Release of cytochrome c from mitochondria is a key initiative step in the apoptotic process, although the mechanisms regulating this event remain elusive. In the present study, using isolated liver mitochondria, we demonstrate that cytochrome c release occurs via distinct mechanisms that are either Ca\(^{2+}\)-dependent or Ca\(^{2+}\)-independent. An increase in mitochondrial matrix Ca\(^{2+}\) promotes the opening of the permeability transition (PT) pore and the release of cytochrome c, an effect that is significantly enhanced when these organelles are incubated in a reaction buffer that is based on a physiologically relevant concentration of K\(^{+}\) (150 mM KCl) versus a buffer composed of mannitol/sucrose/Hepes. Moreover, low concentrations of Ca\(^{2+}\) are sufficient to induce mitochondrial cytochrome c release without measurable manifestations of PT, though inhibitors of PT effectively prevent this release, indicating that the critical threshold for PT varies among mitochondria within a single population of these organelles. In contrast, Ca\(^{2+}\)-independent cytochrome c release is induced by oligomeric Bax protein and occurs without mitochondrial swelling or the release of matrix proteins, although our data also indicate that Bax enhances permeability transition-induced cytochrome c release. Taken together, our results suggest that the intramitochondrial Ca\(^{2+}\) concentration, as well as the reaction buffer composition, are key factors in determining the mode and amount of cytochrome c release. Finally, oligomeric Bax appears to be capable of stimulating cytochrome c release via both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent mechanisms.

Apoptosis, a gene-regulated form of cell death, is involved in cell deletion during organogenesis and in the control of cell proliferation and differentiation in adult tissues, as well as in various diseases (1, 2). The biochemical machinery required for apoptotic cell death is constitutively present in virtually all mammalian cells and can be activated by a variety of extracellular and intracellular signals. Attempts to identify a common, uni-
Xenopus eggs without otherwise affecting the functional integrity of these organelles (23).

The present study was undertaken to provide a more definitive understanding of the mechanisms regulating cytochrome c release in response to Ca\textsuperscript{2+}-dependent and Ca\textsuperscript{2+}-independent effects on isolated liver mitochondria. The results indicate that cytochrome c release triggered by Ca\textsuperscript{2+} is significantly enhanced when mitochondria are incubated in KCl-versus MSH-based reaction buffers. Moreover, this release of cytochrome c can be observed prior to any measurable manifestations of MPT, such as swelling or the release of accumulated Ca\textsuperscript{2+}. In comparison, oligomeric, but not monomeric, Bax triggers a Ca\textsuperscript{2+}-independent release of cytochrome c that is not mitigated by inhibitors of MPT. Importantly, oligomeric Bax was also able to enhance Ca\textsuperscript{2+}-induced cytochrome c release, pointing to a possible dual function for this protein. These data clearly demonstrate that cytochrome c release occurs by distinct Ca\textsuperscript{2+}-dependent and Ca\textsuperscript{2+}-independent mechanisms that are each modulated by oligomeric Bax.

**EXPERIMENTAL PROCEDURES**

**Isolation of Rat Liver Mitochondria**—Male Harlan Sprague-Dawley rats (6 to 8 weeks old) were killed by CO\textsubscript{2} inhalation in accordance with the European directive of protection of vertebrate animals for scientific research. The liver was minced on ice, resuspended in 50 ml of MSH buffer (210 mM mannitol, 70 mM sucrose, 5 mM Hepes, pH 7.5) supplemented with 1 mM EDTA and homogenized with a glass Dounce homogenizer and Teflon pestle. Homogenates were centrifuged at 600 × g for 5 min at 4 °C. The supernatant was decanted and recentrifuged at 5,500 × g for 15 min to form a mitochondrial pellet that was resuspended in MSH buffer without EDTA and centrifuged again at 5,500 × g for 15 min. The final mitochondrial pellet was resuspended in MSH buffer at a protein concentration of 80–100 mg/ml. The purity of the mitochondrial fraction was verified by the absence of both cytosolic (glyceraldehyde-3-phosphate dehydrogenase) and nuclear (poly(ADP-ribose) polymerase) proteins as determined by Western blot analysis.

