Conversion of Procaspase-3 to an Autoactivating Caspase by Fusion to the Caspase-2 Prodomain*

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Caspases are cysteine proteases that play an essential role in apoptosis. Initial activation of caspases defines the key step in apoptotic execution. Based on primary structure, caspases can be divided into two groups, those with long amino-terminal prodomains (class I), and those with relatively short prodomains (class II). On overexpression in mammalian cells, class I caspases can induce cell death that is dependent on their autocatalytic activity. Recent studies suggest that the long prodomains in some class I caspases are able to mediate dimerization of procaspase molecules, thereby promoting autoprocessing. In this communication, we demonstrate that fusion of the prodomain of a class I caspase (Nedd2/caspase-2) with procaspase-3 greatly augments autoprocessing and apoptosis induction by the chimeric caspase-3 molecule. The chimeric caspase-3 molecules were able to form homodimers in Saccharomyces cerevisiae and were efficiently processed in transfected mammalian cells. These results provide direct evidence for a role of a class I caspase prodomain in caspase autoprocessing and establish a basis for functional hierarchy among the two classes of caspases.

Caspases are a family of cysteine proteases that cleave their target protein following an Asp residue. Of the eleven published caspases, caspase-1, -4, -5, and -11 seem primarily involved in the processing of proinflammatory cytokines, whereas others play crucial roles in one or more pathways of apoptosis (reviewed in Refs. 1–5). Caspases are produced as precursor molecules that require processing into two subunits to produce a fully active enzyme (1–5). On the basis of primary structure, proapoptotic caspases can be divided into two classes, class I including caspase-2, -8, -9, and -10 that contain a long amino-terminal prodomain, and class II such as caspase-3, -6, and -7 with a short or absent prodomain. Although numerous studies have provided data on the activation of multiple caspases in apoptosis, the mechanism of activation of the initial caspases in a particular apoptotic pathway is poorly understood. The long prodomain in class I caspases such as caspase-2, -8, and -10 can interact with adaptor molecules that are directly or indirectly recruited to specific death receptors. For example, the prodomain of caspase-8 and -10 contain two death effector domains that interact with the death effector domains in the adaptor molecule FADD, which recruits these caspases to the activated Fas receptor (4–7). In a similar manner, caspase-2 is thought to be recruited to death receptors by binding through the adaptor RAIDD (8). The prodomain of caspase-9 shares a degree of homology with the amino terminus of Apaf-1, a CED-4-like mammalian molecule that can interact with caspase-9 (9). Several recent studies demonstrate that procaspases can be activated through dimerization/oligomerization mediated through their prodomains (10) or induced artificially (11, 12). This suggests that the primary role of adaptor molecules that recruit class I caspases may be to bring procaspase molecules into close proximity with each other to enable dimerization. In vitro cleavage experiments suggest that class II caspases require activated class I caspases for their proteolytic processing (reviewed in Refs. 3–5).

We have recently demonstrated that caspase-2, a class I caspase, can dimerize and autoprocess in Saccharomyces cerevisiae and mammalian cells (10). The prodomain of caspase-2 is essential for the dimerization of the precursor molecule and caspase-2 lacking the prodomain is poorly processed in S. cerevisiae cells (10). Overexpression of procaspase-2 in mammalian cells induces apoptosis because of its autoprocessing activity (13–16). Caspase-3, a class II effector caspase, lacks a long prodomain (17, 18). Unlike procaspase-2, procaspase-3 is a poor inducer of cell death when transfected in mammalian cells (16–18), presumably because of its inability to autoactivate. We reasoned that if the prodomain of a class I caspase was essential for the activation of a procaspase, fusing a class I caspase prodomain onto a class II caspase would generate a procaspase molecule able to dimerize and activate caspase-3. In this communication we show that amino-terminal fusion of the caspase-2 prodomain (C2P)† to procaspase-3 results in remarkable enhancement of its apoptotic activity. We further demonstrate that fusion of C2P to caspase-3 enables the chimeric molecule to dimerize in S. cerevisiae and to autoprocess in vivo. These data provide direct evidence that the primary function of a class I caspase prodomain is to mediate dimerization and that this dimerization is necessary and sufficient for autoprocessing of the caspase precursor. Our results also establish a structural basis for the functional hierarchy among two classes of caspases.

