Cleavage of the Serum Response Factor during Death Receptor-induced Apoptosis Results in an Inhibition of the c-FOS Promoter Transcriptional Activity

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The c-FOS protooncogene is rapidly induced by a wide variety of extracellular stimuli including mitogenic signals. Regulation of c-FOS expression is tightly dependent on the serum response element localized within its promoter. Two transcription factors, the serum response factor (SRF) and the ternary complex factor, bind to the serum response element and play a key role in the regulation of the c-FOS promoter activity. In the present study, we show that two death effectors (CH11 and TRAIL) severely impaired the transcriptional activity of the c-FOS promoter in Jurkat T cells. This inhibition can be accounted for by the specific cleavage by caspase 3 of the SRF both in vitro and in vivo, since acetyl-DEVD-aldehyde prevented SRF cleavage and abolished the inhibitory effect of CH11 and TRAIL on the c-FOS promoter activity. Moreover, phorbol myristate acetate, a potent anti-apoptotic effector, was found to protect SRF completely from cleavage by caspase 3 and also to prevent the inhibition of the c-FOS promoter activity by death effectors. Survival factors play an essential function in the regulation of cell growth mainly by regulating the expression of immediate early gene such as c-FOS. In this line, cleavage of SRF at the onset of apoptosis could abrogate the ability of the cell to induce inappropriate survival pathways. All together, our results are consistent with a role of SRF at the interface between cell survival and death pathways.

Signals generated at the cell surface influenced the rate of activation of numerous genes in cells that in turn affect physiological processes. Notably, the family of immediate early genes, among which we find c-FOS, are rapidly activated, within minutes, in response to mitogenic stimuli such as serum, phorbol esters, lysophosphatidic acid, and growth factors (1–3). Induction of c-FOS is widely dependent on the serum response element (SRE) located within its promoter. Two transcription factors, the serum response factor (SRF) and the ternary complex factor (TCF), bind to the SRE and stimulate the transcriptional activity of the c-FOS promoter (4–6). SRF is ubiquitously expressed and binds as a dimer to the CarG box of the SRE sequence. The central core of SRF contains the DNA binding and dimerization domains, whereas the trans-activation domain critically required for full signal-induced response is located within the N terminus part (3, 7). The TCF belongs to the proteins of the Ets domain family including Elk-1 (8), SRF accessory protein 1 (9), and SAP-2/ERP/NET (10). TCF associates with the SRF via a region located in the dimerization domain of SRF and binds to a purine-rich sequence (CAGGAT) at the 5’ end of the SRE (11). Mutations of the SRE site in the c-FOS promoter dramatically affect the c-FOS response to diverse stimuli demonstrating the key function of SRE in the regulation of c-FOS promoter activity. Until now, two alternative pathways have been described to converge to the SRE and regulate the transcriptional activity of c-FOS promoter. First, activation of the mitogen-activated protein kinase family, extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase, and p38MAPK by mitogenic and stress signals leads to phosphorylation of the TCF proteins and stimulation of their transcriptional activity (12–16). In a second pathway that is independent of TCF, SRF can integrate, via the Rho family of small GTPase, signals from the mitogen lysophosphatidic acid (1).

Apoptosis or programmed cell death is a physiological process to eliminate unwanted or superfluous cells from the organism. It plays a crucial role in embryogenesis, in the development of the nervous system, in adult tissue homeostasis, and in the regulation of the immune system. When deregulated, apoptosis has been involved in the pathogenesis of a number of human diseases including cancers, neurodegenerative disorders, and autoimmune diseases (17). Apoptosis is characterized by cytoplasmic shrinkage, membrane blebbing, chromatin condensation, and nuclear DNA fragmentation and culminates in cellular death. The molecular mechanisms involved in apoptosis regulation have been extensively studied. From these studies, it appears that the activation of caspases is essential for execution of the apoptotic program evoked by death receptor ligands (TRAIL or Fas ligand), by growth factors deprivation, or by genotoxic agents (18–21). Indeed, repression of caspase enzymatic activity, by viral proteins p55 from baculovirus or CrmA from cowpox virus, FLIP, as well as by synthetic inhibitor peptides (acetyl-DEVD-aldehyde) blocks induction of apoptosis mediated by death receptors (22). Other molecules, such as CrmA or FLIP, or death receptors such as Fas, TNF receptor, or CD95, are known to prevent apoptosis by regulating the expression of immediate early genes such as c-FOS (23). Induction of c-FOS in the presence of CRmA and FLIP or CD95 is shown to abrogate the ability of the cell to induce inappropriate survival pathways. This implies that the activation of c-FOS by death effectors may play an essential function in the regulation of cell death. The molecular mechanisms involved in apoptosis regulation have been extensively studied. From these studies, it appears that the activation of caspases is essential for execution of the apoptotic program evoked by death receptor ligands (TRAIL or Fas ligand), by growth factors deprivation, or by genotoxic agents (18–21). Indeed, repression of caspase enzymatic activity, by viral proteins p55 from baculovirus or CrmA from cowpox virus, FLIP, as well as by synthetic inhibitor peptides (acetyl-DEVD-aldehyde) blocks induction of apoptosis.

