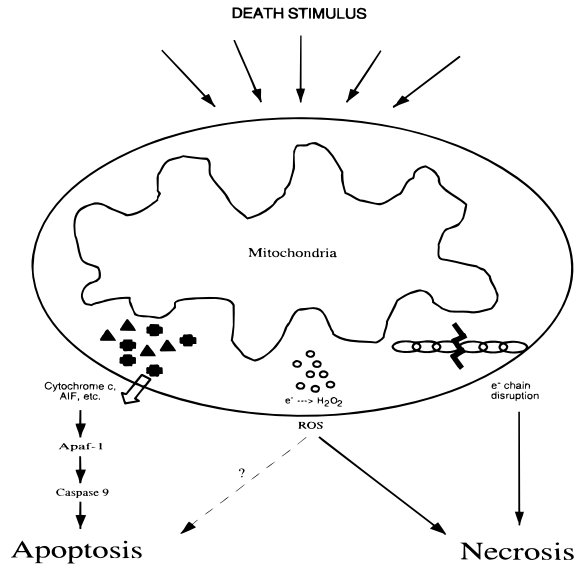

Fig. 1. Mitochondria in cell death. Mitochondria are targets of apoptotic signaling pathways and serve as a repository of apoptogenic molecules. Cytochrome *c*, once released from the mitochondria, acts as a cofactor in the activation of apoptotic proteases, the caspases. ROS play a role in necrotic cell death, but their role in apoptotic cell death remains controversial.

from these receptors can involve both types of caspase activation described above and illustrates clearly how mitochondria serve as amplifiers in propagation of the apoptotic signal. Like other death receptors, one of the founding members of this family, the Fas receptor, oligomerizes in response to binding of its ligand. This oligomerization, in turn, facilitates the formation of a multiprotein complex called the death-inducing signaling complex, or DISC (Medema *et al.*, 1997; Muzio *et al.*, 1996). Within the DISC, the cytoplasmic domain of the Fas receptor is bound to an adaptor molecule (FADD) via protein–protein interaction motifs, termed death domains. Also present in this complex is the zymogenic form of a caspase, caspase-8, bound, via its prodomain region, to the "death-effector domain" of FADD (Medema *et al.*, 1997). Recruitment of procaspase 8 to the DISC is sufficient to allow autoprocessing and consequent activation of protease activity. It is at this point that the road forks and two distinct pathways, described below, can be taken to cell death.

Working in a cell-free apoptotic system consisting of isolated cytoplasm from *Xenopus* eggs and reconstituted nuclei, we noted that purified, active caspase-8 was incapable of inducing a full-blown apoptotic phenotype in the absence of mitochondria (Kuwana *et al.*, 1998). In particular, the apoptotic internucleosomal DNA cleavage was not accompanied by the wholesale nuclear fragmentation normally seen during Fas-induced apoptosis. Reconstitution of mitochondria allowed full apoptosis to proceed, suggesting that active caspase-8 was insufficient, at the doses added, to produce the sort of robust caspase cascade required to trigger apoptosis independently of mitochondria. Further analysis revealed that low doses of caspase-8 could efficiently promote activation of a downstream effector caspase (a caspase 3-like activity), only in the presence of mitochondria. This reinforced the idea that active caspase-8, in this context, was relatively poor at triggering a caspase cleavage cascade. Rather, we found that small amounts of active caspase-8 could trigger mitochondrial cytochrome *c* release; as a consequence, downstream cytoplasmic caspases were potently activated, allowing full apoptosis. This suggested that even small amounts of active caspase-8, generated in response to Fas engagement, could trigger apoptosis once cytochrome *c* was released from its storage site in the intermembrane space of the mitochondria. *In vitro*, at higher doses, caspase-8 could induce some caspase-3 activity in the absence of mitochondria, raising the possibility that the concentration of caspase-8 available for activation at the DISC might determine whether mitochondrial-dependent or -independent (caspase cascade) pathways are utilized during Fas-induced apoptosis (see Fig. 2).

