

An Induced Proximity Model for Caspase-8 Activation*

(Received for publication, October 30, 1997)

Marta Muzio^{‡§}, Brent R. Stockwell[¶], Henning R. Stennicke^{**},
Guy S. Salvesen^{**}, and Vishva M. Dixit[‡] ^{‡‡}

From the [‡]University of Michigan Medical School, Department of Pathology, Ann Arbor, Michigan 48109,
[¶]Howard Hughes Medical Institute, Harvard University, Department of Chemistry and Chemical Biology,
Cambridge, Massachusetts 02138, and the ^{**}Burnham Institute, La Jolla, California 92037

The assembly of the CD-95 (Fas/Apo-1) receptor death-inducing signaling complex occurs in a hierarchical manner; the death domain of CD-95 binds to the corresponding domain in the adapter molecule Fas-associated death domain (FADD) Mort-1, which in turn recruits the zymogen form of the death protease caspase-8 (FLICE/Mach-1) by a homophilic interaction involving the death effector domains. Immediately after recruitment, the single polypeptide FLICE zymogen is proteolytically processed to the active dimeric species composed of large and small catalytic subunits. Since all caspases cleave their substrates after Asp residues and are themselves processed from the single-chain zymogen to the two-chain active enzyme by cleavage at internal Asp residues, it follows that an upstream caspase can process a downstream zymogen. However, since FLICE represents the most apical caspase in the Fas pathway, its mode of activation has been enigmatic. We hypothesized that the FLICE zymogen possesses intrinsic enzymatic activity such that when approximated, it autoprocesses to the active protease. Support for this was provided by (i) the synthesis of chimeric $F_{pk}3FLICE$ molecules that can be oligomerized *in vivo* by the synthetic cell-permeable dimerizer FK1012H2. Cells transfected with $F_{pk}3FLICE$ underwent apoptosis after exposure to FK1012H2; (ii) the creation of a nonprocessable zymogen form of FLICE that retained low but detectable protease activity.

Apoptosis, or programmed cell death, is a cell deletion mechanism that is critical to metazoan survival (1, 2). The cell death machinery is conserved throughout evolution and is composed of several distinct parts including effectors, inhibitors, and activators (1, 3).

Mammalian cysteine proteases (designated caspase for cysteine aspartic acid-specific protease) related to the *Caenorhabditis elegans* cell death gene *CED-3* represent the effector components of the apoptotic machinery participating in a regulated proteolytic cascade (4, 5). Since all caspases cleave their substrates after Asp residues and are themselves processed from the single-chain zymogen to the two-chain active enzyme by

cleavage at internal Asp residues, it follows that an upstream caspase can process a downstream zymogen (6–8).

The assembly of the Fas receptor death-inducing signaling complex occurs in a hierarchical manner; the death domain of CD-95 binds to the corresponding domain in the adapter molecule Fas-associated death domain (FADD) Mort-1, which in turn recruits the zymogen form of the death protease caspase-8 (FLICE/Mach-1) by a homophilic interaction involving the death effector domains (9–12). Immediately after recruitment, the single polypeptide FLICE zymogen is proteolytically processed to the active dimeric species composed of large and small catalytic subunits that amplify the apoptotic signal by activating other downstream caspases (6, 8, 13). However, since FLICE represents the most apical caspase in the Fas pathway, its mode of activation has been enigmatic.

We hypothesized that the FLICE zymogen possesses intrinsic enzymatic activity such that when approximated, it autoprocesses to the active protease. Support for this model was provided by two independent approaches: (i) the synthesis of chimeric $F_{pk}3FLICE$ molecules that can be oligomerized *in vivo* by the synthetic cell-permeable dimerizer FK1012H2. Cells transfected with $F_{pk}3FLICE$ underwent apoptosis after exposure to FK1012H2; (ii) the creation of a nonprocessable zymogen form of FLICE that retained low but detectable protease activity.

MATERIALS AND METHODS

Expression Vectors and Recombinant Proteins— $MF_{pk}3E_u$ vector containing a myristoylation site, three copies of F_{pk} in tandem and a hemagglutinin epitope tag (HA) was originally made by D. Spencer (Baylor College of Medicine). F_{pk} is a double mutant of FKBP12 where residues 89 and 90 have been mutated from GI to PK (14). The catalytic domain of FLICE (encoding Ser-217 to Asp-479) was obtained by polymerase chain reaction and sub-cloned in-frame between the last F_{pk} and the epitope tag at the *SaI* site in $MF_{pk}3E_u$. The same catalytic domain of a mutant version of FLICE, in which Cys-360 was replaced by Ser, was similarly cloned. For production of recombinant purified proteins, the catalytic domain of FLICE or mutant versions of FLICE in which the catalytic Cys-360 was replaced by Ser and/or the cleavage sites Asp-374 and Asp-384 were replaced by Ala, were obtained by polymerase chain reaction and sub-cloned into the pET15b expression vector (Novagen). The proteins were expressed in the BL21 pLysS *Escherichia coli* strain and purified using the QIAexpress Kit (Qiagen) following the manufacturer's instructions.