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1 The abbreviations used are: SRE, serum response element; SRF, serum response factor; TCF, ternary complex factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PMA, phorbol 12-myristate 13-acetate; FCS, fetal calf serum; CHAPS, 3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfonic acid.

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Cleavage of SRF during Induction of Apoptosis

Apoptosis, demonstrating the key role of caspases in the execution of the apoptotic program (22–25). As inhibition of survival pathways may increase susceptibility to death receptor-induced apoptosis, we first studied whether death effectors may affect the expression of c-FOS, which represents a prototype immediate early gene. By using a luciferase reporter plasmid containing a fragment of the c-FOS promoter, we showed that induction of apoptosis by TRAIL and CH11, an anti-Fas monoclonal antibody, inhibits the transcriptional activity of the c-FOS promoter. Interestingly, we identified, in vivo and in vitro, a caspase-dependent cleavage of the serum response factor (SRF) during CH11 and TRAIL-induced apoptosis. Moreover, we showed that PMA, which prevents apoptosis of Jurkat T cells, abrogates both the cleavage of SRF and the inhibitory effects of CH11 and TRAIL on the transcriptional activity of the c-FOS promoter. Taken together, our results point to the cleavage of SRF during apoptosis and reveal a striking parallel between SRF cleavage, c-FOS gene repression, and apoptosis. Based on these observations, we propose that during apoptosis the expression of c-FOS, a key gene in cell growth and survival, is impaired, ensuing SRF cleavage by caspase 3.

EXPERIMENTAL PROCEDURES

Materials—Phorbol 12-myristate 13-acetate (PMA), sodium fluoride, sodium orthovanadate, aprotonin, and leupeptin were purchased from Sigma and acetyl-DEVD-allylde from Bachem. T4 polynucleotide kinase was from Biolabs, and RNase and proteinase K were from Roche Molecular Biochemicals. RPMI and fetal calf serum (FCS) were from Life Technologies, Inc. Peroxidase-conjugated anti-rabbit and antimouse antibodies were from Dakopatts. TRAIL, anti-C-terminal SRF (G20), and anti-Erk1 (120) antibodies were purchased from Santa Cruz Biotechnology; anti-Fas monoclonal antibody (CH11) was from Eurodex, and anti-caspase 3 monoclonal antibody was from Transduction Laboratories. Anti-N-terminal SRF antibody was obtained by injection of a rabbit with a peptide (NH2-GSLNRTPTGRPG-COOH) corresponding to the N-terminal part of SRF.

Cell Cultures—Jurkat T cells (clone Jd) were grown at 37 °C under 5% CO2 in RPMI supplemented with 5% FCS and 100 μg/ml penicillin/streptomycin. Jurkat cells, used for electroporation experiments (Jd tag), a kind gift from Dr. Crabtree (Stanford, CA), express the large T antigen of SV40 and were grown under the same conditions in RPMI with 10% FCS.