EXPERIMENTAL PROCEDURES

Green Fluorescent Protein (GFP) Fusion Constructs—Details of the caspase-GFP fusion constructs used in this study have been described elsewhere (19). Briefly, caspase coding regions were amplified by polymerase chain reaction using Pfu polymerase and fused in frame to the

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† The abbreviations used are: C2P, prodomain of caspase-2/Nedd2; GFP, Aequorea victoria green fluorescent protein; AD, activation domain; BD, DNA-binding domain; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.
expression vectors has been described previously (16). The Bcl-2 expres-
sor with either CrmA, P35, MIHA, or Bcl-2 expression vectors were cotransfected with either CrmA, P35, MIHA, or Bcl-2 expression vectors 18 h post-transfection. In some cases caspase-GFP expression vectors of polymerase chain reaction using GFP and C2P-caspase-3(C163G)-GFP vectors, respectively, by 30 cycles and the caspase-3(C163G) mutant were amplified from the caspase-3-GFP plasmids, respectively, by site-directed mutagenesis. The C2P-caspase-3(C163G)-GFP and C2P-caspase-3AB(C163G)-GFP constructs were generated from C2P-caspase-3-GFP and C2P-caspase-3AB-GFP plasmids, respectively, by site-directed mutagenesis. The C2P-caspase-3-GFP and C2P-caspase-3AB(C163G)-GFP constructs lack the first 9 amino-terminal residues of the caspase-3 precursor (19). Cell Culture and Transient Transfection—NIH-3T3 cells were main-
tained in Dulbecco’s modified Eagle’s medium with 10% fetal calf ser-
um. For cell death assays, 2 x 10^5 cells were plated at 35-mm dishes the day before transfection. Expression vectors (2 µg of total DNA) were transfected into cells using the Fugene reagent (Boehringer Mannheim) according to the manufacturer’s protocol. Cells were observed, scored for apoptotic morphology and photographed using a fluorescence micro-
scope (Olympus BH2-RFCA) 18 h post-transfection. For immunoblot-
ning, cells were plated at a density of 6 x 10^5 cells per 60-mm dish and transfection with a total of 6 µg of plasmid DNA. Cells were harvested 18 h post-transfection. In some cases caspase-GFP expression vectors were cotransfected with either CrmA, P35, MIHA, or Bcl-2 expression vectors by cotransfection at a ratio of 1:3. Construction of CrmA, P35, and MIHA expression vectors has been described previously (16). The Bcl-2 expres-
sion vector was kindly provided by Dr David Vaux.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting—Pro-
tiens were resolved on SDS-polyacrylamide gel electrophoresis gels and transferred to polyvinylidine difluoride membrane (DuPont). Blots were probed with an anti-GFP monoclonal antibody (Boehringer Mannheim). Following incubation with appropriate horseradish peroxidase-coupled second antibody, signals were detected using the ECL system (Amersham Pharmacia Biotech).

Construction of Two-hybrid Vectors—The coding regions of caspase-3 and the caspase-3(C163G) mutant were amplified from the caspase-3-
GFP and C2P-caspase-3(C163G)-GFP vectors, respectively, by 30 cycles of polymerase chain reaction using Pfu polymerase (Strata-
gene) cloning into yeast Gal4 DNA binding domain (Gal4DB) vector pAS2.1 (CLONTECH), an upstream oligonucleotide containing an EcoRI site (primer A) and a downstream primer containing a BamHI site (primer B) were used, and the amplified product cloned in frame into EcoRI/BamHI digested pAS2.1. For cloning into the Gal4 activation domain (Gal4AD) vector pACT2 (CLONTECH), an upstream primer containing a BamHI restriction site (primer C) and the downstream primer B were used, and the amplified product cloned in frame into BamHI digestion site in pACT2. Prime A, 5'-CGGGATTTCGAGAAACACTGAGAAGCTCAA; primer B, 5'-GGTGGAAATGAGGTAATAAAATAGGTTCTTT; primer C, 5'-CGGGATCCGGAGAGACCTGAAAAACTCTA (regions complementary to the caspase-3 sequence are underlined).