Cells and Transfections—Human embryonic kidney 293 and HeLa cells were cultured as described previously. Cell death assays were performed as described (9). HeLa cells were transfected using the lipofectAMINE procedure (Life Technologies, Inc.) according to the manufacturer's instructions. 293 cells were transfected using calcium phosphate precipitation.

Western and Coimmunoprecipitation Analysis—For immunoblotting analysis, cells (5×10^5) were lysed in 50 μ l of buffer (50 mM KCl, 50 mM HEPES, 5 mM EGTA, 2 mM $MgCl_2$, protease inhibitor mixture) followed by three cycles of freeze thaw. The membrane-rich fraction was pelleted, resuspended in 8 M urea and, after boiling in sample buffer, resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, and probed with anti-HA antibody. For coim-

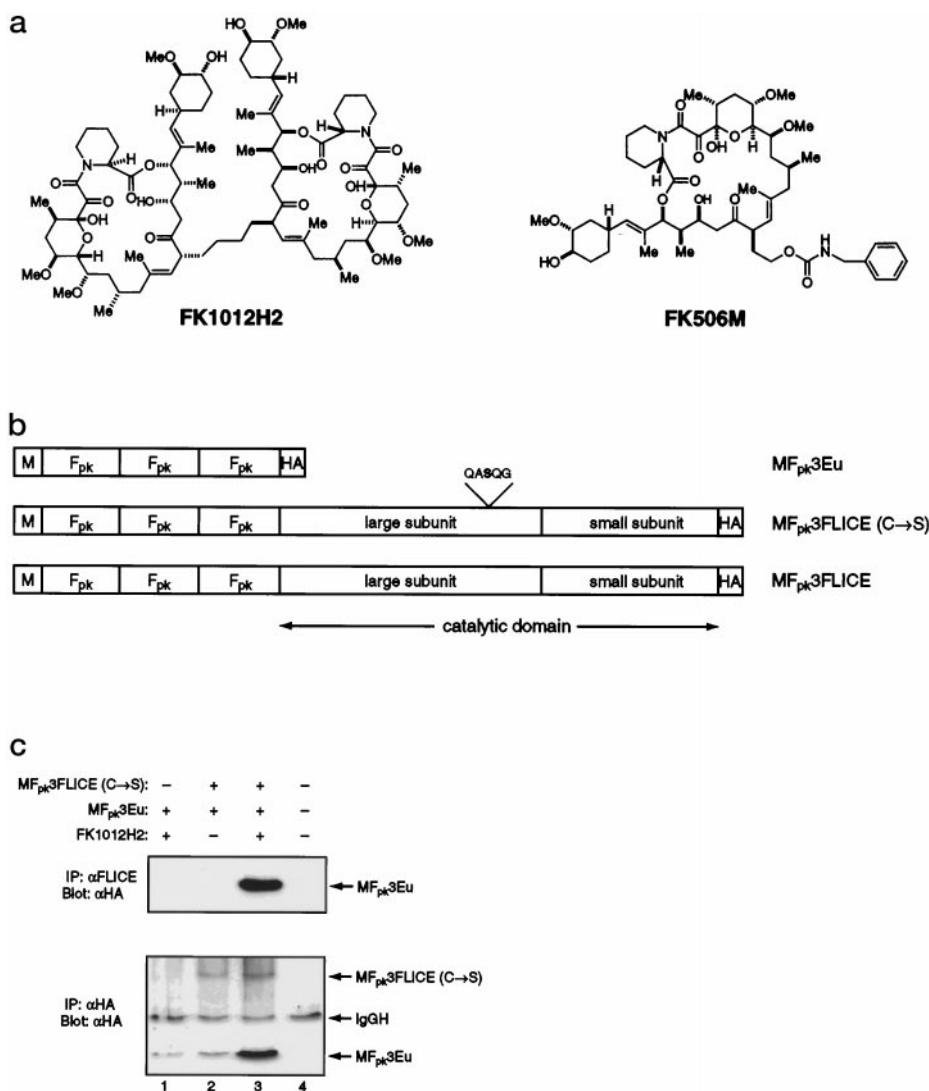
* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[‡] Supported by a Human Frontier Science Program Organization (HFSPO) fellowship.