**Measurement of Functional Activity of Isolated Mitochondria**—Mitochondria (1 mg/ml) were incubated in MSH buffer or a buffer containing 150 mM KCl, 1 mM KH\textsubscript{2}PO\textsubscript{4}, 5 mM succinate, and 5 mM Tris-HCl, pH 7.4, for 8 min at 4 °C. The supernatant was decanted and recentrifuged at 5,500 × g for 15 min to form a mitochondrial pellet that was resuspended in MSH buffer without EDTA and centrifuged again at 5,500 × g for 15 min. The final mitochondrial pellet was resuspended in MSH buffer at a protein concentration of 80–100 mg/ml. The purity of the mitochondrial fraction was verified by the absence of both cytosolic (glyceraldehyde-3-phosphate dehydrogenase) and nuclear (poly(ADP-ribose) polymerase) proteins as determined by Western blot analysis.

**Measurement of Functional Activity of Isolated Mitochondria**—Mitochondria (1 mg/ml) were incubated in MSH buffer or a buffer containing 150 mM KCl, 1 mM KH\textsubscript{2}PO\textsubscript{4}, 5 mM succinate, and 5 mM Tris-HCl, pH 7.4, at 25 °C. Rotenone (2 μM) was added to maintain pyridine nucleotides in a reduced form. Estimation of ΔΨ was performed using an electrode sensitive to the lipophilic cation tetraphenylphosphonium (TPP\textsuperscript{+}). Energy mitochondria rapidly accumulate TPP\textsuperscript{+} from the incubation buffer and release this cation as ΔΨ decays. Ca\textsuperscript{2+} fluxes across the inner mitochondrial membrane were monitored using a Ca\textsuperscript{2+}-sensitive electrode (model 97–20; Orion Research, Inc., Beverly, MA). Mitochondrial swelling was monitored continuously as changes in A\textsubscript{490}. Oxygen consumption by isolated rat liver mitochondria was measured using a Clark-type oxygen electrode (Yellow Spring Instrument Co., Yellow Springs, OH) at 25 °C. Mitochondria with a respiratory control ratio (defined as the rate of respiration in the presence of ADP divided by the rate obtained following the expenditure of ADP) above 4 were used for all experiments. In some instances, mitochondrial respiration was uncoupled by the addition of 1 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP). Fresh mitochondria were prepared for each experiment and used within 4 h. At the end of the incubation period, mitochondrial suspensions were centrifuged at 10,000 × g for 5 min, and the resulting supernatants and/or pellets were used for Western blot analysis.

**Western Blot Analysis**—Samples were mixed with Laemmli’s loading buffer, boiled for 5 min, and subjected to 15% SDS polyacrylamide gel electrophoresis at 130 V followed by electrophoretic transfer to nitrocellulose membranes for 2 h at 100 V. Membranes were blocked for 1 h with 5% nonfat milk in phosphate-buffered saline at room temperature and subsequently probed overnight with an anti-cytochrome c (1:2,500), anti-adenylation kinase-2 (1:2,000), anti-adenylation kinase-3 (1:2,000), anti-glyceraldehyde-3-phosphate dehydrogenase (1:5,000), or anti-polypeptide (ADP-ribose) polymerase (1:1,000) antibody. The membranes were rinsed and incubated with a horseradish peroxidase-conjugated secondary antibody. Following the secondary antibody incubation, the membranes were rinsed, and bound antibodies were detected using enhanced chemiluminescence according to the manufacturer’s instructions.

**Expression and Purification of Bax**—Expression and purification of full-length Bax protein was performed as described previously (24). Briefly, the full-length human Bax cDNA sequence was amplified by standard polymerase chain reaction techniques. The polymerase chain reaction DNA fragment was isolated using the QIAquick kit (Qiagen) and subcloned into the NeoI and HindIII sites of the plasmid pBAD. The plasmid was transformed into Escherichia coli, and transformants were isolated by selection for ampicillin resistance. Cultures of the resistant colony were grown to an A\textsubscript{650} of ~7. After induction, the culture was further incubated for several hours, and cells were harvested by centrifugation. The cells were resuspended in lysis buffer (100 mM HEPES-NaOH, pH 8.0, 100 mM NaCl, 1 mM MgCl\textsubscript{2}, 0.1% 2-mercaptoethanol, 1% Triton X-100, a mixture of protease inhibitors, 30 μg/ml DNase I, and 50 μg/ml lysozyme) and broken by sonicication. After centrifugation, Bax was recovered in the supernatant. Because the protein was expressed with a His tag at the N terminus, the purification by affinity chromatography on nickel-nitrilotriacetic acid-agarose (Qiagen) followed by ion-exchange chromatography on Q-Sepharose (Amersham Pharmacia Biotech) was performed according to the manufacturer’s instructions. The protein was at least 95.8% pure as determined by SDS polyacrylamide gel electrophoresis. Multimerization of recombinant Bax to a stable complex, which did not dissociate into smaller species, was performed in the presence of 1% octyl glucoside (24).

