Caspase-3 Is Required for α-Fodrin Cleavage but Dispensable for Cleavage of Other Death Substrates in Apoptosis*

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Although the commonly activated death protease caspase-3 appears not to be essential for apoptosis during development except in the brain, it was not shown whether substrates known to be cleaved by caspase-3 are still proteolyzed in its absence. We have addressed this question with MCF-7 breast carcinoma cells that we recently showed lack caspase-3 owing to the functional deletion of the CASP-3 gene. Tumor necrosis factor- or staurosporine-induced apoptosis of caspase-3-deficient MCF-7 cells resulted in cleavage of the death substrates PARP, Rb, PAK2, DNA-PKcs, gelsolin, and DFF-45, but not α-fodrin. In contrast, all these substrates including α-fodrin were cleaved in apoptotic HeLa cells expressing caspase-3. Introduction of CASP-3 cDNA, but not CASP-10 cDNA, into MCF-7 cells restored α-fodrin cleavage. In addition, tumor necrosis factor- or staurosporine-induced apoptosis of MCF-7 cells stably expressing pro-caspase-3 also resulted in α-fodrin cleavage. Although the specific caspase inhibitory peptides Z-VAD-fmk and Z-DEVD-fmk prevented apoptosis of MCF-7 cells, we were unable to detect activation of caspases 2 and 7, which are known to be inhibited by Z-DEVD-fmk. Together our results suggest that caspase-3 is essential for cleavage of α-fodrin, but dispensable for the cleavage of PARP, Rb, PAK2, DNA-PKcs, gelsolin, and DFF-45 and imply that one or more caspases other than caspases 2, 3, and 7 is activated and plays a crucial role in the cleavage of these substrates in MCF-7 cells.

Programmed cell death (apoptosis) is an essential mechanism for controlling cell numbers in metazoan organisms (1). Among the many known effectors and regulators of apoptosis, a distinct class of aspartyl proteases (ICE-like proteases or caspases) stands out as being crucial for apoptosis in almost all cell types examined (2–4). Caspases are present as inactive zymogens containing an N-terminal prodomain and large and small catalytic subunits. They are activated either by autocatalytic processing mainly in inflammation, whereas members of group II (caspases 2, 3, and 7) and group III (caspases 6, 8, 9, and 10) are grouped into three subfamilies based on their substrate specificities (4–6). Group I caspases (caspases 1, 4, and 5) prefer the tetrapeptide sequence WEHD and are believed to play a role mainly in inflammation, whereas members of group II (caspases 2, 3, and 7) and group III (caspases 6, 8, 9, and 10) with the optimal peptide recognition motifs DExD and (I/L/V)ExD, respectively, are mainly involved in apoptosis (4–6). The fact that caspases 8 and 10 each contain two N-terminal located death effector domains (DED) that enable them to associate with death receptors, places these two caspases most upstream in the apoptotic activation pathway (7–9). In contrast, caspase-3 is believed to play the role of the executioner most downstream in the apoptotic pathways as it is commonly activated in cells by various death stimuli (10–13). Many different substrates are apparently cleaved by caspase-3, notably DNA fragmentation factor (DFF-45) (14), the actin regulatory protein gelsolin (15), the DNA repair enzymes poly(ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), structural proteins such as α-fodrin (16, 17), the signaling enzymes protein kinase Cδ (PKCδ), cytosolic phospholipase A2 (cPLA2), sterol-regulatory element-binding proteins, and p21-activated kinase 2 (PAK2) (4, 18–20). Although it seems likely that the selective cutting of these and other key proteins is detrimental to the cell, it is unclear which of the substrates must be cleaved to commit the cell to die and to ensure apoptosis. Elucidating the substrate specificities of the individual proteases in vivo is another largely unsolved problem and is complicated by the fact that most caspases cleave more than one caspase precursor and/or substrate in vitro (4, 19, 20).