[¶] A Howard Hughes Medical Institute predoctoral fellow supported in part by NIGMS, National Institutes of Health, Grant GM-52067 (to S. L. Schreiber).

^{‡‡} Present address and to whom correspondence should be addressed: Genentech Inc., 1 DNA Way, South San Francisco, CA 94080. Tel.: 415-225-1000; Fax: 415-225-6000; E-mail: dixit@gene.com.

FIG. 1. Induced oligomerization of F_{pk} containing proteins. *a*, structure of the synthetic ligands; FK1012H2 is a dimeric variant of FK506M. *b*, schematic representation of F_{pk} -containing chimeric proteins; *M* indicates a myristoylation site; HA, hemagglutinin epitope tag; F_{pk} 3, three mutant FKBP polypeptides in tandem. MF_{pk} 3FLICE contains three F_{pk} polypeptides fused to the catalytic domain of FLICE. MF_{pk} 3FLICE(C \rightarrow S) is a mutant version of MF_{pk} 3FLICE where the catalytic Cys-360 has been replaced by Ser (9). *c*, FK1012H2-induced oligomerization of F_{pk} -containing proteins. 293 cells were transiently transfected with MF_{pk} 3Eu alone (lane 1), MF_{pk} 3Eu and MF_{pk} 3FLICE(C \rightarrow S) (lanes 2 and 3), or vector alone (lane 4). After treatment with FK1012H2 (lanes 1 and 3), cells were lysed and anti-FLICE (upper panel) or anti-HA (lower panel) immunoprecipitates (IP) were immunoblotted with anti-HA monoclonal antibody. In the absence of FK1012H2 (lane 2), no complex is detectable between MF_{pk} 3FLICE(C \rightarrow S) and MF_{pk} 3Eu. In the presence of the dimerizer FK1012H2 (lane 3), complex formation is evident.



munoprecipitation analysis, cells (2×10^6) were lysed in 1 ml of buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris, 20 mM HEPES, protease inhibitor mixture) and incubated either with anti-HA¹ antibodies or anti-FLICE (small subunit-specific) rabbit antiserum. Immune complexes were precipitated by the addition of protein G-Sepharose (Sigma). After extensive washing, the Sepharose beads were boiled in sample buffer, and the eluted proteins were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with anti-HA antibody.

Enzymatic Analysis of Recombinant Mutant Proteins—The enzymatic reaction was carried out at 37 °C in 20 mM PIPES, 0.1 mM substrate, 100 mM NaCl, 10 mM dithiothreitol (fresh), 1 mM EDTA, 0.1% CHAPS, and 10% sucrose, pH 7.2. The initial rates of hydrolysis were measured by release of AFC (7-amino-4-methyl coumarin) from the substrate by the enzymes at appropriate emission and excitation wavelengths using a Perkin-Elmer LS50B fluorimeter equipped with a thermostated plate reader. Titrations of wild type and FLICE-DD [arrow] AA were carried out by preincubating the enzyme with varying concentrations of Z-DEVD-FMK or CrmA in assay buffer for 30 min at room temperature. The inhibitor was added at concentrations spanning from 0 to significantly above the concentration of active enzyme. After the preincubation, Z-DEVD-AFC was added in assay buffer to a final substrate concentration of 0.1 mM. The relative amount of uninhibited enzyme was evaluated from the initial rates of hydrolysis of the substrate as described above, and the concentration of active enzyme was

calculated by extrapolating data points to their intersection with the x axis. The caspase-8 concentration used in the titration assays were 0.2 micromolar for the native enzyme and 20 micromolar for the DD \rightarrow AA mutant.

Affinity Labeling of Recombinant Mutant Proteins—100 ng of purified proteins were incubated with or without N-(biotinyl-Asp-Glu-Val-Asp-[(2,6-dimethylbenzoyl)oxy]methyl ketone (BIO-DEVD-AMK) (15) at a concentration of 0.5 μ M in a final volume of 50 μ l of buffer (0.1% CHAPS, 10 mM dithiothreitol, 10 mM Tris, pH 7.5) for 15 min at 25 °C. For competition experiments, increasing amounts of a nonbiotinylated version of the DEVD tetrapeptide (DEVD-CMK; Bachem) were added to the reaction. Samples were boiled in sample buffer in the absence of reductant, resolved by SDS-polyacrylamide gel electrophoresis under nonreducing conditions, blotted onto nitrocellulose, blocked in phosphate-buffered saline containing 3% bovine serum albumin and 0.1% Tween 20, incubated with streptavidin-conjugated horseradish peroxidase (ICN) in phosphate-buffered saline, 0.1% bovine serum albumin, 0.1% Tween, washed with 50 mM Tris, pH 7.5, 0.25% gelatin, 0.05% Tween 20, 150 mM NaCl, 5 mM EDTA, and developed by ECL. Alternatively, the membrane was analyzed by immunoblotting with anti-FLICE antiserum specific for the small catalytic subunit.