Reporter Plasmids, Transfections, and Luciferase Assays—The FOS luciferase reporter was constructed by a 771+42 fragment surrounding the transcriptional start site of the human FOS gene kindly provided by Dr. R. Treisman. The SRE luciferase reporter plasmid contains the SRE motif upstream from the minimal thymidine kinase promoter. Jd tag cells were transiently transfected by electroporation. Ten million Jd tag cells were resuspended in 400 μl of RPMI and placed in a 0.4-cm gap cuvette with 10 μg of total plasmid DNA. Electroporation was performed with a double electric shock (260 V, 960 microfarads) using the gene pulsar system (Bio-Rad). Each 10 million cells were separated into four wells, and 36 h after transfection, cells were exposed to 100 ng/ml TRAIL, 100 ng/ml CH11, and/or 100 ng/ml PMA for 4 h. When indicated, cells were incubated directly after electroporation with 100 μM acetyl-DEVD-allylde. Then soluble extracts were harvested in 50 μl of lysis reporter buffer (Promega) and assayed for luciferase activity. Luciferase activity was normalized by protein amount.

Western Blot Assays—Jurkat T cells (4 × 106) were stimulated with 100 ng/ml TRAIL, 100 ng/ml CH11, and/or 100 ng/ml PMA for the times indicated in the figure legends. Then the cells were lysed in buffer containing 50 mM Hepes, pH 7.4, 150 mM NaCl, 20 mM EDTA, 100 μM NaF, 10 mM Na2VO4, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, 20 μg/ml aprotonin, and 1% Nonidet P-40. Proteins (100 μg) were separated on 10% SDS-polyacrylamide gels and transferred to polyvinyllidine difluoride membranes (Immobilon, Millipore). SRF was detected with polyclonal antibodies (Santa Cruz Biotechnology) at 1/3000 in saturation buffer and with a secondary peroxidase-conjugated anti-rabbit antibody at 1/10,000 dilution. Caspase 3 was detected with monoclonal antibody at a 1/4000 dilution in saturation buffer and with a secondary anti-mouse antibody at a 1/5000 dilution as described previously (26). Proteins were visualized with the Amersham Pharmacia Biotech ECL system.

DNA Fragmentation—Cells (106) were cultured in RPMI medium in 12-well dishes with 100 ng/ml TRAIL, 100 ng/ml CH11, and/or 100 ng/ml PMA. When indicated, cells were exposed to 100 μM acetyl-DEVD-allylde for 24 h. The cells were then collected and lysed with 200 μl of lysis buffer containing 10 mM Tris, pH 7.5, 1 mM EDTA, and 0.2% Triton X-100. Samples were treated with 100 μg/ml RNase for 30 min at 37 °C and then with 100 μg/ml proteinase K for 30 min at 37 °C. Cellular DNA was isopropl alcohol-precipitated, dried, and resuspended in Tris-EDTA buffer for 30 min at 55 °C. Next, DNA was analyzed by electrophoresis on 1.2% agarose gels containing ethidium bromide as described previously (27).

In Vitro Transcription/Translation Assay and in Vitro Cleavage of Caspase 3—In vitro translation of SRF from pMHSRF, kindly provided by Dr. Harel-Bellan, was carried out using the SP6 transcription-translation system from Promega. SRF translation was monitored using 125I-methionine (Amersham Pharmacia Biotech) and SDS-polyacrylamide gel electrophoresis analysis. In vitro translated SRF (2.5 μl) was incubated with 25 ng of recombinant caspase 3 in the presence or absence of 10 μM acetyl-DEVD-allylde for 6 h at 37 °C in buffer containing 25 mM Hepes, pH 7.5, 0.1% CHAPS, and 2 mM dithiothreitol. Reactions were separated on a 10% SDS-polyacrylamide gel and analyzed by autoradiography or were used to perform gel mobility shift assays.