Further evidence for the *in vivo* existence of both mitochondria-dependent and -independent pathways of Fas-induced apoptosis was recently reported by Peters and colleagues (Scaffadi *et al.*, 1998). This group identified two cell types, which used, almost exclusively, one of two Fas signaling pathways. In cell type I, Fas receptor engagement induces a vast activation of caspase-8, such that this molecule can directly activate downstream caspases. In cell type II, Fas receptor engagement induces a level of caspase-8 processing insufficient to directly activate downstream caspases. Therefore, in these cells, amplification of Fas signaling through mitochondrial release of cytochrome *c* is required to induce apoptosis.

Bcl-2 FAMILY MEMBERS: ROAD BLOCKS AND CROSSING GUARDS FOR CYTOCHROME *c*

While we found that caspase-8 could induce mitochondrial release of cytochrome *c* in a cell-free system, this effect was not direct; in the absence of cytosolic factors, caspase-8 had no effect on isolated mitochondria. These data suggested that caspase-8 must cleave

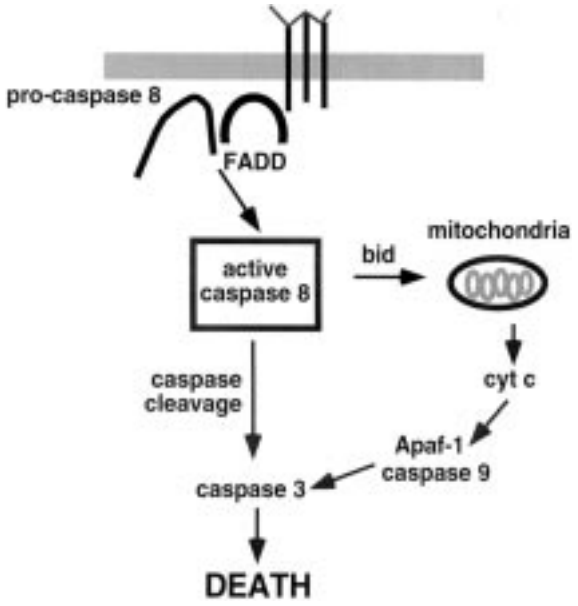

Fig. 2. The Fas receptor and pathways to cell death. After engagement of Fas by Fas ligand, the adaptor protein FADD binds to the intracellular domain of the oligomerized receptor. FADD then engages procaspase-8, leading to its activation. In some cell types, active caspase-8 can initiate a caspase cleavage cascade to trigger cell death. However, caspase-8 can also trigger mitochondrial cytochrome *c* release through cleavage of the Bcl-2 relative, bid. Therefore, the mitochondria can serve as an amplifier of the apoptotic signal generated by Fas engagement. In cell types with low levels of caspase-8 activation, the mitochondria-dependent pathway predominates.

one or more cytosolic factors to trigger mitochondrial cytochrome *c* release. Indeed, two groups have independently identified Bid, a proapoptotic member of the Bcl-2 family of apoptotic regulators, as the caspase-8 substrate critical for this effect (Li *et al.*, 1998; Luo *et al.*, 1998). Following caspase-8-induced cleavage in response to Fas or tumor necrosis factor (TNF), the C-terminal portion of Bid was shown to translocate from the cytoplasm to the mitochondria, triggering subsequent cytochrome *c* release.

Although caspase cleavage of Bid triggers cytochrome *c* release, it is clear that there must be non-caspase-dependent ways to activate cytochrome *c* release. Under a number of different circumstances, cytochrome *c* release precedes any detectable activation of caspases, making cytochrome *c* the primary trigger for caspase activation. For example, we have found that a central regulator of apoptosis in *Drosophila melanogaster*, reaper, will induce mitochondrial cytochrome *c* release in our cell-free system in the absence of caspase activation (Evans *et al.*, 1997). Reaper, like caspase-8 does not appear to act directly on the mitochondria. Cytosolic factors, in addition to reaper, are required for cytochrome *c* release, although these factors do not appear to be caspases/caspase substrates. In an attempt to identify such factors, we recently reported identification of a novel protein, Scythe, which is required for mitochondrial cytochrome *c* release and apoptosis induced *in vitro* by reaper (Thress *et al.*, 1998). While a truncated variant of Scythe can induce apoptosis independently of reaper, it, too, requires additional cytosolic factors to induce cytochrome *c* release; reaper and full-length Scythe, acting together, are also incapable of triggering cytochrome *c* release in the absence of accessory cytosolic factors. While we do not know the proximal trigger of cytochrome *c* release in response to reaper/Scythe, bcl-2 family members are excellent candidates.