The coding regions of the caspase-3 molecules containing the caspase-2 prodomain were amplified from the previously described vectors2 C2P-caspase-3(C163G)-GFP, C2P-caspase-3AB(C163G)-GFP, and C2P-caspase-3(C163G) was amplified using the following oligonucleotide primers: primer D, 5'-GGAATTCGAGAAACACTGAGAAGCTCAA; primer E, 5'-GGGATCCGGAGAGACCTGAAAAACTCTA (regions complementary to the caspase-2 sequence are underlined) and primer F described above. The amplified products were digested with BamHI and cloned in frame into Small/BamHI digested pAS2.1 and pACT2.

Yeast Two-Hybrid Experiments—These assays were essentially car-
ried out as described previously (10). Caspase-3 constructs in Gal4AD and Gal4BD vectors were cotransfected into S. cerevisiae strain Y190 and colonies containing both vectors were selected on SD medium lacking leucine and tryptophan. Transformed colonies were directly screened for β-galactosidase activity in a colony-lift filter assay using 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal).

RESULTS AND DISCUSSION

Fusion of Caspase-2 Prodomain to Procaspase-3 Augments Its Cell Killing Activity—Overexpression of procaspase-3 re-
ults in apoptosis in many cell types, whereas overexpression of procaspase-3 leads to relatively little cell death (13–18). To check if a class I prodomain fused to procaspase-3 can enhance its cell killing activity, we generated expression constructs in which the C2P was placed in frame at the amino terminus of procaspase-3 (Fig. 1A). We constructed two chimeric expression vectors in which either the full-length procaspase-3 or pro-
caspase-3 lacking the first 9 amino-terminal residues (Δ9) was fused to the prodomain of caspase-2. Because one of the cleav-
age sites in procaspase-3 occurs following Asp-9 (20), the small pro-
domain region in caspase-3 and 3 amino-terminal residues in pro-
caspase-3. This deletes caspase cleavage sites between C2P and caspase-3. B, apoptosis-inducing activity of various GFP fusion constructs in tran-
siently transfected NIH-3T3 cells. Various constructs were transfected into NIH-3T3 cells. After an 18 h incubation, cells were fixed and observed for apoptotic morphology using a fluorescence microscope. Data, shown as percent of morphologically apoptotic cells among all GFP-positive cells, are mean values from an experiment performed in duplicate. Similiar results were obtained in multiple independent experiments.

FIG. 1. C2P fusion onto caspase-3 precursor greatly enhances cell killing activity of caspase-3. A, structure of the various caspase-
GFP fusion proteins used in this study. All constructs were generated by fusing GFP at the carboxyl-terminal of caspase molecules in pEGFP-
N1. The Δ9 chimeric constructs lack 3 carboxyl-terminal residues of the caspase-2 prodomain and 9 amino-terminal residues in procaspase-3. This deletes caspase cleavage sites between C2P and caspase-3. B, apoptosis-inducing activity of various GFP fusion constructs in tran-
siently transfected NIH-3T3 cells. Various constructs were transfected into NIH-3T3 cells. After an 18 h incubation, cells were fixed and observed for apoptotic morphology using a fluorescence microscope. Data, shown as percent of morphologically apoptotic cells among all GFP-positive cells, are mean values from an experiment performed in duplicate.
>95%), whereas caspase-3-GFP induced apoptosis in around 15% cells (Fig. 1B). Most of the caspase-3-GFP transfected cells showed normal morphology and, as reported by us recently (19), the caspase-3-GFP fusion protein was localized largely in the cytoplasm (Fig. 2C). The cells transfected with the pro-caspase-2-GFP construct showed very weak fluorescence, presumably because of destruction of GFP in apoptotic cells (Fig. 2B). Although caspase-3-GFP induced apoptosis in some of the transfected cells, apoptotic cells retained fluorescence suggesting that these cells represent early apoptotic cells (data not shown). The chimeric C2P-caspase-3-GFP molecules, with or without the first 9 amino acids of procaspase-3, were potent killers of transfected NIH-3T3 cells when compared with wild-type procaspase-3 with an apoptotic activity comparable with procaspase-2 (Figs. 1B, 2D, and 2E). C2P-caspase-3(C163G)-GFP, the catalytically inactive caspase-3 mutant fused to the caspase-2 prodomain, was unable to induce cell death, suggesting that autocatalytic activity of C2P-caspase-3 was necessary for cell killing (Figs. 1B and 2F). As reported recently (19), the C2P-caspase-3(C163G)-GFP mutant chimeric protein localized to discreet “dot-like” structures in both cytoplasmic and nuclear compartments of the transfected cells (Fig. 2F). These results suggest that addition of the caspase-2 prodomain to the amino terminus of caspase-3 can confer strong apoptosis-inducing activity onto caspase-3. However, we do not yet know whether concentration in specific dot-like regions contributes to the apoptotic activity of the chimeric C2P-caspase-3 by promoting oligomerization and processing.

