FEBS Letters 427 (1998) 198–202 FEBS 20190

# Caspases disrupt mitochondrial membrane barrier function

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Received 18 March 1998

**Abstract Mitochondrial intermembrane proteins including cytochrome *c* are known to activate caspases. Accordingly, a disruption of the mitochondrial membrane barrier function with release of cytochrome into the cytosol has been shown to precede caspase activation in a number of different models of apoptosis. Here, we addressed the question of whether caspases themselves can affect mitochondrial membrane function. Recombinant caspases were added to purified mitochondria and were found to affect the permeability of both mitochondrial membranes. Thus, caspases cause a dissipation of the mitochondrial inner transmembrane potential. In addition, caspases cause intermembrane proteins including cytochrome *c* and AIF (apoptosis-inducing factor) to be released through the outer mitochondrial membrane. These observations suggest that caspases and mitochondria can engage in a circular self-amplification loop. An increase in mitochondrial membrane permeability would cause the release of caspase activators, and caspases, once activated, would in turn increase the mitochondrial membrane permeability. Such a self-amplifying system could accelerate the apoptotic process and/or coordinate the apoptotic response between different mitochondria within the same cell.**

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*Key words:* Caspase; Mitochondrion; Permeability transition; Programmed cell death

## 1. Introduction

In most models of apoptosis, the permeability of the inner and/or outer mitochondrial membranes increases early during the death process leading to the dissipation of the inner transmembrane potential ($\Delta\Psi_m$) and/or the release of intermembrane proteins into the cytosol [1,2]. The exact chronological and cause-effect relationship between the increase in inner versus outer mitochondrial membrane permeability is not understood and may depend on the cell type and/or apoptosis inducer [3–11]. In many cases, opening of the mitochondrial megachannel (or permeability transition pore) appears to be critical for the apoptotic process because inhibition of megachannel opening prevents cell death [1,12–15]. Opening of the megachannel in isolated mitochondria causes several changes in mitochondria which are also found in apoptosis: $\Delta\Psi_m$ dissipation, matrix swelling, and rupture of the outer mitochondrial membrane resulting in the release of intermembrane proteins [1,9,11,16–19]. Opening of the megachannel is controlled by the apoptosis inhibitory proteins Bcl-2 and Bcl-$X_L$ [12,20,21].

Several mitochondrial intermembrane proteins have been shown to activate caspases, a class of proteases whose activation is indispensable for the acquisition of apoptotic morphology [22]. Thus, the 15 kDa protein cytochrome *c* can interact with the ATP-binding protein Apaf-1 to form a complex which proteolytically activates pro-caspase 9 (or favors its autoactivation) [23,24]. Another intermembrane protein, the ~50 kDa AIF (apoptosis-inducing factor), also may have a caspase-activating potential [8]. In contrast to cytochrome *c* and caspases, AIF suffices to activate nuclear endonucleases in the absence of further cytosolic proteins [6]. While cytochrome *c* and caspases act via a caspase-activated DNase, also called DNA fragmentation factor (DFF) [25], addition of purified AIF itself can provoke nuclear apoptosis in vitro [6]. In accord with its putative role in caspase activation, pharmacological inhibition of the megachannel can prevent the activation of caspases [14,26]. Moreover, Bcl-2 and Bcl-$X_L$, which inhibit the megachannel [12,20,21] and favor the retention of intermembrane proteins in mitochondria [4–8], inhibit the activation of downstream caspases [27,28].

Altogether, it appears that the disruption of mitochondrial membrane function occurs mostly upstream of caspase activation [8,26]. Nonetheless, in some models of apoptosis, caspase activation is an early feature of signal transduction. Thus, crosslinking or ligation of CD95/Fas/APO-1 has been shown to cause the recruitment of pro-caspase 8 into a receptor complex [29,30], thereby stimulating the primary (that is pre-mitochondrial) activation of caspases [8,28]. In CD95/Fas/APO-1-induced apoptosis, inhibition of this 'upstream caspase' by transfection with the selective caspase inhibitor *crm*A or synthetic peptides prevents the mitochondrial manifestations of apoptosis [8,31,32], underlining that they do indeed function upstream of mitochondria. Stimulated by these findings, we decided to evaluate the effects of caspases on isolated mitochondria in vitro. Here, we show that a number of different recombinant caspases can act on mitochondria to disrupt their membrane barrier function.