**Statistical Analysis**—Data are presented as means ± S.D., and significance was determined using a Student’s t test. A value of p < 0.05 was considered to be significant.

**RESULTS**

**Mitochondrial Permeability Transition and Cytochrome c Release**—MPT is a consequence of Ca\textsuperscript{2+} overload (15, 16), and the sensitivity of mitochondria to permeability transition (PT) can be enhanced by different factors. Among these factors are an elevated level of P\textsubscript{i}, oxidative stress, the depletion of adenine nucleotides, and the oxidation of pyridine nucleotides. Fig. 1 is representative of a typical response of mitochondria to PT-inducing agents. In particular, when P\textsubscript{i} or organic hydroperoxide is added to Ca\textsuperscript{2+}-loaded isolated mitochondria (Fig. 1), these organelles swell (panel A), ΔΨ decays (panel B), and accumulated Ca\textsuperscript{2+} is released (panel C). MPT-induced drops in ΔΨ result in an acceleration of respiration (panel D), and all of these manifestations of MPT can be prevented by cyclosporin A (CSA) (panels A–D).

Although the rate of Ca\textsuperscript{2+} release during MPT is similar in KCl versus MSH incubation buffers (Fig. 2A), the amount of cytochrome c released is significantly more pronounced when mitochondria are incubated in the more physiologically rele-
a greater loss of cytochrome c was observed in MSH buffer (Fig. 2D). Taken together, these data indicate that although PT-induced uncoupling of mitochondria occurs in both buffers, respiration is suppressed only in KCl buffer, an effect most likely because of a greater loss of cytochrome c.

**Low Doses of Ca^{2+} Stimulate MPT and Cytochrome c Release in a Subpopulation of Mitochondria**—In the aforementioned experiments, cytochrome c release was a consequence of PT induction, accompanied by swelling of mitochondria and rupture of the outer membrane. The next step was to determine whether cytochrome c release could occur when Ca^{2+} loading was insufficient to induce observable manifestations of MPT. As seen in Fig. 3A, Ca^{2+} loading of mitochondria alone did not induce changes characteristic of MPT (cf. Fig. 1C), and this cation was retained by mitochondria unless CCCP, an uncoupler of oxidative phosphorylation, was added. In addition, Ca^{2+}-loaded mitochondria exhibited controlled respiration (Fig. 3B), although increasing the Ca^{2+} retention time from 1 to 5 min prominently diminished the rate of CCCP-directed uncoupled respiration (Panel B, trace a versus trace b). Taking into consideration that the observed decrease in the rate of uncoupled respiration might be because of damage of the respiratory chain and/or the release of cytochrome c, the presence of this protein in the incubation buffer was analyzed. As seen in Fig. 3C, the accumulation of Ca^{2+} by mitochondria in the absence of observable PT was sufficient to stimulate a release of cytochrome c, an effect that was considerably more pronounced the longer mitochondria retained accumulated Ca^{2+}.
Inhibitors of MPT, such as ADP and Mg$^{2+}$, suppressed this release (Fig. 3C) and restored the rate of uncoupled respiration (Fig. 3D). Additional evidence of cytochrome c release in the absence of observable MPT is presented in Fig. 4 where the sequential addition of Ca$^{2+}$ pulses to isolated mitochondria led to a stepwise decrease in the overall optical density (Fig. 4A, traces 1–5) without inducing large amplitude swelling characteristic of PT. Meanwhile, the amount of cytochrome c released from mitochondria was enhanced as Ca$^{2+}$ loading increased (Fig. 4B). This release reflected PT induction in a subpopulation of mitochondria, which was further supported by the fact that co-treatment of isolated mitochondria with 1 μM CsA eliminated cytochrome c release induced by Ca$^{2+}$ additions ranging between 20 and 80 nmol/mg protein (data not shown). Cytochrome c release ultimately reached a pinnacle at 80 nmol Ca$^{2+}$/mg protein (Fig. 4B, lanes 6 and 7) as all mitochondria underwent PT (Fig. 4A).