**Inhibition of Chimeric Caspase-3 Activity by MIHA and P35**—Many viral and cellular proteins have distinct inhibitory effects on biological and biochemical activities of caspases (1–5). Therefore, it was of interest to determine whether fusion of the caspase-2 prodomain to caspase-3 would affect interaction of the chimeric molecule with cellular and viral inhibitors of apoptosis. We compared the inhibitory effects of three caspase inhibitors (CrmA, P35, and MIHA/XIAP) and Bcl-2 on apoptosis induced by caspase-2-GFP and caspase-3-GFP containing the caspase-2 prodomain (C2P-caspase-3-GFP). Caspase expression constructs were cotransfected with expression constructs containing one of the inhibitors at a ratio of 1:3, and transfected NIH-3T3 cells scored 18 h later. As shown in Fig. 3, only P35 significantly inhibited caspase-2-GFP induced apoptosis (around 20%). However, C2P-caspase-3-GFP induced apoptosis was strongly inhibited by MIHA and P35, and partially inhibited by Bcl-2 and CrmA (Fig. 3). The observation that C2P-caspase-3-GFP-mediated apoptosis is suppressed by P35 and XIAP, but not by CrmA, is consistent with previously reported inhibitory profiles of caspase-3 (16–18, 20, 21), suggesting that the C2P-domain fusion does not alter the basic kinetic characteristics of caspase-3.

**Chimeric Prodomain Caspase-3 Undergoes Autoproteolytic Processing in Vivo**—To check whether the addition of the caspase-2 prodomain can mediate in vivo processing of caspase-3, we carried out immunoblotting of the extracts from transfected NIH-3T3 cells. As shown in Fig. 4, this protein did not show any appreciable processing in NIH-3T3 cells transfected with caspase-3-GFP (59 kDa). However, in cells transfected with C2P-caspase-3-GFP, no full-length (77 kDa) protein was detected. Instead, a band of 39 kDa, representing procaspase-3(C163G)-GFP, was present (Fig. 4), demonstrating that the chimeric molecule containing the caspase-2 prodomain was much more readily processed when compared with wild-type caspase-3. On the other hand, the catalytically inactive C2P-caspase-3(C163G)-GFP protein was detected as a single unprocessed band of 77 kDa (Fig. 4) suggesting that autocatalytic activity of the chimeric caspase is required for its process-
Autoactivation of Chimeric Caspase-3

Fig. 3. Different inhibitory profiles of p35 and MIHA on apoptosis induced by caspase-2 and chimeric C2P-caspase-3. NIH-3T3 cells were cotransfected with caspase-GFP constructs and either a Bcl-2, CrmA, P35, or MIHA expression vector at a 1:3 ratio. Cells were examined for apoptotic morphology and scored using a fluorescence microscope 18 h post-transfection. Data, shown as percent of morphologically apoptotic cells among all GFP-positive cells, are mean values from an experiment performed in duplicate. Similar results were obtained in multiple independent experiments.

Fig. 4. Chimeric C2P-caspase-3 protein is efficiently processed in transfected NIH-3T3 cells. NIH-3T3 cells transfected with various expression constructs were harvested 18 h post-transfection, and cell extracts were subjected to immunoblot analysis using an anti-GFP antibody. Caspase-3(G163G) (59 kDa) protein does not show significant processing, whereas C2P-caspase-3-GFP (77 kDa) is detected as a 39-kDa band representing p12-GFP. The first lane in the figure (caspase-3-GFP) was derived from a different immunoblot than the rest.