## 2. Materials and methods

### 2.1. Materials

Recombinant caspases produced as active enzymes as previously described [33,34] were kindly provided by Nancy Thornberry (Merck, Rahway, NJ, USA) or by Guy Salvesen (Burnham Institute, La Jolla, CA, USA). Caspase activity is defined as the amount of enzyme required to cleave 1 μmol of the fluorogenic substrate Ac-DEVD-amc (caspase 2, 3, 6, 7, 8), Ac-YVAD-amc (caspase 1), or Ac-WEHD.amc

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*Abbreviations:* AIF, apoptosis-inducing factor; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; $\Delta\Psi_m$, mitochondrial transmembrane potential; diamide, diazenedicarboxylic acid bis-5-*N,N*-dimethylamide; $DiOC_6(3)$, 3,3′-dihexyloxacarbocyanine iodide; PT, permeability transition; PTPC, permeability transition pore complex; Z-VAD.fmk, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone

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*PII* S0014-5793(98)00424-4

(caspase 4, 5) per hour. All caspase substrates and inhibitors (Ac-DEVD.cmk, Ac-YVAD.cmk, Z-VAD.fmk) were purchased from Bachem (Basel, Switzerland). All remaining reagents were from Sigma (St. Louis, MO), unless specified differently. Mitochondria from mouse liver were purified on a Percoll (Pharmacia, Uppsala, Sweden) gradient, following published protocols [35].

*2.2. Determination of cytochrome c release*

Isolated mitochondria (1 mg mitochondrial protein per ml; 50 μl/tube) were incubated in a buffer containing (400 mM mannitol; 50 mM Tris-HCl; 0.5% (w/v) bovine serum albumin; 10 mM $KH_2PO_4$; pH 7.2) in the presence of 0.1–1 U recombinant caspases for 30 min at room temperature. Thereafter, mitochondria were spun down (12 000 × *g*, 3 min, 4°C). Aliquots of the supernatant (10 μl) were subjected to PAGE gel electrophoresis (12%), Western blot on nitrocellulose, and immunodetection of cytochrome *c* using a specific monoclonal antibody (clone 7H8.2C12, Pharmingen), as described [23].

*2.3. Oxygen uptake and membrane potential*

Membrane potential and oxygen uptake were simultaneously measured in an oxygen-electrode chamber (Hansatech, UK) using tetraphenylphosphonium cation ($TPP^+$) as a membrane potential probe. The transmembrane potential ($\Delta\Psi_m$) was measured at 25°C in a final volume of 1.5 ml respiratory medium consisting of 0.3 M sucrose, 10 mM KCl, 5 mM $MgCl_2$, 10 mM TES, 10 mM $KH_2PO_4$ (pH 7.2). The $TPP^+$ binding was corrected according to Hashimoto and Rottenberg [36].