Mice with a homozygous deletion in the CASP-3 gene show hyperplasia and abnormalities only in the brain, demonstrating that caspase-3 is essential for normal brain development (21). Furthermore, using the MCF-7 breast carcinoma cell line that lacks caspase-3 due to the functional deletion of the CASP-3 gene (22), we and others have recently demonstrated that caspase-3 is required for DNA fragmentation and blebbing (22) and for apoptosis induced by cytochrome c (23), but is not essential for TNF-, staurosporine- (22), or Fas-induced apoptosis (23). However, with the exception of PARP (21), it was not shown whether substrates known to be cleaved by caspase-3 are still proteolyzed in its absence, which other caspases are activated in apoptotic caspase-3-deficient cells, and whether various cleavage events are necessary for the cell to die. These questions are addressed in more detail in this paper.

EXPERIMENTAL PROCEDURES

Cell Lines, Reagents, and Antibodies—All cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum, 10 mM glutamine, and 50 μg/ml streptomycin and penicillin/ml (24). The human breast carcinoma cell line MCF-7 was obtained from the ATCC. The caspase inhibitory peptides benzyloxyoxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (Z-VAD-fmk) and Z-Asp-Glu-Val-Asp-fmk (Z-DEVD-fmk) were obtained from CLONTECH, and the protease inhibitors

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§ The abbreviations used are: ICE, interleukin 1α-converting enzyme; TNF, tumor necrosis factor; DED, death effector domains; PARP, poly(ADP-ribose) polymerase; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; PKC, protein kinase C; Chx, cycloheximide; PIPES, 1,4-piperazinediethanesulfonic acid; CHAPS, 1,4-piperazinediethanolamine; pNA, p-nitroanilide; Rb, retinoblastoma protein.

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aprotinin, bacitracin, antipain, leupeptin, and phenylmethylsulfonyl fluoride as well as staurosporine were purchased from Sigma. TNF was recombinant human TNF-α with a specific activity of 4 × 10^5 units/mg of protein. Purified, active recombinant human caspases 3, 6, 7, and 8 were purchased from Pharmingen. The following antibodies were used: monoclonal antibodies to gelsolin were from Sigma. The monoclonal antibodies to CPP32 (caspase-3), ICh-1 (caspase-2), and gelsolin were purchased from Transduction Laboratories Inc. The polyclonal antibodies raised against ICE (caspase-1), TX (caspase-4), McH4 (caspase-10), McH5 (caspase-8), DNA-PKcs, and PAK2, as well as the monoclonal antibodies to Rb (Rb-IP8), were bought from Santa Cruz Biotechnology. Polycomb 7 and caspase-9 antibodies were generated by immunizing rabbits with the synthetic peptides KPPRSSFVPPSFL-SKKKKN and MDEADRRLLRR corresponding to the N terminus of the putative p20 subunit of caspase-7 and to the N terminus of the prodomain of caspase-9, respectively (25, 26). The monoclonal anti-non-erythroid spectrin (α-fodrin; mAb1622) antibodies were from Chemicon International Inc., and the monoclonal anti-PARP antibodies were obtained from G. G. Poirier. The polyclonal antibodies raised against DFF-45 were from X. Wang. Preparations of Nuclear Extracts, Cell Extracts, and Western Blotting—
Nuclear and cell extracts were prepared as described (24, 27) in the presence of 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml each of aprotinin, bacitracin, antipain, and leupeptin. To confirm equal loadings, protein concentrations were determined with the Bio-Rad protein assay. Aprotinin, bacitracin, antipain, and leupeptin. To confirm equal loadings, protein concentrations were determined with the Bio-Rad protein assay. For detection of Rb, PARP, DNA-PKcs, and α-fodrin cleavage, nuclear extracts and cell extracts were separated in 0.1% SDS, 6.5% polyacrylamide gels and subjected to Western blotting as described (27). The cell extracts for the detection of PAK2, gelsolin, DFF-45, and the various caspases were fractionated in 0.1% SDS, 12.5% polyacrylamide gels. The proteins were visualized by the Amersham Pharmacia Biotech ECL kit. Western blot analysis of the extracts for the detection of PAK2, gelsolin, DFF-45, and the various caspases was performed with the ApoAlertTM CPP32 Colorimetric Assay Kit (CLONTECH) according to the protocol of the manufacturer. For the protease cleavage assays, Rb was synthesized in the presence of [35S]methionine as described (29). Cleavage reactions were performed for 2 h at 37 °C using 3 μl of in vitro translated Rb and 200 ng of active caspases in 30 μl containing 20 mM PIPES (pH 7.2), 100 mM NaCl, 10 mM dithiothreitol, 1 mM EDTA, 0.1% CHAPS, and 10% sucrose, and analyzed in 0.1% SDS-6.5% polyacrylamide gels followed by autoradiography.