RESULTS AND DISCUSSION

To determine if FLICE oligomerization *in vivo* could result in activation, chimeric F_{pk} FLICE expression constructs were engineered. F_{pk} (molecular mass 12 kDa), a double mutant of FKBP (FK binding protein FK506) (16) contains a single binding site for the cell-permeable immunosuppressive drug FK506. The FKBP-FK506 complex is a potent inhibitor of calcineurin, a protein phosphatase that plays a key role in signal-

¹ The abbreviations used are: HA, hemagglutinin; PIPES, 1,4-piperazinediethanesulfonic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; BIO-DEVD-AMK, N-(biotinyl-Asp-Glu-Val-Asp-[(2,6-dimethylbenzoyl)oxy]methyl ketone; -CMK, chloromethyl ketone; -FMK, fluoromethyl ketone; -AFC, 7-amino-4-trifluoromethyl coumarin; Z-, carbobenzyloxy.

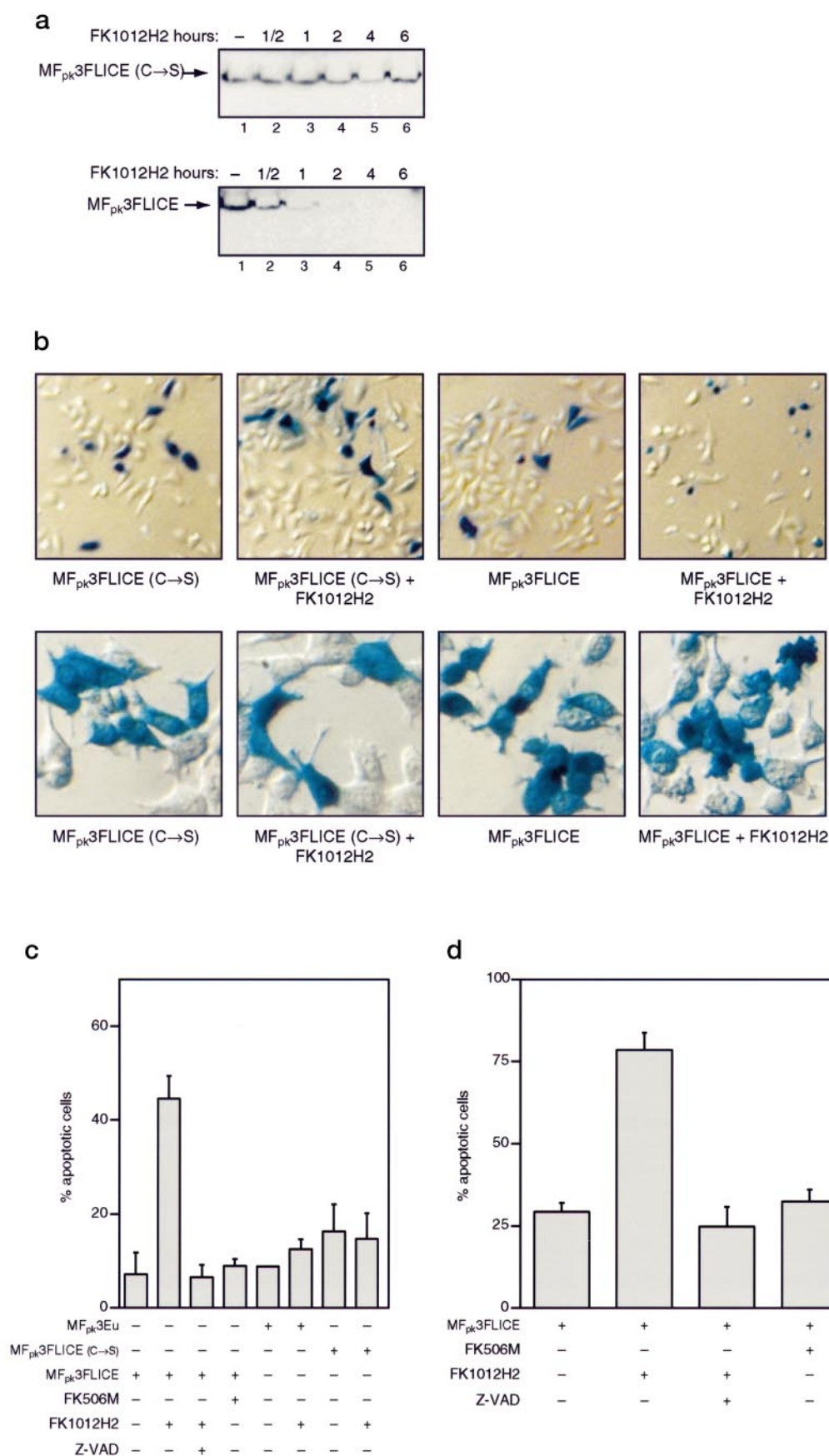


FIG. 2. FK1012H2-induced oligomerization of FLICE triggers self-processing and apoptosis. *a*, 293 cells were transiently transfected with MF_{pk}3FLICE(C → S) (upper panel) or MF_{pk}3FLICE (lower panel); 36 h post-transfection cells were treated with FK1012H2 (250 nM) for the indicated times. Cell extracts equalized for protein content were analyzed by immunoblotting with anti-HA monoclonal antibody. *b*, 293 and HeLa cells were transiently transfected with MF_{pk}3FLICE(C → S) or MF_{pk}3FLICE with pCMV-β-galactosidase. 36 h post-transfection cells were left untreated or treated with FK1012H2 (250 nM) for 3 h, fixed, stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal), and