*2.4. Determination of AIF release*

Mitochondria were resuspended in cell-free system (CFS) buffer (220 mM mannitol, 68 mM sucrose, 2 mM NaCl, 2.5 mM $KH_2PO_4$, 0.5 mM EGTA, 2 mM $MgCl_2$, 5 mM pyruvate, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol, 10 mM HEPES-NaOH, pH 7.2) in the presence or absence of recombinant caspase and caspase inhibitors (100 μM Ac-DEVD.cmk or Ac-YVAD.cmk) for 15 min, followed by centrifugation as above (12 000 × *g*, 3 min, 4°C). The supernatant was subject to a second centrifugation (150 000 × *g*, 30 min, 4°C) to remove particles. Nuclei from HeLa or 2B411 T cell hybridoma cells purified on a sucrose gradient [37] were cultured at a density of $10^3$ nuclei/μl in the presence of mitochondrial supernatants for 90 min at 37°C in CFS buffer. Alternatively, purified cytochrome *c* from bovine heart (Sigma) was added. Without prior fixation, nuclei were stained with propidium iodide (PI) (10 μg/ml; Sigma; ≥ 5 min at room temperature), followed by cytofluorometric analysis in a EPICS Profile II Analyzer (Coulter, Hialeah, FL), while gating the forward and side scatters on single-nucleus events [38].

## 3. Results and discussion

*3.1. Recombinant caspases cause cytochrome c release from isolated mitochondria*

As cytochrome *c* release is a critical event in several in vitro models of apoptosis [5,7,23,24], we have evaluated the capacity of caspases to provoke release of this protein, which is normally confined to the mitochondrial intermembrane space. Mitochondria were incubated with recombinant, enzymatically active caspases, followed by immunodetection of cytochrome *c* release into the supernatant. As demonstrated in Fig. 1, all tested caspases (1, 2, 5, 6, 7, 8, 9) did indeed cause the liberation of cytochrome *c* from the mitochondrial intermembrane space.

*3.2. Caspases cause the release of AIF from isolated mitochondria*

AIF is biologically defined as an activity that causes DNA fragmentation in isolated nuclei in vitro [6,38]. As shown in Fig. 2, the supernatant of mitochondria with the megachannel-opening agent atractyloside (but not the supernatant of control mitochondria) contains an activity which provokes

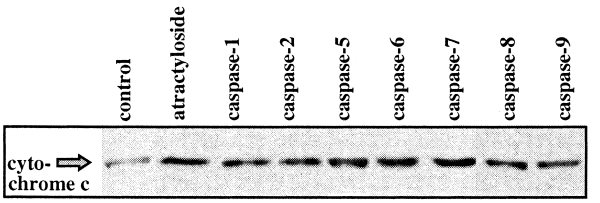



Fig. 1. Recombinant caspases cause cytochrome *c* release from isolated mitochondria. Mitochondria were incubated in the presence or absence of different recombinant caspases (20 U/ml), as described in Section 2, followed by centrifugation and recovery of the supernatant. The supernatants were then subjected to SDS-PAGE, Western blot and immunodetection of cytochrome *c* using a specific monoclonal antibody. Incubation with atractyloside (5 mM) served as an internal control of maximum cytochrome *c* release. The immunodetection yielded one single band corresponding to a molecular weight of 15 kDa.

loss of DNA in isolated HeLa nuclei, as quantified by staining with the DNA-intercalating dye PI and cytofluorometry. In contrast, purified cytochrome *c* did not affect the DNA content of isolated HeLa nuclei, thus confirming the assertion that AIF is not identical with cytochrome *c* [6,8]. Incubation of mitochondria with recombinant caspases causes the release of an AIF activity. In this experimental system, recombinant caspases (1, 2, 4, 6, 8) added in the absence of mitochondria have no effect on the DNA content of purified nuclei (not shown). Inhibition of their activity, *after* recovery of the mitochondrial supernatant, using specific tetrapeptide inhibitors does not abolish the AIF activity contained in the mitochondrial supernatant. Thus, in conditions in which Ac-YVAD.cmk or Ac-DEVD.cmk fully prevent the caspase-mediated cleavage of the fluorogenic substrates Ac-YVAD.afc or Ac-DEVD.afc (not shown), the AIF activity recovered in the supernatant remains active (Fig. 2). AIF activity is only affected by the tripeptide inhibitor Z-VAD.fmk, which results in its partial inhibition, as previously described [6]. These data indicate that caspases, though themselves inactive on isolated nuclei, can provoke the release of AIF from mitochondria.