**Bax-induced Cytochrome c Release Can Occur via Both MPT-independent and MPT-dependent Mechanisms**—Next, we tested the effect of recombinant Bax protein on cytochrome c release. Bax has been reported to facilitate cytochrome c release in response to different stimuli, although the precise mechanism responsible for this event remains unclear. Both monomeric and oligomeric forms of Bax (Fig. 5A) were generated for our study because of reports indicating that oligomerization of Bax is a critical event for integration of this protein into membranes (22). It should be noted that our monomeric form of Bax also contained the oligomeric form (Fig. 5A, lane 2), although it is clear from the data that these different pools of recombinant Bax had strikingly dissimilar effects on cytochrome c release.

The addition of oligomeric Bax to isolated mitochondria prominently induced the release of cytochrome c (Fig. 5B). This release did not depend on MPT, because the incubation buffer used for these experiments contained 1 mM EGTA, a concentration sufficient to chelate available Ca$^{2+}$ and hence prevent MPT. In contrast to the results obtained with oligomeric Bax, cytochrome c release was marginal in the presence of the monomeric form of Bax (Fig. 5B), which is in agreement with other data indicating that only oligomeric Bax is able to induce cytochrome c release (25). The slight release observed in the presence of monomeric Bax was most likely because of the presence of some of the oligomeric form in this preparation (Fig. 5A, lane 2).

In addition to stimulating an MPT-independent release of cytochrome c, recombinant oligomeric Bax also facilitated Ca$^{2+}$-induced MPT as assessed by both the release of accumulated Ca$^{2+}$ (Fig. 5C) and a drop in mitochondrial membrane potential (Fig. 5D). In contrast, the monomeric form of this protein had no effect on either parameter as compared with control mitochondria (Fig. 5, C and D). Western blot analysis of
cytochrome c release in samples taken 6 min after the addition of Ca\textsuperscript{2+} revealed that oligomeric Bax enhanced MPT-dependent cytochrome c release (Fig. 5E). Finally, an attempt to compare the ability of oligomeric Bax to enhance MPT-independent and MPT-dependent release of cytochrome c revealed, as we expected, that cytochrome c release was significantly more pronounced in samples taken from Bax-treated mitochondria that were stimulated to undergo PT (Fig. 5F).

To determine whether the combined or individual effects of Bax and Ca\textsuperscript{2+} on cytochrome c release were unique for this apoptotic protein, we also evaluated the release of other mitochondrial proteins. Results indicated that the intermembrane space protein adenylate kinase-2 was released into the cytosol during MPT-independent and MPT-dependent modes of protein release, an effect that was consistent with our cytochrome c release data (Fig. 6, A and B). In contrast, the mitochondrial matrix protein adenylate kinase-3 was released only via PT, although oligomeric Bax significantly enhanced this result by potentiating the onset of PT (Fig. 6C). Taken together, our data indicate that the effect of Bax alone is not specific for cytochrome c release and that, in some cases, a different intermembrane space protein is also released. However, treatment of isolated mitochondria with Bax alone does not appear to compromise matrix integrity, because this protein did not initiate or enhance the release of adenylate kinase-3 unless PT was induced.