TABLE I

| Gal4BD construct in pAS2.1 vector | Gal4AD construct in pACT2 vector | β-galactosidase activity |
|----------------------------------|---------------------------------|-------------------------|
| Empty vector                    | Empty vector                    | —                       |
| p53                              | SV40 large T                    | +                       |
| Caspase-3                        | Caspase-3                        | ±                       |
| Caspase-3(C163G)                 | Caspase-3(C163G)                | ±                       |
| C2P                              | C2P                             | —                       |
| C2P                              | Empty vector                    | —                       |
| Empty vector                     | C2P                             | +                       |
| Empty vector                     | Empty vector                    | —                       |
| C2P-caspase-3Δ9                  | C2P-caspase-3Δ9                 | +                       |
| C2P-caspase-3Δ9                  | Empty vector                    | —                       |
| Empty vector                     | C2P-caspase-3(C163G)            | —                       |
| Empty vector                     | C2P-caspase-3(C163G)            | —                       |
| C2P-caspase-3Δ9(C163G)           | Empty vector                    | —                       |
| C2P-caspase-3Δ9(C163G)           | Empty vector                    | —                       |
| C2P-caspase-3Δ9(C163G)           | Empty vector                    | —                       |
| C2P-caspase-3Δ9(C163G)           | Empty vector                    | —                       |
| C2P-caspase-3Δ9(C163G)           | Empty vector                    | —                       |
| C2P-caspase-3Δ9(C163G)           | Empty vector                    | —                       |

Conclusions—Our results provide strong support for the notion that the primary function of the prodomain in class I caspases is to promote dimerization and that dimerization is crucial for their activity. Interestingly, in all transfection experiments, we noticed that the intensity of the processed protein band (39 kDa) representing the p12-GFP protein was always much lower than that of the unprocessed protein, suggesting that the processed caspase subunits may be unstable. This may explain, at least in part, why the GFP fluorescence is very weak in apoptotic NIH-3T3 cells transfected with C2P-caspase-3-GFP or caspase-2-GFP (Fig. 2). In NIH-3T3 cells cotransfected with C2P-caspase-3-GFP and either MIHA or P35, almost complete suppression of processing of the chimeric caspase molecule was seen (Fig. 4), suggesting that MIHA and P35 inhibit C2P-caspase-3-GFP-induced apoptosis by inhibiting procaspase processing. Bcl-2 is known to act upstream of caspase-3 by inhibiting the activation of caspase-9, the class I caspase required for the processing of caspase-3 (9, 22–24). However, Bcl-2 did not inhibit the processing of the C2P-caspase-3 chimeric molecule (Fig. 4). CrmA, which has only a marginal effect on C2P-caspase-3-mediated apoptosis (Fig. 3), also did not inhibit the processing of the chimeric molecule. We also compared the effects of Bcl-2, CrmA, MIHA, and P35 on caspase-2-GFP processing. Consistent with cell killing experiments, none of these molecules completely blocked procaspase-2 processing, as a processed doublet of 39/41 kDa, representing the p12-GFP/p14-GFP was seen in all cases (data not shown). As expected, caspase-2(C320G)-GFP protein was not significantly processed in transfected cells (data not shown). These results provide compelling evidence that the addition of a class I caspase prodomain to a class II caspase can confer autoprocessing ability on class II caspases.

Homodimerization of C2P-Caspase-3 Chimeric Molecules in S. cerevisiae—Procaspase-2 molecules can dimerize through their prodomains to mediate autocatalysis (10). To check if the chimeric caspase-3 molecule is able to dimerize, we utilized the yeast two-hybrid system. Caspase-3 constructs with or without the caspase-2 prodomain were fused to Gal4 DNA-binding and activation domains in S. cerevisiae vectors. To avoid processing of the molecules in transformed cells, we also used C163G mutants of caspase-3. As shown in Table I wild-type caspase-3 exhibited some β-galactosidase activity. However, a small number of colonies (<10%) transformed with caspase-3(C163G) mutant exhibited some β-galactosidase activity. This may be because of relatively large amounts of protein expression in some transformed cells carrying high plasmid copy number, which may lead to dimerization. In contrast to caspase-3 and caspase-3(C163G), nearly all (>95%) colonies transformed with the C2P, or the chimeric proteins C2P-caspase-3(C163G) and C2P-caspase-3Δ9(C163G) exhibited β-galactosidase activity (Table I). The chimera containing the wild-type caspase-3 lacking the first 9 amino acid residues (C2P-caspase-3Δ9) was also able to form homodimers, albeit with a lower efficiency (~70% colonies positive for β-galactosidase). These results suggest that addition of a caspase-2 prodomain onto caspase-3 can greatly enhance the ability of caspase-3 molecules to dimerize.