*3.3. Recombinant caspases disrupt the $\Delta\Psi_m$*

Since caspases cause the release of cytochrome *c* and AIF through the outer mitochondrial membrane, we determined their effect on the inner transmembrane electrochemical gradient. Mitochondria treated with recombinant caspases were resuspended in a buffer containing succinate and rotenone, followed by determination of the $\Delta\Psi_m$ with a $TPP^+$-sensitive electrode. As shown in Fig. 3, caspases do indeed provoke a reduction of the $\Delta\Psi_m$, and this effect is inhibited by pre-incubation of caspases with an excess of tetrapeptide inhibitors. Pre-incubation of mitochondria with a low dose of recombinant caspase 1 gradually increases the sensitivity of mitochondria to $Ca^{2+}$-induced permanent disruption of the $\Delta\Psi_m$, which is a sign of mitochondrial permeability transition. Thus, untreated mouse liver mitochondria require 5 additions of 75 nmol $Ca^{2+}$ (per mg protein) to induce for durable $\Delta\Psi_m$ dissipation ([11] and Fig. 4A). In contrast, mitochondria pretreated with caspase 1 during 10 min only required 2 additions of 75 nmol $Ca^{2+}$ to obtain the same effect (Fig. 2C). After 30 min of pre-incubation, caspase 1 itself sufficed to cause a permanent disruption of the $\Delta\Psi_m$ (Fig. 2D). Addition of exogenous cytochrome *c* after antimycin A (which inhibits the respiratory complex II) failed to enhance oxygen con-

sumption by untreated control mitochondria (insert in Fig. 1A), although it did so in mitochondria treated with caspase 1 and/or calcium to achieve a permanent $\Delta\Psi_m$ dissipation (Fig. 4B–D). This indicates that the outer mitochondrial membrane has become permeable to exogenous cytochrome *c* after $Ca^{2+}$ and/or caspase 1 have acted on mitochondria. Analogous results were obtained when caspase 3 instead of caspase 1 was used (not shown). Of note, it appeared that cytochrome *c* did not restore the $\Delta\Psi_m$ in conditions in which it increased the oxygen consumption of mitochondria, suggesting that mitochondria are durably uncoupled after caspase treatment (Fig. 4D).

### 3.4. Concluding remarks

In this work we show that several different recombinant caspases share the capacity to perturb the mitochondrial membrane barrier function, at the levels of both the outer and the inner mitochondrial membranes. Thus, the outer membrane becomes permeable to the intermembrane proteins cytochrome *c* and AIF (Figs. 1, 2 and 4). Furthermore, the proton gradient normally built up on the inner membrane dissipates upon incubation with caspases (Figs. 3 and 4). In the experimental conditions described in this paper, it appears that loss of barrier function of the inner and the outer membranes occur in a quasi-simultaneous fashion (Figs. 1–4 and data not shown). The membrane-permeabilizing effect of caspases is probably due to effects on specific protein substrates in the mitochondrial membranes, based on the observation that neutralization of caspase proteolytic activity with tetrapeptide inhibitors abolished their effects on mitochondria (Fig. 3). Moreover, we have previously shown that caspases can increase the membrane permeability of proteoliposomes