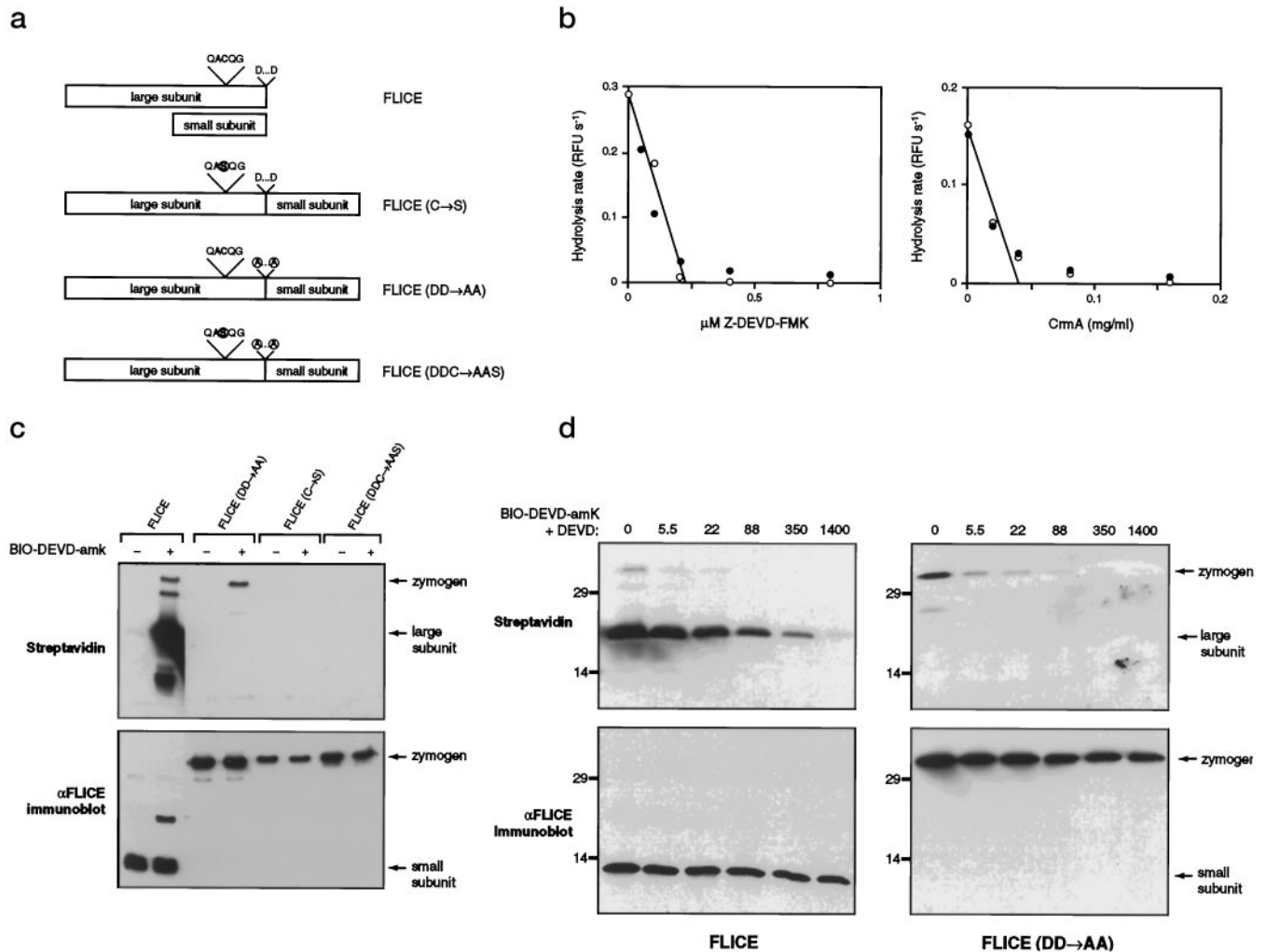


FIG. 3. Titration and affinity labeling of recombinant FLICE. *a*, schematic representation of the different mutant versions of FLICE; mutated residues are circled. *b*, FLICE(DD → AA) enzyme was titrated with the inhibitors Z-DEVD-FMK (left panel) and CrmA (right panel). Equivalent amounts of activity were added for the wild type (open circles) and mutant enzymes (solid circles), corresponding to a 100-fold higher protein concentration for the mutant enzyme. *c*, recombinant purified native active FLICE composed predominantly of the large and small catalytic subunits, and indicated mutated zymogen forms were compared for their ability to bind the biotinylated irreversible tetrapeptide inhibitor DEVD-AMK (BIO-DEVD-AMK) as detected by Western blot analysis using horseradish peroxidase HRP-streptavidin (upper panel). Immunoblot analysis with an anti-FLICE (small catalytic subunit-specific) antiserum of the same samples is shown as a control (lower panel). As expected, the large catalytic subunit of processed native FLICE that contains the active-site cysteine avidly bound the biotinylated inhibitor. The nonprocessable zymogen form of FLICE(DD → AA) that also contains the active-site cysteine bound the inhibitor, albeit to a lesser extent, consistent with a model in which the zymogen possesses enzymatic activity. As predicted, mutant forms lacking the catalytic cysteine (C → S and DDC → AAS) did not bind the inhibitor. *d*, specificity of the interaction between BIO-DEVD-AMK and FLICE or FLICE(DD-*AA*) was confirmed by competing the binding with increasing amounts of a nonbiotinylated version of the DEVD tetrapeptide (5.5–1,400-fold excess of DEVD with respect to BIO-DEVD). Samples were analyzed as in *c*.

ing. An FK506 dimer (FK1012H2) was previously synthesized by introducing a cross-linker into the domain of FK506 necessary for inhibition of calcineurin. FK1012H2, although unable to bind calcineurin, still retains its ability to bind and dimerize F_{pk} polypeptides given its bivalent nature (17–19) (Fig. 1*a*).