**DISCUSSION**

Although it was originally believed that MPT induction was the root mechanism responsible for cytochrome c release in response to different cytotoxic stimuli, more recently this notion has been challenged, and the precise mechanisms regulating the release of this protein are unclear. In fact, many of the early results on mechanisms of cytochrome c release were generated using cell-free systems wherein isolated mitochondria and nuclei were treated with different PT pore activators, which, in turn, led to mitochondrial swelling, the release of cytochrome c (and other proteins), and subsequent changes in nuclear morphology that were characteristic of apoptosis. However, ample evidence from more recent studies suggests that although MPT is likely to be a mechanism responsible for cytochrome c release, it is no longer regarded as the mechanism (26). In particular, Martinou et al. (27) demonstrated that mitochondria of neurons undergoing apoptosis, induced by neuronal growth factor depletion, actually reduced in size and resumed their normal function when reincubated with neuronal growth factor. A more recent study from our laboratory demonstrated that etoposide stimulated cytochrome c release from isolated mitochondria, despite the presence of 1 mM EGTA (a known inhibitor of MPT) in the reaction buffer (28). Thus, the current study was undertaken to examine more comprehensively the mechanisms regulating the release of this important apoptotic protein and the potential effect recombinant Bax protein has on this event. We clearly demonstrate here, using isolated liver mitochondria, that cytochrome c release occurs via distinct mechanisms that can be divided into two groups, Ca\textsuperscript{2+}-dependent and Ca\textsuperscript{2+}-independent.

As mentioned previously, it is well known that Ca\textsuperscript{2+} is the basic PT pore activator (16). Coincidentally, Ca\textsuperscript{2+}-dependent cytochrome c release occurs when mitochondria experience Ca\textsuperscript{2+} overload, resulting in the induction of PT. Yet a number of reports in the literature make claims of MPT-dependent cytochrome c release in the absence of Ca\textsuperscript{2+}; in fact, many of these studies base this claim on the fact that CsA, a pharmacological inhibitor of MPT, effectively blocks the release of this apoptogenic protein (29, 30). For example, Kroemer and coworkers (29) recently reported the ability of Bid (also a proapoptotic Bcl-2 family protein) to induce cytochrome c release by opening the permeability transition pore, an effect they base solely on the fact that it was preventable by CsA and other inhibitors of PT. Although this may be the case, a far more accurate and convincing demonstration of MPT would have included the presence of Ca\textsuperscript{2+}-mediated large amplitude swelling. In short, claims of cytochrome c release via MPT in the absence of Ca\textsuperscript{2+} are at best improbable. This was our rationale for employing a multiparameter mitochondrial functional analysis, including measurements of swelling, decreases in Δψ, changes in respiration, and the release of accumulated Ca\textsuperscript{2+}, to more accurately link changes in mitochondrial integrity and the release of cytochrome c.

Consistent with data reported by other investigators, we demonstrate the ability of mitochondria undergoing PT to release cytochrome c (31, 32). The fact that this effect was more pronounced in KCl versus MSH buffers is likely to be related to the understanding that cytochrome c binding to the inner mitochondrial membrane is weaker in buffers with higher ionic strength (33). In addition to cytochrome c release occurring during full-blown MPT, our results indicate that this protein is released even when Ca\textsuperscript{2+} loading is below the threshold needed to elicit observable manifestations of PT, though different inhibitors of MPT, including ADP and Mg\textsuperscript{2+}, were able to suppress cytochrome c release. Thus, it appears that low Ca\textsuperscript{2+} loading is sufficient to induce PT in a subpopulation of mitochondria, and as recently described by Rizzuto et al. (34) the released Ca\textsuperscript{2+} is subsequently taken up by neighboring polarized mitochondria. This effect can likely be explained by the inherent heterogeneity of mitochondria (35) that make up any particular population of these organelles in terms of their sensitivity to PT. In other words, all mitochondria are not created equally.

The mechanisms responsible for Ca\textsuperscript{2+}-independent cytochrome c release are less clear, although increasing evidence indicates that certain proapoptotic members of the Bcl-2 family of proteins, notably Bax, are able to participate in this process. One possible, and increasingly popular, mechanism is that in the absence of MPT, or any changes in mitochondrial volume, oligomeric Bax inserts and forms a channel in the outer membrane large enough to allow the release of cytochrome c (20, 36). As our data suggest, monomeric Bax is incapable of stimulating cytochrome c release, which is consistent with reports indicating that oligomerization of Bax is required prior to its insertion in the membrane. It should be noted that in addition to the ability of Bax to stimulate cytochrome c release in the absence
of any changes in mitochondrial volume, this protein also possesses the ability to enhance MPT-mediated release of cytochrome c. In this case, Bax may interact with the permeability transition pore proteins ANT or voltage-dependent anion channel and hasten the opening of the pore. In fact, it was previously reported that Bax interacts directly with ANT and facilitates ANT opening induced by atractyloside (an ANT inhibitor) and that ANT-deficient yeast are resistant to Bax-induced cell death, suggesting that ANT may also be a functional target of Bax (26, 37). At the same time, it was demonstrated that Bax and Bak directly target and open voltage-dependent anion channel in liposomes and induce changes in ∆ψ (21). Interestingly, it should be noted that more recent results from our laboratory indicate that Ca2+-independent cytochrome c release can also occur via mitochondrial swelling stimulated by alterations in osmotic balance.2