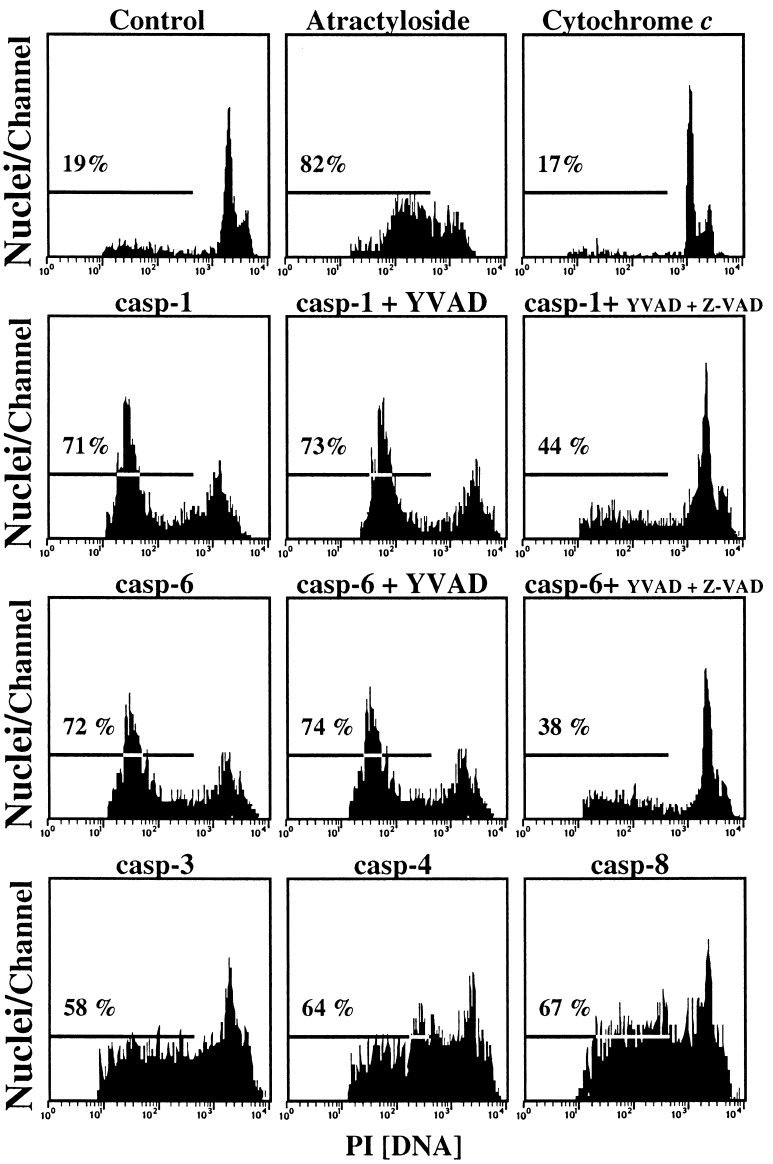



Fig. 2. Recombinant caspases cause the release of AIF from isolated mitochondria. Mitochondria were incubated in the absence (control) or in the presence of atractyloside (5 mM, 30 min) or the indicated caspase (20 U/ml), followed by centrifugation and recovery of the supernatant. The supernatants were pre-incubated during 10 min in the presence or absence of Ac-YVAD.cmk, Ac-DEVD.cmk and/or Z-VAD.fmk (100 μM), as indicated, before they were added to purified HeLa cell nuclei. Nuclei and mitochondrial supernatants were cultured during 90 min at 37°C, followed by determination of the DNA content by staining with PI and cytofluorometric analysis. Results are also shown for nuclei kept in the presence of 100 μg/ml cytochrome *c*.

containing mitochondrial proteins, whereas they have no effects on protein-free liposomes [20]. The nature of the caspase substrates contained in the mitochondrial membrane, however, remains to be clarified. As a possibility, proteins of the Bcl-2 family may be the target of caspases. Thus, Bcl-2 is a caspase 3 substrate, and Bcl-$X_L$ is a caspase 1 substrate [39,40]. However, liver mitochondria contain little if any Bcl-2 [41], suggesting that caspase 3 and its homologues must act on other proteins.

As outlined in Section 1, proteins which are normally secluded by the mitochondrial outer membrane can act as potent caspase activators when they are released into the cytosol [5,7,8,23,24]. Although data obtained in several cell-free systems of apoptosis induction would suggest that caspase activation usually occurs downstream of mitochondrial membrane permeabilization [5,7,8,23,24], the data presented here indicate that caspases can also act upstream of mitochondria. It is certainly conceivable that mitochondria and caspases engage in a circular amplification loop of mutual activation, thereby locking the cell in an irreversible stage of death commitment. We have previously observed that during apoptosis different mitochondria located in the same cell lose their $\Delta\Psi_m$ in a quasi-simultaneous fashion [42], suggesting that some sort of inter-mitochondrial coordination exists. An amplification loop between caspases and mitochondria might fulfill such a coordinating function.