Bcl-2 family members may be pro- or antiapoptotic and it is possible that they all exert their effects at the mitochondria. All Bcl-2 family members, both proapoptotic (e. g., Bax, Bid, Bad) and antiapoptotic (e. g., Bcl-2, Bcl-xL), either constitutively reside on or are translocated to mitochondria membranes (Adams and Cory, 1998). Moreover, Bcl-2 has been shown to exert its antiapoptotic effects, at least in part, through preventing mitochondrial cytochrome *c* release (Yand *et al.*, 1997; Kluck *et al.*, 1997a); the proapoptotic Bax can cause cytochrome *c* release which is antagonized by Bcl-2 (Gross *et al.*, 1998; Rosse *et al.*, 1998).

WHICH EXIT FOR CYTOCHROME *c*?

It is not yet entirely clear how Bcl-2 family members regulate cytochrome *c* release. The crystal structure of one Bcl-2 family member, Bcl-xL, revealed marked structural homology to a family of bacterial toxins known to have distinct pore-forming capabilities (Muchmore *et al.*, 1996). Indeed, Bcl-2 family members have been shown to be capable of forming ion-conducting pores (Antonsson *et al.*, 1997; Minn *et al.*, 1997). This has prompted the suggestion that Bcl-2 family members might themselves form pores in the mitochondrial membrane through which cytochrome *c* might pass (Reed, 1997). However, the Bcl-2, Bax, and Bcl-XL pores formed in synthetic membranes appear to be too small to allow for passage of anything as large as cytochrome *c*.

An alternative and attractive hypothesis for the release of cytochrome involves the collapse of the

mitochondrial inner transmembrane potential by the opening of large conductance channels known as permeability transition pores (PTP) (Kroemer *et al.*, 1997). In the normal, nonapoptotic cell, there exists a H^+ gradient across the inner mitochondrial membrane, which results in a transmembrane potential. Different apoptotic signals, however, have been shown to disrupt this potential by initiating the opening of PTP (Petit *et al.*, 1996; Zamzami *et al.*, 1995). Pore opening leads to osmotic expansion of the matrix of the mitochondria because of its high solute concentration. Continued matrix swelling eventually leads to mitochondrial membrane rupture and release of apoptogenic molecules, such as cytochrome *c* (Petit *et al.*, 1998). What, then, is the connection between the Bcl-2 superfamily and the PTP? A recent report, has shown that the proapoptotic regulator Bax can associate both physically and functionally with a component of the PTP, adenine nucleotide translocator (ANT). Cooperation between these two proteins was found to be both necessary and sufficient to cause channel formation in artificial membranes (Marzo *et al.*, 1998). These and other data suggest that proteins of the PTP may be able to synergize with proapoptotic members of the Bcl-2 family to increase mitochondrial permeability and cell death. These data, while compelling, are certainly not the complete story. Indeed, several reports have shown that cytochrome *c* release and caspase activation can occur *prior* to a loss in mitochondrial transmembrane potential (Bossy-Wetzel *et al.*, 1998; Kluck *et al.*, 1997b, Vander Heiden *et al.*, 1997).