Recently, evidence was provided that cardiolipin, which is present exclusively in mitochondrial membranes, mediates the targeting of truncated Bid to mitochondria through a previously unknown three-helix domain in truncated Bid and that this interaction is critical for cytochrome c release (38). The involvement of cardiolipin in the release of cytochrome c was also documented by data showing that elevated levels of mitochondrial phospholipid hydroperoxide glutathione peroxidase in cells triggered to undergo apoptosis completely suppressed the release of cytochrome c (39). Taken together, these findings suggest that cytochrome c release may occur by a two-step process, wherein this protein is first liberated from cardiolipin and then released via specific or nonspecific pores/channels in the outer mitochondrial membrane.

In summary, we have demonstrated that mitochondria release cytochrome c via distinct Ca2+-dependent or Ca2+-independent mechanisms. In the first case, mitochondrial Ca2+ overload causes swelling, rupture of the outer mitochondrial membrane, and the nonspecific release of cytochrome c. In contrast, Ca2+-independent protein release is regulated by the oligomeric form of the proapoptotic protein Bax, which is specific for intermembrane space proteins, and occurs without MPT. Importantly, oligomeric Bax also enhances MPT-induced protein release. Which, if any, of these mechanisms predominates under physiological conditions remains unclear. However, it is tempting to speculate that under pathological conditions when the level of intracellular Ca2+ increases, such as during ischemia-reperfusion injury, swelling, rupture, and the release of mitochondrial proteins occurs by PT, whereas during "authorized" cell death or receptor-mediated killing this release occurs without swelling and proceeds via a Bax- and/or truncated Bid-regulated pathway.