RESULTS

Only Caspase-8 Is Activated in TNF-treated, but Not in Staurosporine-treated MCF-7 Cells—We have recently demonstrated that the human MCF-7 breast carcinoma cell line is devoid of caspase-3 owing to the functional deletion of the CASP-3 gene (22). Despite the lack of caspase-3, MCF-7 cells are still sensitive to a variety of apoptosis inducers such as transforming growth factor-β1 (30), Fas (23), and TNF or staurosporine (22) indicating that other caspases may be activated.

To examine whether MCF-7 cells specifically lack only pro-caspase-3 and to determine which pro-caspases are activated in TNF-CHX- or staurosporine-induced apoptosis, control and apoptotic cell lysates of MCF-7 cells were probed with antibodies to various caspases. As a control, lysates of similarly treated HeLa D98 and H21 cells which are highly or only marginally sensitive to TNF, respectively (24), were included. Besides caspase-3, MCF-7 cells also lack detectable levels of pro-caspase-1 which, however, is present in both HeLa cell lines (Fig. 1). All other pro-caspases tested (pro-caspases 2, 5, 7, 8, 9, and 10) were present in all three cell lines examined (Fig. 1). There was some variation in the protein levels of pro-caspases 2 and 7 in HeLa and MCF-7 cells, but pro-caspases 5, 9, and 10 were equally expressed in all three cell types (Fig. 1). In addition to the observed activation of caspase-3 in HeLa D98 and H21 cells following a death stimulus (Fig. 1), only caspase-8, but none of the other tested caspases, was activated by both death stimuli to various degrees as demonstrated by the appearance of a singlet band or doublet of approximately 24–26 kDa (Fig. 1, lanes 2, 3, 5, 6, and 8). These proteins which were present only in apoptotic lysates most likely represent intermediate cleavage products of caspase-8, as demonstrated previously in vitro and in vivo (31). Interestingly, in all three cell lines examined, caspase-8 was efficiently and similarly activated by TNF/Chx (Fig. 1, lanes 2, 5, and 8), despite the fact that only 18% of the HeLa H21 cells were killed by this death stimulus (Fig. 1). In contrast, all three cell lines were killed to a similar extent following a 16-h staurosporine treatment (Fig. 1), which, however, resulted only in a marginal and almost undetectable activation of caspase-8 in HeLa H21 and in MCF-7 cells, respectively (Fig. 1, lanes 6 and 9). These data are

![Fig. 1. Caspase-8, but none of the other tested caspases, is activated in apoptotic MCF-7 cells. Western blot analysis of the expression of the indicated pro-caspases (or caspase-8 fragments) in lysates of untreated HeLa D98, HeLa H21, or MCF-7 cells (lanes 1, 4, and 7). HeLa cells treated for 4 h (lanes 2 and 5) or MCF-7 cells treated for 20 h (lane 8) with TNF/Chx, and cells treated for 16 h with staurosporine (lanes 3, 6, and 9).](http://www.jbc.org/content/15541/1/15541.full.html)
in agreement with reports demonstrating an important role for caspase-8 predominantly in TNF- or Fas-mediated apoptosis (8, 9, 31), but also suggest that caspase-8 (like caspase-3) may not always be essential for apoptosis, even though both caspases are commonly activated by a variety of death stimuli (10, 13, 31, 32).