Conclusions—Our results provide strong support for the notion that the primary function of the prodomain in class I caspases is to promote dimerization and that dimerization is
necessary and sufficient for autoprocessing. These results also support the idea that recruitment of class I caspases through adaptor molecules mediates dimerization and activation of upstream procaspases. Procaspase-8 has been shown to possess some intrinsic protease activity that probably mediates its autoprocessing (11). Because procaspase-3 in transfected cells is not processed, intrinsic protease activity in procaspase-3 may be responsible for its weak apoptosis-inducing activity when overexpressed in mammalian cells. A recently published study shows that artificially induced procaspase-3 oligomerization was sufficient for its activation (25), supporting our findings. Because most living cells express moderate levels of many procaspases, the intrinsic procaspase activity is unlikely to have any deleterious effect, until dimerization further augments processing and generation of fully activated caspase.

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REFERENCES
1. Kumar, S. (1995) Trends Biochem. Sci. 20, 198–202
2. Martin, S. J., and Green, D. R. (1995) Cell 82, 349–352
3. Kumar, S., and Lavin, M. F. (1996) Cell Death Diff. 3, 255–267
4. Nicholson, D. W., and Thornberry, N. A. (1997) Trends Biochem. Sci. 22, 299–303
5. Chinnaiyan, A. C., and Dixit, V. M. (1997) Semin. Immunol. 9, 69–76
6. Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O'Rourke, K., Shevchenko, A., Scaffidi, C., Bretz, J. D., Zhang, M., Ng, J., Gentz, R., Mann, N., Kramer, P. H., Peter, M. E., and Dixit, V. M. (1996) Cell 85, 817–827
7. Boldin, M. P., Goncharov, T. M., Goltsev, Y. V., and Wallach, D. (1996) Cell 85, 803–815
8. Duan, H., and Dixit, V. M. (1997) Nature 385, 86–89
9. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S., Ahmad, M., Alnemri, E. S., and Wang, X. (1997) Cell 91, 479–489
10. Butt, A. J., Harvey, N. L., Parasivam, G., Kumar, S. (1998) J. Biol. Chem. 273, 6763–6768
11. Muzio, M., Stockwell, B. R., Stennicke, H. R., Salvesen, G. S., and Dixit, V. M. (1998) J. Biol. Chem. 273, 2926–2930
12. Yang, X., Chang, H. Y., and Baltimore, D. (1998) Mol. Cell 1, 319–325
13. Kumar, S., Kinoshita, M., Noda, M., Copeland, N. G., and Jenkins, N. A. (1994) Genes Dev. 8, 1613–1626
14. Wang, L., Miura, M., Bergeron, L., Zhu, H., and Yuan, J. (1994) Cell 78, 739–750
15. Kumar, S., Kinoshita, M., Dorstyn, L., and Noda, M. (1997) Cell Death Differ. 4, 378–387
16. Dorstyn, L., and Kumar, S. (1997) Cell Death Differ. 4, 579–579
17. Tewari, M., Quan, L. T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1995) Cell 81, 801–809
18. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding C. K., Gallant, M., Garreau, Y., Griffin, P. R., Labelle, M., Lazenbik, Y. A., Munday, N. A., Raju, S. A., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K. (1995) Nature 376, 37–43
19. Colussi, P. A., Harvey, N. L., and Kumar, S. (1998) J. Biol. Chem. 273, 24428–24432
20. Xue, D., and Horvitz, H. R. (1995) Nature 377, 248–251
21. Deveraux, Q. L., Takahashi, R., Salvesen, G. S., Reed, J. C. (1997) Nature 388, 304–304
22. Chinnaiyan, A. M., Orth, K., O'Rourke, K., Duan, H., Poirier, G. G., and Dixit, V. M. (1996) J. Biol. Chem. 271, 4573–4576
23. Pan, G., O'Rourke, K., and Dixit, V. M. (1998) J. Biol. Chem. 273, 5841–5845
24. Srinivasula, S. M., Ahmed, M., Fernandez-Alnemri, T., and Alnemri, E. S. (1998) Mol. Cell 1, 949–957
25. MacCorkle, R. A., Freeman, K. W., and Spencer, D. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3655–3660