Additional experimentation will be required to determine the precise mechanisms of cytochrome *c* release. For example, the caspase-8 substrate Bid may modulate the PTP or other aspects of mitochondrial function. If, indeed, apoptotic stimuli like reaper utilize Bcl-2 family members to trigger mitochondrial cytochrome *c* release, then there must be mechanisms other than cleavage that can activate these molecules. Perhaps proteins like reaper/Scythe can modulate the conformation of Bcl-2 family members, thereby mimicking their cleavage by caspases. Identification of regulators which modulate the ability of Bcl-2 family members to trigger or inhibit cytochrome *c* release and identification of additional factors, which modulate cytochrome *c* release, will no doubt continue to be an active focus of research for those interested in how, when, and under what circumstances cells die by apoptosis.

REFERENCES

Adams, J. M., and Cory, S. (1998). *Science* **281**, 1322–1326.

Antonsson, B., Conti, F., Ciavatta, A., Montessuit, S., Lewis, S., Martinou, I.,Bemasconi, L., Bernard, A., Mermod, J. J., Mazzei, G., Maundrell, K., Gambale, F., Sadoul, R., and Martinou, J. C. (1997). *Science* **277**, 370–372.

Boldin, M. P., Varfolomeev, E. E., Pancer, Z., Mett, I. L., Camonis, J. H., and Wallach, D. (1995). *J. Biol. Chem.* **270**, 7795–7798.

Boldin, M. P., Goncharov, T. M., Goltsev, Y. V., and Wallach, D. (1996). *Cell* **85** 803–815.

Bossy-Wetzel, E., Newmeyer, D. D., and Green, D. R. (1998). *EMBO J.* **17**, 37–49.

Bredesen, D. E. (1995). *Ann. Neurol.* **38**, 839–851.

Chinnaiyan, A., K. O'Rourke, M. Tewari, and V. Dixit (1995). *Cell* **81**, 505–512.

Duan, H., and Dixit, V. M. (1997). *Nature* (*London*) **385**, 86–89.

Eguchi, Y., Shimizu, S., and Tsujimoto, Y. (1997). *Cancer Res.* **57**, 1835–1840.

Evans, E. K., Kuwana, T., Strum, S. L., Smith, J. J., Newmeyer, D. D., and Kornbluth, S. (1997). *EMBO J.* **16**, 7372–7381.

Fridovich, I. (1978). *Science* **201**, 875–880.

Greenlund, L. J., Deckwerth, T. L., and Johnson, E. M., Jr. (1995). *Neuron* **14**, 303–315.

Gross, A., Jockel, J., Wei, M. C., and Korsmeyer, S. J. (1998). *EMBO J.* **17**, 3878–3885.

Halliwell, B., and Gutteridge, J. M. (1990). *Methods Enzymol.* **186**, 1–85.

Jacobson, M. D., and Raff, M. C. (1995). *Nature* (*London*) **374**, 814–816.

Jurgensmeier, J. M., Xie, Z., Deveraux, Q., Ellerby, L., Bredesen, D., and Reed, J. C. (1998). *Proc. Natl. Acad. Sci. U.S.* **95**, 4997–5002.

Kluck, R. M., Bossy-Wetzel, E., Green, D. R., and Newmeyer, D. D. (1997a) *Science* **275**, 1132–1136.

Kluck, R. M., Martin, S. J., Hoffman, B. M., Zhou, J. S., Green, D. R., and Newmeyer, D. D. (1997b). *EMBO J.* **16**, 4639–4649.

Kroemer, G., Petit, P., Zamzami, N., Vayssiere, J. L., and Mignotte, B. (1995). *FASEB J.* **9**, 1277–1287.

Kroemer, G. Zamzami, N., and Susin, S. A. (1997). *Immunol Today* **18**, 44–51.

Kuwana, T., J. J. Smith, M. Muzio, V. Dixit, D. D. Newmeyer, and S. Kornbluth.(1998). *J. Biol. Chem.* **273**, 16589–16594.

Li, H., Zhu, H., Xu, C. J., and Yuan, J. (1998). *Cell* **94**, 491–501.

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., and Wang, X. (1997). *Cell* **91**, 479–489.

Liu, X., Kim, C. N., Yang, J., Jemmerson, R., and Wang, X. (1996). *Cell* **86**, 147–157.

Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998). *Cell* **94**, 481–490.