To mimic recruitment of FLICE to its receptor signaling complex (11, 12), the prodomain of FLICE was substituted with a myristoylation signal followed by three tandem repeats of F_{pk} , (MF_{pk}3FLICE). A catalytically inactive version was constructed by mutating the active-site Cys-360 to Ser (MF_{pk}3FLICE(C → S)). An additional control was the construct MF_{pk}3E_u that encoded only the myristoylation signal, three

F_{pk} repeats in tandem, and an HA epitope tag (Fig. 1*b*). To confirm the ability of FK1012H2 to induce oligomerization of F_{pk} -containing proteins, human 293 cells were transiently transfected with the MF_{pk}3E_u construct and the catalytically inactive chimera MF_{pk}3FLICE(C → S). The MF_{pk}3FLICE immunoprecipitates contained associating MF_{pk}3E_u only in the presence of FK1012H2 (Fig. 1*c*), confirming the validity of the dimerization approach.

Ectopic expression of the catalytically active chimera MF_{pk}3FLICE resulted in the production of a protein of predicted molecular mass (69 kDa) that was membrane-associated. The addition of the synthetic ligand FK1012H2 induced

photographed under phase contrast microscopy. *c*, 293 cells were transiently transfected with MF_{pk}3E_u, MF_{pk}3FLICE, or MF_{pk}3FLICE(C → S) and pCMV-β-galactosidase. 36 h post transfection cells were treated with FK1012H2 (250 nM) or FK506 M (250 nM) with or without the addition of the broad-spectrum caspase inhibitor Z-VAD-FMK (20 μM). After 3 h, cells were fixed, stained with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) and examined by phase contrast microscopy. Data (mean ± S.E.) shown are the percentage of round and blebbing apoptotic cells as a function of total number of blue cells counted (*n* > 3). *d*, HeLa cells were treated and analyzed as in *c*.

rapid disappearance of the catalytically competent MF_{pk}3FLICE chimera. Emergence of the active subunits, however, was not detectable under these experimental conditions. As predicted, the catalytically inactive derivative was efficiently expressed, but on exposure to FK1012H2, did not undergo processing even after prolonged incubation (Fig. 2a).