When integrating the data from this study and from preceding papers, it is tempting to envisage a scenario in which the disruption of mitochondrial barrier function determines the decision between survival and death, whereas the activation of downstream caspases, which is necessary for the acquisition of apoptotic morphology, would determine the modality of death (necrosis versus apoptosis). Normally, disruption of mitochondrial barrier function and caspase activation would be tied together by mutually stimulatory effects of caspases and mitochondria. Only in circumstances in which caspase activation is blocked (e.g. by cysteine oxidation or nitrosylation, ATP depletion, or synthetic inhibitors such as Z-VAD.fmk), the primary disruption of mitochondrial barrier function would fail to activate the apoptotic default death pathway and instead provoke a non-apoptotic, cytolytic type of cell death. This scenario is compatible with most of the published data [26,43–47] and would allow for the operative reinterpretation of the sometimes spurious difference between necrotic and apoptotic cell death.

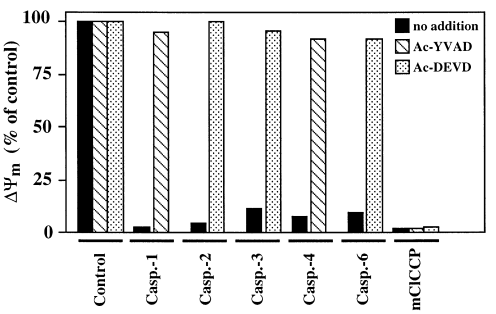



Fig. 3. Recombinant caspases cause disruption of the $\Delta\Psi_m$. Mitochondria were incubated in the presence of the indicated caspase (10 U/ml) pre-treated (10 min, 100 μM) with the indicated caspase inhibitor. The $\Delta\Psi_m$ was measured using $TPP^+$, as detailed in Section 2, as percentage of untreated controls. The zero value was defined using the protonophore CCCP (100 μM) as an internal control.

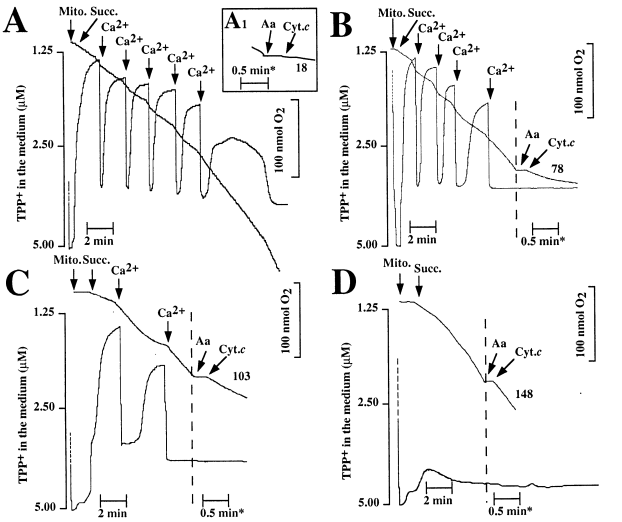



Fig. 4. Effect of caspase 1 on the mitochondrial permeability transition (MPT) induced by $Ca^{2+}$. Isolated mouse liver mitochondria were monitored constantly for oxygen consumption (line starting at the bottom) and $\Delta\Psi_m$ (line starting at the top). Simultaneous recording of the oxygen consumption and of the $\Delta\Psi_m$ was achieved with a Clark electrode and a $TPP^+$ electrode, respectively. Successive addition of $Ca^{2+}$ (75 $Ca^{2+}$ nmol/mg mitochondrial protein), as indicated by arrows. This experiment was performed on control mitochondria (A) or on mitochondria pre-incubated at room temperature for 5, 10, or 30 min in the presence of caspase 1 (2 U/ml). Preincubation in the absence of caspase has no effect (not shown). The oxygen consumption was measured after addition of antimycin A (Aa) and exogenous reduced cytochrome *c* (Cyt c) before (insert A1) or after induction of MPT (A–D). Numbers refer to the oxygen consumption (nmol $O_2$/min/mg protein) determined in these conditions. Not that the time scale in B, C, D changes.