**Cleavage of Death Substrates but Not of α-Fodrin in Caspase-3-deficient MCF-7 Cells**—Having established that MCF-7 cells lack pro-caspases 1 and 3, but express other apoptosis-related pro-caspases, we examined control and apoptotic cell lysates of HeLa D98, HeLa H21, and MCF-7 cells for the status of various death substrates known to be cleaved by caspase-3 or a caspase-3-like protease (4, 19). The same experimental conditions were applied as described in Fig. 1. The cleavages of PARP, the retinoblastoma protein (Rb), α-fodrin, and DNA-PKcs could be readily assessed by the appearance of the corresponding fragments in apoptotic lysates, whereas proteolysis of PAK2, gelsolin, and DFF-45 was judged by the decrease in the intensity of the full-length proteins. Two different antibodies to PAK2 and gelsolin both gave the same results, but no fragments were detected. Nevertheless, with the exception of α-fodrin, all substrates tested including PARP, Rb, PAK2, gelsolin, and DFF-45 were still cleaved in caspase-1- and caspase-3-deficient MCF-7 cells (Fig. 2, lanes 8 and 9). Except for gelsolin which was only cleaved in staurosporine-treated, but not in TNF/Cx-treated, MCF-7 cells (a pattern also observed in D98 cells; Fig. 2, lanes 2 and 3), all substrates were cleaved to a similar extent in MCF-7 cells treated with TNF/Cx or staurosporine (Fig. 2, lanes 8 and 9). This is in contrast with the cleavage patterns observed in the two HeLa cell lines, in which most of the substrates were cleaved more efficiently in TNF/Cx-treated cells (Fig. 2, lanes 1–6). Together with our results demonstrating that of all the caspases tested only caspase-8 was activated in MCF-7 cells by TNF/Cx but not by staurosporine (Fig. 1), these data provide evidence for the existence of other as yet unknown caspases that cleave PARP, Rb, PAK2, DNA-PKcs, gelsolin, and DFF-45.

Interestingly, α-fodrin, known to be cleaved by caspase-3 but not by caspases 1 and 2 (16, 17), was the only substrate tested that was resistant to caspase cleavage in apoptotic lysates of TNF/Cx- or staurosporine-treated MCF-7 cells, based on the absence of the typical 150-kDa fragment (Fig. 2, lanes 8 and 9). Although both death stimuli induced the formation of a 150-kDa α-fodrin fragment in all three cell lines including MCF-7 cells (Fig. 2, lanes 2, 3, 5, 6, 8, and 9), this fragment was recently shown to be the product of the thiol protease calpain, an enzyme unrelated to caspases (33). Taken together, these results imply that of all the substrates tested, only cleavage of α-fodrin appears to require the activation of caspase-3.

**Caspase-3 Induces Apoptosis in MCF-7 Cells, and α-Fodrin and PARP, but Not Rb, Are Cleaved**—To assess whether certain cleavage events are necessary for apoptosis, MCF-7 cells were transiently transfected with a pCMV-β-galactosidase construct with expression vector pcDNA3 encoding full-length pro-CASP-3, pro-CASP-10, or with vector alone. The cells were fixed and stained for β-galactosidase expression 40 h following transfection. Western blot analysis of the expression of the indicated death substrates was performed with lysates of MCF-7 cells transfected with vector alone (lane 3), CASP-3 (lane 4), or CASP-10 (lane 5). As controls for the cleavages, nuclear extracts (PARP and Rb) or cell lysates (caspase-3 and α-fodrin) from untransfected MCF-7 cells either untreated (lane 1) or treated for 20 h with TNF/Cx (lane 2) were included.
clearly demonstrate that cleavage of fodrin cleavage, MCF-7 cells stably expressing pro-caspase-3 (22) lanes 4, -10 (Fig. 3). Caspase-3 no Rb cleavage was observed in MCF-7 cells transfected with the vector alone (MCF-7.0.19, lanes 1–3). Cells were either untreated (lanes 1, 4, 7, and 10) or were treated for 16 h with TNF/Chx (lanes 2, 5, 8, and 11) or for 16 h with staurosporine (lanes 3, 6, 9, and 12).