We next observed if MF_{pk}3FLICE oligomerization resulted in the expected apoptotic demise of transfected cells. Human 293 and HeLa cells were transiently transfected with MF_{pk}3FLICE-(C → S) or MF_{pk}3FLICE expression constructs together with a reporter plasmid encoding β-galactosidase. Thirty six h after transfection, cells were left untreated or treated with 250 nM FK1012H2, stained, and analyzed by phase contrast microscopy. As shown in Fig. 2b, oligomerization of MF_{pk}3FLICE but not MF_{pk}3FLICE(C → S) induced phenotypic alterations characteristic of apoptosis. Cells shrank, displayed membrane blebbing, and detached from the dish. Additionally, the apoptotic substrate poly(ADP-ribose) polymerase was cleaved to its signature 85-kDa form, and genomic DNA was cleaved into characteristic internucleosomal size fragments (data not shown), both biochemical hallmarks of apoptosis. Significantly, treatment of Fpk or Fpk-FLICE(C → S)-transfected cells with higher doses of FK1012H2 for an extended period of time did not induce apoptosis (16, 17, 19, 20) (data not shown). After 3 h of exposure to the synthetic ligand, 50–80% MF_{pk}3FLICE-transfected cells started blebbing, condensing, and detaching from the dish. This was completely abrogated by the broad-spectrum caspase inhibitor Z-VAD-FMK that has previously been shown to inhibit FLICE-induced apoptosis (Fig. 2, c and d) (12). Importantly, the monomeric form of the ligand, FK506M, did not induce apoptosis, confirming that the results observed were oligomerization-dependent (Fig. 2, c and d).

These data demonstrate that specific clustering of FLICE zymogen causes apoptosis through caspase activation. We hypothesized that this activation occurs through self-processing of the clustered FLICE due to an intrinsic proteolytic activity of the zymogen. To further address this hypothesis, different recombinant versions of FLICE were generated (Fig. 3a); as shown previously (6, 8, 11), overexpression of catalytically competent FLICE constructs in *E. coli* generated an active heterodimeric enzyme composed of large and small catalytic subunits. Enzymatically inactive FLICE (catalytic Cys-360 mutated to Ser; FLICE(C → S)) did not undergo processing, consistent with a requirement for intrinsic enzymatic activity. Importantly, an altered form of FLICE that retained the catalytic cysteine but was mutated at the internal cleavage sites (Asp-374 and Asp-384; FLICE(DD → AA)) also did not undergo auto-processing and was expressed as a single polypeptide zymogen as confirmed by protein staining and anti-FLICE immunoblot analysis (data not shown and Fig. 3c). The processing mutant FLICE in which the catalytic cysteine was additionally inactivated (FLICE (DDCAAS)) was also expressed as a single polypeptide zymogen. These recombinant forms of FLICE were used to establish whether the zymogen did indeed possess enzymatic activity.

The activity of the cleavage site mutant version FLICE(DD → AA) was determined by its ability to hydrolyze the tetrapeptide caspase substrate Z-DEVD-AFC (21), and the portion of active material was determined by titration with the protein inhibitor CrmA (22, 23) and the covalent peptide based inhibitor, Z-DEVD-FMK (21). A 100-fold more FLICE(DD → AA) was required on a protein basis to give the same rates of substrate hydrolysis as wild type FLICE (Fig. 3b). Thus, from these results it is apparent that the bacterially expressed

FLICE(DD → AA) mutant equivalent to the zymogen form has approximately 1% of the activity of the wild type enzyme. As expected, the catalytic mutant FLICE(C → S) possessed no enzymatic activity (data not shown).

To interpret the enzymatic analysis, we tested the ability of the different versions of FLICE to bind the biotinylated irreversible inhibitor BIO-DEVD-AMK, which covalently binds to the active-site cysteine (15). As expected, the large subunit of processed native FLICE that contains the catalytic cysteine bound BIO-DEVD-AMK (Fig. 3c). Additionally, the unprocessed cleavage site mutant (FLICE(DD → AA)) also bound BIO-DEVD-AMK, indicating that the 1% activity resides in the single chain. FLICE(DDC → AAS), as anticipated, did not show any specific binding to BIO-DEVD-AMK. The specificity of the bands indicated was confirmed by competing the binding with a nonbiotinylated version of the DEVD tetrapeptide (Fig. 3d).

Taken together, these data are consistent with a model wherein FLICE zymogen possesses intrinsic low level caspase activity that upon approximation mediated by the adapter molecule Fas-associated death domain (FADD) attains a sufficient concentration to activate the apoptosis pathway. This study provides a remarkably simple solution to the chicken and egg conundrum of how the initiating caspase (FLICE) is proteolytically processed.