In conclusion, the observation that caspases can perturb the mitochondrial membrane barrier function has far-reaching implications for our understanding of apoptotic signaling.

*Acknowledgements:* We thank Drs. N. Thornberry (Merck, Rahway, NJ, USA) for recombinant caspases 1, 2, and 4 and Dr. G. Salvesen (The Burnham Institute, La Jolla, CA, USA) for caspases 3 and 6. This work has been supported by grants from the ANRS, ARC, CNRS, FRM, INSERM, LFC (to G.K.). I. Marzo and S.A. Susin receive fellowships from the Spanish Ministry of Science and from the European Commission, respectively.

## References

[1] Kroemer, G., Dallaporta, B. and Resche-Rigon, M. (1998) Annu. Rev. Physiol. 60, 619–642.
[2] Penninger, J. and Kroemer, G. (1998) Adv. Immunol. 68, 51–144.
[3] Petit, P.X., Susin, S.A., Zamzami, N., Mignotte, B. and Kroemer, G. (1996) FEBS Lett. 396, 7–14.
[4] Zamzami, N., Susin, S.A., Marchetti, P., Hirsch, T., Gómez-Monterrey, I., Castedo, M. and Kroemer, G. (1996) J. Exp. Med. 183, 1533–1544.
[5] Yang, J. et al. (1997) Science 275, 1129–1132.
[6] Susin, S.A. et al. (1996) J. Exp. Med. 184, 1331–1342.
[7] Kluck, R.M., Bossy-Wetzel, E., Green, D.R. and Newmeyer, D.D. (1997) Science 275, 1132–1136.
[8] Susin, S.A. et al. (1997) J. Exp. Med. 186, 25–37.
[9] vander Heiden, M.G., Chandal, N.S., Williamson, E.K., Shcumacker, P.T. and Thompson, C.B. (1997) Cell 91, 627–637.
[10] Bossy-Wetzel, E., Newmeyer, D.D. and Green, D.R. (1998) EMBO J. 17, 37–49.