the presence of some inactive caspase-3 precursor remaining in CASP-3 transfected MCF-7 cells (Fig. 3B, upper panel, lane 4). Regardless of the explanation, these results indicate that caspase-3 can activate a death pathway in MCF-7 cells. More importantly, Western blot analysis showed that the overexpression of pro-caspase-3 (but not pro-caspase-10) led to the cleavage of α-fodrin giving rise to the typical 120-kDa fragment (Fig. 3B, compare lanes 4 and 5). Surprisingly, Rb was not cleaved in the presence of caspase-3 (Fig. 3B, lane 4), although the cleavage site in Rb (DEAD ↓ G) appears to be close to a typical caspase-3 recognition motif (29, 34). Likewise, little or no Rb cleavage was observed in MCF-7 cells transfected with CASP-10 (Fig. 3B, lane 5). PARP, on the other hand, was cleaved into the signature 85-kDa apoptotic fragment in MCF-7 cells transfected with either the CASP-3 or CASP-10 cDNAs (Fig. 3B, lanes 4 and 5).

To further investigate whether caspase-3 is required for α-fodrin cleavage, MCF-7 cells stably expressing pro-caspase-3 (22) were treated with TNF/Chx or staurosporine, and α-fodrin cleavage was monitored by Western blotting. Both death stimuli resulted in the activation of caspase-3 in three individual MCF-7 clones stably expressing pro-caspase-3 (Fig. 4, upper panels). Consistent with the caspase-3 activation profile obtained in apoptotic HeLa cells (Fig. 1), TNF/Chx treatment of the three pro-caspase-3-expressing MCF-7 clones resulted in a more efficient activation of this protease than the treatment with staurosporine (Fig. 4, upper panels). More importantly, processing of pro-caspase-3 in these cells was accompanied by the cleavage of α-fodrin into the typical 120-kDa fragment (Fig. 4, lower panels). Similar to the results obtained with parental caspase-3-deficient MCF-7 cells (Figs. 2 and 3B), TNF/Chx or staurosporine treatment of vector-transfected MCF-7 cells did not result in the appearance of the 120-kDa α-fodrin fragment (Fig. 4, lower panel, lanes 1–3). Taken together, these results clearly demonstrate that cleavage of α-fodrin requires caspase-3 activity.

Rb Is Cleaved In Vitro by Caspase-7—To investigate which caspase is responsible for Rb cleavage, the non-phosphorylated form of Rb was synthesized in vitro in the presence of [35S]methionine and incubated with various recombinant active caspases. In agreement with our result that induction of apoptosis by transient expression of pro-caspase-3 does not result in the cleavage of Rb (Fig. 3B), this experiment revealed that only caspase-7, but not caspases 3, 6, or 8, cleaved Rb (Fig. 5). The observed cleavage pattern of Rb by caspase-7 was similar to that obtained in vivo and in vitro in nuclear extracts of apoptotic cells (29), suggesting that caspase-7 or a closely related protease may be responsible for cleavage of Rb in vivo.