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REFERENCES

1. Kerr, J. F., Wyllie, A. H., and Currie, A. R. (1972) Br. J. Cancer. 26, 239–257
2. Wyllie, A. H., Kerr, J. F., and Currie, A. R. (1980) Int. Rev. Cytol. 68, 351–306
3. Petit, P. X., Zamzami, N., Vayssiere, J. L., Mignotte, B., Kroemer, G., and Castedo, M. (1997) Mol. Cell. Biochem. 174, 185–198
4. Bossy-Wetzel, E., and Green, D. R. (1999) Mutat. Res. 434, 243–251
5. Skulachev, V. P. (1999) Mol. Aspects Med. 20, 139–184
6. Thores, K., Korshbuth, S., and Smith, J. J. (1999) J. Bioenerg. Biomembr. 31, 321–326
7. Robertson, J. D., and Orrenius, S. (2000) Crit. Rev. Toxicol. 30, 609–627
8. Cai, J., Yang, J., and Jones, D. P. (1998) Biochim. Biophys. Acta 1359, 139–149
9. Kim, B. R., and Reed, J. C. (1998) Science 281, 1299–1312
10. Daugas, E., Susin, S. A., Zamzami, N., Ferri, K. F., Irinopoulou, T., Larochette, N., Prevost, M. C., Leber, B., Andrews, D., Penninger, J., and Kroemer, G. (2000) FASEB J. 14, 729–739
11. Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. L., Jones, D. P., and Wang, X. (1997) Science 275, 1129–1132
12. Hampton, M. B., Zhivotovsky, B., Slater, A. F., Burgess, D. H., and Orrenius, S. (1998) Biochem. J. 329, 95–99
13. Lemeshko, V. V., and Slekh, V. E. (1993) Mech. Ageing Dev. 68, 221–233
14. Bernardi, P. (1999) Physiol. Rev. 79, 1127–1155
15. Halestrap, A. P., Kerr, P. M., Javadi, S., and Woodfield, K. Y. (1998) Biochim. Biophys. Acta 1366, 79–94
16. Crompton, M. (1999) Biochem. J. 341, 233–249
17. Doran, E., and Halestrap, A. P. (2000) Biochem. J. 348, 343–350
18. Tsujimoto, Y., and Shimizu, S. (2000) FEBS Lett. 466, 6–10
19. Eskes, R., Antonsson, B., Osen-Sand, A., Montessuit, S., Richter, C., Sadowi, R., Mazzei, G., Nichols, A., and Martinou, J. C. (1998) J. Cell Biol. 143, 217–224
20. Jurgenesmeier, J. M., Xie, Z., Deveraux, Q., Ellerby, L., Bredesen, D., and Reed, J. C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4997–5002
21. Shimizu, S., Narita, M., and Shimotohono, Y. (1999) Nature 399, 483–487
22. Eskes, R., Desagher, S., Antonsson, B., and Martinou, J. C. (2000) Mol. Cell. Biol. 20, 929–935
23. von Ahsen, O., Renken, C., Perkins, G., Kluck, R. M., Bossy-Wetzel, E., and Newmeyer, D. D. (2000) J. Cell Biol. 150, 1227–1236
24. Montessuit, S., Mazzei, G., Magnenat, E., and Antonsson, B. (1999) Protein Expression Purif. 15, 202–206
25. Gross, A., Jockel, J., Wei, M. C., and Korsmeyer S. J. (1998) EMBO J. 17, 3875–3885
26. Marzo, I., Brenner, C., Zamzami, N., Jurgenesmeier, J. M., Susin, S. A., Vieira, H. L., Prevost, M. C., Xie, Z., Matsuyama, S., Reed, J. C., and Kroemer, G. (1998) Science 281, 2027–2035
27. Martinou, I., Desagher, S., Eskes, R., Antonsson, B., Andre, E., Fakan, S., and Martinou, J. C. (1999) J. Cell Biol. 144, 883–889
28. Robertson, J. D., Gogvadze, V., Zhivotovsky, B., and Orrenius, S. (2000) J. Biol. Chem. 275, 32438–32443
29. Zamzami, N., El Hamel, C., Maise, C., Brenner, C., Muñoz-Pinedo, C., Belzui, A-S., Costantini, P., Vieira, H., Loeffler, M., Molle, G., and Kroemer, G. (2000) Oncogene 19, 6342–6350
30. Hirsch, T., Decaudin, D., Susin, S. A., Marchetti, P., Larochette, N., Resche-Rigon, M., and Kroemer, G. (1998) Exp. Cell Res. 241, 436–434
31. Kanton, S. P., and Piantadosi, C. A. (1997) Biochem. Biophys. Res. Commun. 232, 669–711
32. Yang, J. C., and Cortopassi, G. A. (1998) Free Radic. Biol. Med. 24, 624–631
33. Cortese, J. D., Voglino, A. L., and Hackenbrock, C. R. (1996) Biochemistry 35, 6402–6409
34. Rizzuto, R., Bernardi, P., and Pozzan, T. (2000) J. Physiol. 529, 37–47
35. Beatrice, M. C., Stiers, D. L., and Pfeiffer, D. R. (1982) J. Biol. Chem. 257, 7161–7171
36. Antonsson, B., Montessuit, S., Lauper, S., Eskes, R., and Martinou, J. C. (2000) Biochem. J. 345, 271–278
37. Brenner, C., Cadou, H., Vieira, H. L., Zamzami, N., Marzo, I., Xie, Z., Leber, B., Andrews, D., Duchoi, H., Reed, J. C., and Kroemer, G. (2000) Oncogene 19, 929–936
38. Lutter, M., Fang, M., Lan, X., Nishijima, M., Xie, X. S., and Wang, X. (2000) Nat. Cell Biol. 2, 764–761
39. Nomura, K., Imai, H., Kurokawa, T., Kobayashi, T., and Nakagawa, Y. (2000) Biochem. J. 351, 183–193

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Cytochrome c Release Occurs via $\text{Ca}^{2+}$-dependent and $\text{Ca}^{2+}$-independent Mechanisms That Are Regulated by Bax

Vladimir Gogvadze, John D. Robertson, Boris Zhivotovsky and Sten Orrenius

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