[11] Petit, P.X., Goubern, M., Diolez, P., Susin, S.A., Zamzami, N. and Kroemer, G. (1998) FEBS Lett. (in press).
[12] Zamzami, N., Marchetti, P., Castedo, M., Hirsch, T., Susin, S.A., Masse, B. and Kroemer, G. (1996) FEBS Lett. 384, 53–57.
[13] Marchetti, P., Hirsch, T., Zamzami, N., Castedo, M., Decaudin, D., Susin, S.A., Masse, B. and Kroemer, G. (1996) J. Immunol. 157, 4830–4836.
[14] Marchetti, P. et al. (1996) J. Exp. Med. 184, 1155–1160.
[15] Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W. and Vogelstein, B. (1997) Nature 389, 300–305.
[16] Zoratti, M. and Szabò, I. (1995) Biochem. Biophys. Acta 1241, 139–176.
[17] Bernardi, P. (1996) Biochim. Biophys. Acta 1275, 5–9.
[18] Kantrow, S.P. and Piantadosi, C.A. (1997) Biochem. Biophys. Res. Commun. 232, 669–671.
[19] Chernyak, B.V. (1997) FEBS Lett. 418, 131–134.
[20] Marzo, I. et al. (1998) J. Exp. Med. (in press).
[21] Zamzami, N. et al. (1998) Oncogene (in press).
[22] Nicholson, D.W. and Thornberry, N.A. (1997) Trends Biochem. Sci. 22, 299–306.
[23] Liu, X., Kim, C.N., Yang, J., Jemmerson, R. and Wang, X. (1996) Cell 86, 147–157.
[24] Zhou, H., Henzel, W.J., Liu, X., Lutschg, A. and Wang, X.D. (1997) Cell 90, 405–413.
[25] Liu, X., Zou, H., Slaughter, C. and Wang, X. (1997) Cell 89, 175–184.
[26] Hirsch, T., Marchetti, P., Susin, S.A., Dallaporta, B., Zamzami, N., Marzo, I., Geuskens, M. and Kroemer, G. (1997) Oncogene 15, 1573–1582.
[27] Shimizu, S., Eguchi, Y., Kamiike, W., Matsuda, H. and Tsujimoto, Y. (1996) Oncogene 12, 2251–2257.
[28] Medema, J.P., Scaffidi, C., Krammer, P.J. and Peter, M.E. (1998) J. Biol. Chem. 273, 3388–3393.
[29] Muzio, M. et al. (1996) Cell 85, 817–827.
[30] Medema, J.P., Scaffidi, C., Kirschkel, F.C., Shevchenko, A., Mann, M., Krammer, P.H. and Peter, M.E. (1997) EMBO J. 16, 2794–2804.
[31] Castedo, M., Hirsch, T., Susin, S.A., Zamzami, N., Marchetti, P., Macho, A. and Kroemer, G. (1996) J. Immunol. 157, 512–521.
[32] Hirata, H., Takahashi, A., Kobayashi, S., Yonehara, S., Sawai, H., Okazaki, T., Yamamoto, K. and Sasada, M. (1998) J. Exp. Med. 187, 587–600.
[33] Mittl, P.R.E., Dimarco, S., Krebs, J.F., Bai, X., Karanewsky, D.S., Priestle, J.P., Tomaselli, K.J. and Grutter, M.G. (1997) J. Biol. Chem. 272, 6539–6547.
[34] Fernandes-Alnemri, T. et al. (1996) Proc. Natl. Acad. Sci. USA 93, 7464–7469.
[35] Petit, P.X., O'Connor, J.E., Grunwald, D. and Brown, S.C. (1990) Eur. J. Biochem. 220, 389–397.
[36] Hashimoto, K. and Rottenberg, H. (1983) Biochemistry 1983, 5738–5745.
[37] Wood, E.R. and Earnshaw, W.C. (1990) J. Cell Biol. 111, 2839–2850.
[38] Susin, S.A. et al. (1997) Exp. Cell Res. 236, 397–403.
[39] Cheng, E.H.Y., Kirsch, D.G., Clem, R.J., Ravi, R., Kastan, M.B., Bedi, A., Ueno, K. and Hardwick, J.M. (1997) Science 278, 1966–1968.
[40] Clem, R.J. et al. (1998) Proc. Natl. Acad. Sci. USA 95, 554–559.
[41] Reed, J.C. (1994) J. Cell Biol. 124, 1–6.
[42] Zamzami, N. et al. (1995) J. Exp. Med. 182, 367–377.
[43] Xiang, J., Chao, D.T. and Korsmeyer, S.J. (1996) Proc. Natl. Acad. Sci. USA 93, 14559–14563.
[44] Boise, L.H. and Thompson, C.B. (1997) Proc. Natl. Acad. Sci. USA 94, 3759–3764.
[45] Leist, M., Single, B., Castoldi, A.F., Kühnle, S. and Nicotera, P. (1997) J. Exp. Med. 185, 1481–1486.
[46] Nieminen, A.L., Byrne, A.M., Herman, B. and Lemasters, J.I. (1997) Am. J. Physiol. 41, C1286–C1294.
[47] Brunet, C.L., Gunby, R.H., Benson, R.S.P., Hickman, J.A., Watson, A.J.M. and Brady, G. (1998) Cell Death Differ. 5, 107–115.