Lack of DEVD Activity in Caspase-3-deficient MCF-7 Cells—Based on their substrate specificities, caspase-3 together with caspases 2 and 7 belongs to the group II subfamily of caspases that preferentially cleave the peptide sequence DExD found in many death substrates (4–6). These caspases are believed to be the final executioners whereas (except for caspase-6) caspases of group III (caspases 6, 8, 9, and 10) with an optimal recognition sequence (I/L/V)ExD appear to play a more apical role in apoptosis. Of all the caspases tested, only caspase-8 was activated by TNF/Chx in caspase-3-deficient MCF-7 cells (Fig. 1, lane 8). However, with the exception of α-fodrin, substrates known to be cleaved by caspase-3 or caspase-3-like proteases (PARP, Rb, PAK2, DNA-PKcs, gelsolin, and DFF-45) were still proteolyzed in apoptotic MCF-7 cells (Fig. 2), suggesting that other DEVD-specific caspases cleave these substrates. To examine this possibility, we assayed lysates of untreated and TNF/Chx- or staurosporine-treated HeLa D98, HeLa H21, and MCF-7 cells for DEVD activity, which is based on the specific cleavage of the substrate DEVD-paranitroanilide (DEVD-pNA) by caspase-3 (or a caspase-3-like protease). Surprisingly, MCF-7 cells lack any detectable DEVD-pNA-cleaving activity following treatment with TNF/Chx or staurosporine, whereas both death stimuli induced substantial DEVD-pNA-cleaving activity in HeLa D98 and H21 cells, with TNF/Chx being the most potent inducer (Fig. 6). Specific Caspase Inhibitors Prevent Apoptosis in MCF-7 Cells—Next we investigated the apparent paradox that substrates known to be cleaved by DEVD-specific caspases are still proteolyzed in MCF-7 cells, which lack detectable DEVD-pNA-cleaving activity. For this purpose, MCF-7 cells and HeLa D98 cells were incubated with TNF/Chx or staurosporine in the absence or presence of cell-permeable, irreversible caspase inhibitory peptides (35). Z-VAD-fmk, a caspase inhibitor of broad specificity, completely prevented TNF-induced apoptosis of MCF-7 and HeLa D98 cells (Fig. 7). In addition, Z-DEVD-fmk, a selective inhibitor of the caspase-3 subfamily also inhibited TNF-induced apoptosis of both cell lines, although the protection was not surprisingly less efficient than that obtained with Z-VAD-fmk (Fig. 7). In contrast, both peptides were less efficient in protecting the two cell lines from staurosporine-induced apoptosis (Fig. 7). Interestingly, both Z-VAD-fmk and Z-DEVD-fmk protected HeLa D98 cells from TNF- or staurosporine-induced apoptosis in a dose-dependent fashion with maximum protection obtained in the 22–66 μM range, whereas in MCF-7 cells even the lowest concentration (7 μM) of either
Z-VAD-fmk and Z-DEVD-fmk. HeLa D98 and H21 cells but not in MCF-7 cells. Lysates of untreated (control) cells or cells treated for 4 h (D98) or 20 h (H21 and MCF-7) with TNF/Chx or for 20 h (all cells) with staurosporine were assayed for DEVD activity as described under “Experimental Procedures.” The values are derived from one representative experiment of four performed in duplicates.

FIG. 6. Detectable DEVD-pNA-cleaving activity in apoptotic HeLa D98 and MCF-7 cells by Z-VAD-fmk and Z-DEVD-fmk. HeLa D98 cells and MCF-7 cells were treated for 4 h or 20 h, respectively, with TNF/Chx (T/C) or for 20 h with staurosporine in the absence (control) or presence of various indicated concentrations of Z-VAD-fmk or Z-DEVD-fmk. Apoptosis was measured as described above. The values are derived from one representative experiment of three performed in triplicate.

FIG. 7. Inhibition of apoptosis of HeLa D98 and MCF-7 cells by Z-VAD-fmk and Z-DEVD-fmk. HeLa D98 cells and MCF-7 cells were treated for 4 h or 20 h, respectively, with TNF/Chx (T/C) or for 20 h with staurosporine in the absence (control) or presence of various indicated concentrations of Z-VAD-fmk or Z-DEVD-fmk. Apoptosis was measured as described above. The values are derived from one representative experiment of three performed in duplicate.