Acknowledgments— We are grateful to S. L. Schreiber for helpful suggestions and discussions. We thank David Spencer for MF_{pk}3E_u, Linda Clayton for BIO-DEVD, Ian Jones for his expertise in preparing the figures, and the following members of the Dixit lab for encouragement and discussions: Divya Chaudhary, Arul Chinnaiyan, Hangjun Duan, Shimin Hu, Eric Humke, Justin McCarthy, Karen O'Rourke, James Pan, and Claudius Vincenz.

REFERENCES

1. Steller, H. (1995) *Science* **267**, 1445–1449
2. Raff, M. C. (1992) *Nature* **356**, 397–400
3. Chinnaiyan, A., and Dixit, V. (1996) *Curr. Biol.* **6**, 555–562
4. Martin, S., and Green, D. (1995) *Cell* **82**, 349–352
5. Henkart, P. (1996) *Immunity* **4**, 195–201
6. Muzio, M., Salvesen, G. S., and Dixit, V. M. (1997) *J. Biol. Chem.* **272**, 2952–2956
7. Orth, K., O'Rourke, K., Salvesen, G., and Dixit, V. (1996) *J. Biol. Chem.* **271**, 20977–20980
8. Srinivasula, S., Ahmad, M., Fernandes-Alnemri, T., Litwack, G., and Alnemri, E. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 14486–14491
9. Chinnaiyan, A., O'Rourke, K., Tewari, M., and Dixit, V. (1995) *Cell* **81**, 505–512
10. Boldin, M., Varfolomeev, E., Pancer, Z., Mett, I., Camonis, J., and Wallach, D. (1995) *J. Biol. Chem.* **270**, 7795–7798
11. Boldin, M., Goncharov, T., Goltsev, Y., and Wallach, D. (1996) *Cell* **85**, 803–815
12. Muzio, M., Chinnaiyan, A., Kischkel, F., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J., Zhang, M., Gentz, R., Mann, M., Krammer, P., Peter, M., and Dixit, V. (1996) *Cell* **85**, 817–827
13. Medema, J., Scaffidi, C., Kischkel, F. C., Shevchenko, A., Mann, M., Krammer, P. H., and Peter, M. E. (1997) *EMBO J.* **16**, 2794–2804
14. Spencer, D., Wandless, T., Schreiber, S., and Crabtree, G. (1993) *Science* **262**, 1019–1024
15. Clayton, L., Ghendler, Y., Mizoguchi, E., Patch, R., Ocain, T., Orth, K., Bhan, A., Dixit, V., and Reinherz, E. (1997) *EMBO J.* **16**, 2282–2293
16. Yang, D., Rosen, M. K., and Schreiber, S. L. (1993) *J. Am. Chem. Soc.* **115**, 819–820
17. Hofmann, K., and Tschopp, J. (1995) *FEBS Lett.* **371**, 321
18. Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995) *Science* **269**, 1424–1427
19. Heguy, A., Baldari, C., Macchia, G., Telford, J. L., and Melli, M. (1992) *J. Biol. Chem.* **267**, 2605–2609
20. Spencer, D., Belshaw, P., Chen, L., Ho, S., Randazzo, F., Crabtree, G., and Schreiber, S. (1996) *Curr. Biol.* **6**, 839–847
21. Nicholson, D., All, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., Munday, N. A., Raju, S. M., Smulson, M. E., Yamin, T., Yu, V. L., and Miller, D. K. (1995) *Nature* **376**, 37–43
22. Zhou, Q., Snipas, S., Orth, K., Muzio, M., Dixit, V. M., and Salvesen, G. S. (1997) *J. Biol. Chem.* **272**, 7797–7800
23. Ray, C., Black, R. A., Kronheim, S. R., Greenstreet, T. A., Sleath, P. R., Salvesen, G. S., and Pickup, D. J. (1992) *Cell* **69**, 597–604

CELL BIOLOGY AND METABOLISM:
**An Induced Proximity Model for Caspase-8
Activation**

Marta Muzio, Brent R. Stockwell, Henning R.
Stennicke, Guy S. Salvesen and Vishva M.
Dixit
J. Biol. Chem. 1998, 273:2926-2930.
doi: 10.1074/jbc.273.5.2926

Access the most updated version of this article at <http://www.jbc.org/content/273/5/2926>

Find articles, minireviews, Reflections and Classics on similar topics on the [JBC Affinity Sites](#).

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 23 references, 11 of which can be accessed free at
<http://www.jbc.org/content/273/5/2926.full.html#ref-list-1>