Martins, L. M. and Earnshaw, W. C. (1997). *Trends Cell Biol.* **7**, 111–114.

Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J. M., Susin, S. A., Vieira, H. L., Prevost, M. C., Xie, Z., Matsuyama, S., Reed, J. C. and Kroemer, G. (1998). *Science* **281**, 2027–2031.

Medema, J. P,. Scaffadi, C., Kischkel, F. C., Shevchenko, A., Mann, M., Krammer, P. H., and Peter, M. E. (1997). *EMBO J.* **16**, 2794–2804.

Mignotte, B., and Vayssiere, J. L. (1998). *Eur. J. Biochem.* **252**, 1–15.

Minn, A. J., Velez, P., Schendel, S. L., Liang, H., Muchmore, S. W., Fesik, S. W., Fill, M., and Thompson, C. B. (1997). *Nature* (*London*) **385**, 353–357.

Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S. L., Ng, S. L., and Fesik, S. W. (1996). *Nature* (*London*) **381**, 335–341.

Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C. Bretz, J. D., Zhang, M. Gentz, R., Mann, M., Krammer, P. H., Peter, M., and Dixit, V. M. (1996). *Cell* **85**, 817–827.

Petit, P. X., Goubern, M., Diolez, P., Susin, S. A., Zamzami, N., and Kroemer, G.(1998). *FEBS Lett.* **426**, 111–116.

Petit, P. X., Susin, S. A., Zamzami, N., Mignotte, B., and Kroemer, G. (1996). *FEBS Lett.* **396**, 7–13.

Quillet-Mary, A., Jaffrezou, J. P., Mansat, V., Bordier, C., Naval, J., and Laurent, G. (1997). *J. Biol. Chem.* **272**, 21388–21395.

Reed, J. C. (1997). *Cell* **91**, 559–562.

Rosse, T., Olivier, R., Monney, L., Rager, M., Conus, S., Fellay, I. Jansen, B., and Borner, C. (1998). *Nature* (*London*) **391**, 496–499.

Scaffadi, C., Fulda, S., Srinivasan, A., Friesen, C., Li, F., Tomaselli, K. J., Debatin, K., Krameer, P. H., and Peter, M. E. (1998). *EMBO J.* **17**, 1675–1687.

Shimizu, S., Eguchi, Y., Kosaka, H., Kamiike, W., Matsuda, H., and Tsujimoto, Y. (1995). *Nature* (*London*) **374**, 811–813.

Susin, S. A., Zamzami, N., Castedo, M., Hirsch, T., Marchetti, P., Macho, A., Daugas, E., Geuskens, M. and Kroemer, G. (1996). *J. Exp. Med.* **184**, 1331–1341.

Susin, S. A., Zamzami, N., Castedo, M., Daugas, E., Wang, H. G., Geley, S., Fassy, F., Reed, J. C., and Kroemer, G. (1997). *J. Exp. Med.* **186**, 25–37.

Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., Snow, B. E., Brothers, G. M., Mangion, J., Jacotot, E., Constantini, P., Loeffler, M., Larochette, N., Goodlett, D. R., Aebersold, R., Siderovski, D. P., Penninger, J. M., Kroemer, G., *Nature* **397**, 441–446 (1999).

Thress, K., Henzel, W., Shillinglaw, W., and Kornbluth, S. (1998). *EMBO J.* **17**, 6135–6143.

Vander Heiden, M. G., Chandel, N. S., Williamson, E. K., Schumacker, P. T., and Thompson, C. B. (1997). *Cell* **91**, 627–637.

Wyllie, A. H., Kerr, J. F., and Currie, A. R. (1980). *Intern. Rev. Cytol.* **68**, 251–306.

Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P., and Wang, X. (1997). *Science* **275**, 1129–1132.

Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssiere, J. L., Petit, P. X. and Kroemer, G. (1995). *J. Exp. Med.* **181**, 1661–1672.

Zou, H., Henzel, W. J., Liu, X., Lutschg, A., and Wang, X. (1997). *Cell* **90**, 405–413.