DISCUSSION

In mice it was inferred that caspase-3 is not required for developmentally regulated apoptosis in tissues and organs other than the brain, but except for PARP cleavage in thymocytes, it was not shown whether other death substrates known to be cleaved by caspase-3 are still proteolyzed in its absence (21). We addressed this question with the MCF-7 cell line which readily undergoes apoptosis following treatment with various death stimuli (22, 23, 30) despite the functional deletion of the CASP-3 gene (22). α-Fodrin was the only substrate tested not to be cleaved into the 120-kDa fragment in caspase-3-deficient apoptotic MCF-7 cells, confirming that generation of this fragment is not required for apoptosis induced by TNF (17) or staurosporine. In these cells, α-fodrin was only cleaved into the 150-kDa fragment (most likely by calpain; Ref. 33), showing that proteolysis by a caspase and calpain are independent events. Introduction of the CASP-3 (but not CASP-10) gene into MCF-7 cells resulted in the cleavage of α-fodrin into the typical 120-kDa fragment. As it was recently shown in vitro that cleavage of this substrate was mediated by caspase-3 but not by caspases 1 or 2 (17), we conclude that the presence and activation of caspase-3 is required for cleavage of α-fodrin in vivo. Whether caspase-3 cleaves α-fodrin directly or via the activation of another caspase is presently unknown.

Remarkably, all other substrates tested including PARP, Rb, DNA-PKcs, PAK2, gelsolin, and DFF-45 were still cleaved in caspase-3-deficient apoptotic MCF-7 cells. Considering the fact that MCF-7 cells die without blebbing and DNA fragmentation (22, 30), these results were surprising as the reported caspase-3-mediated cleavages of gelsolin and PAK2 were implicated in the morphological changes associated with apoptosis (15, 18), and the cleavage of DFF-45 and its recently discovered mouse homolog ICAD by caspase-3 were shown to be important events in the initiation of DNA fragmentation (14, 36, 37). As introduction of the CASP-3 gene into MCF-7 cells resulted in DNA fragmentation and blebbing following TNF treatment (22), our present results indicate that besides the cleavages of PAK2, gelsolin, and DFF-45, additional caspase-3-dependent cleavage events are necessary for these processes to occur at least in MCF-7 cells. The cleavage of α-fodrin could contribute to the morphological changes, because fodrin is a major component of the cortical cytoskeleton of most eukaryotic cells (38), it has binding sites for actin, calmodulin, and microtubules (39), and its proteolysis has been recently suggested to contribute to the structural rearrangements including blebbing during apoptosis (16, 17). Our data presented here and in a previous study (22) strongly support this hypothesis, although additional studies with a non-cleavable α-fodrin protein are necessary to clarify this issue.

We have also shown that of all the caspases tested (caspases 1, 2, 3, 5, 7, 8, 9, and 10), only caspases 3 and 8 were detectably activated in TNF- or staurosporine-treated HeLa cells, and only caspase-8 was activated in TNF-treated MCF-7 cells. The available antibodies to caspases 4 and 6 were not of a sufficiently high quality for us to determine whether or not these caspases were present and activated. However, caspase-4 is believed to play a role in inflammation, and there is no evidence that it is involved in apoptosis (3, 40). Caspase-6, on the other hand, appears to cleave only the nuclear lamins during apoptosis (41–43). Moreover, the cleavage site specificities of caspases 1 or 2 (17), we conclude that the presence and activation of caspase-3 is required for cleavage of α-fodrin in vivo. Whether caspase-3 cleaves α-fodrin directly or via the activation of another caspase is presently unknown.
confirms its status as a universal marker of apoptosis, which is consistent with recent although still controversial findings that DNA repair and so to hasten apoptosis (3, 4, 19). This is transfection, which is in sharp contrast to TNF- or Fas-induced 3 and 10 are not involved in Rb cleavage, and, furthermore, the from which two main conclusions can be drawn. First, caspases led to PARP cleavage and cell death, but Rb was not cleaved, not required for apoptosis mediated by caspases. Together with earlier studies showing that PARP, Rb, DNA-PKcs, PAK2, gelsolin, and DFF-45 are cleaved by a caspase inhibitory peptide of broad specificity, and to a lesser..

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