Cellular Extracts and Gel Mobility Shift Assay—Jurkat T cells (4 × 106) were stimulated with 100 ng/ml TRAIL, 100 ng/ml CH11, and/or 100 ng/ml PMA. When indicated, cells were preincubated with 100 μM acetyl-DEVD-allylde for 24 h. Cells were then lysed in Hepes buffer containing 350 mM NaCl, 20% glycerol, 1% Nonidet P-40, 1 mM MgCl2, 0.5 mM EDTA, 0.1 mM EGTA. Double-stranded synthetic SRF sequence
(5′-GGATGTCATATTAGGACATCT-3′) was γ-32P-ATP (Amersham Pharmacia Biotech) end-labeled using T4 polynucleotide kinase. Ten μg of cellular proteins or the in vitro translated SRF reaction mix described above was preincubated in a binding buffer containing 10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 4% glycerol, 80 μg/ml salmon sperm DNA, 15 μg/ml poly(dI-dC) for 10 min on ice. Then, 30,000–50,000 cpm of 32P-labeled probe were added to the binding reaction for 20 min at room temperature. For competition experiments, a 50-fold excess of unlabeled oligonucleotides was added during preincubation. For supershift assays, 0.5 μl of anti-SRF or 1 μl of anti-Elk-1 antibodies were added to cellular extracts in the binding reaction buffer. DNA-protein complexes were resolved by electrophoresis on 5% polyacrylamide gels (37.5/1 acrylamide/bisacrylamide) in TBE buffer (22.5 mM Tris borate, 0.5 mM EDTA, pH 8) for 3 h at 150 V.

RESULTS

Activation of Death Receptors Inhibits the Transcriptional Activity of the c-FOS Promoter—Initially, we sought to investigate the effects of death receptor activation on c-FOS expression in T lymphocytes. To this end, Jurkat T cells were transiently transfected with luciferase reporter plasmids, containing either a c-FOS promoter fragment or an SRE motif upon a thymidine kinase minimal promoter. The cells were exposed to TRAIL or CH11, an anti-Fas monoclonal antibody that mimics the apoptotic effects of Fas ligand. Used as a positive control, PMA resulted in a 6-fold stimulation of the c-FOS and SRE luciferase reporter plasmids (Fig. 1, A and B). By contrast, we observed that TRAIL or CH11 markedly decreased the c-FOS promoter activity (~50–60%) (Fig. 1A). Similarly, TRAIL or CH11 decreased the SRE-driven promoter activity indicating that TRAIL or CH11 effects were targeted to the SRE motif of the c-FOS promoter (Fig. 1B). At that same time, the activity of the RSV promoter was not impaired by TRAIL or CH11, indicating that a decrease in c-FOS and SRE transcriptional activities was not due to an overall effect on transcription (Fig. 1C). As activation of death receptors by their respective ligand induces apoptosis via activation of caspases, we tested the hypothesis that caspase activities were involved in inhibition of the c-FOS promoter activity by death effectors. Interestingly, the inhibitory effects of TRAIL or CH11 on c-FOS promoter activity were abrogated by acetyl-DEVD-aldehyde, an inhibitor of the CPP32-like caspase family, and this inhibitor did not affect the stimulation evoked by PMA (Fig. 1D).

In conclusion, these results demonstrate that TRAIL or CH11 induce a specific inhibition of the transcriptional activity...
of the c-FOS promoter. Additionally, our data also suggest that the inhibitory effect of TRAIL or CH11 is targeted to the SRE sequence within the c-FOS promoter.

**Induction of Apoptosis Is Accompanied by the Cleavage of SRE-binding Proteins—**To investigate further the mechanism by which TRAIL or CH11 inhibited the transcriptional activity of the c-FOS promoter, we used the c-FOS SRE sequence as a probe in band shift assays (Fig. 2A). Cellular extracts prepared from Jurkat T cells formed two complexes with the radiolabeled SRE. The major complex (I) was formed by a homodimeric SRF bound to the SRE motif. Moreover, SRF is able to form a ternary complex with the ubiquitously expressed transcription factors of the TCF family (complex II) (8, 11). When Jurkat T cells were exposed to TRAIL or CH11 for 2 h, we observed the formation of two additional complexes, with faster electrophoretic mobility, that we called complexes III and IV. Induction of complexes III and IV by TRAIL or CH11 was abolished when Jurkat T cells were preincubated with the caspase inhibitor, acetyl-DEVD-aldehyde. This observation demonstrates the pivotal role of caspase activities in the generation of complexes III and IV. On the other hand, an anti-Elk1 antibody did not affect complexes III and IV migration. These results demonstrate that complexes III and IV contain the SRF protein. Additionally, we performed supershift assays using a combination of antibodies directed against the N- or C-terminal part of SRF. Although the anti-N-terminal SRF antibody failed to react with the SRE motif since this complex that is recognized by the anti-N-terminal SRF antibody is not displaced by the anti-C-terminal SRF antibody. Complex IV is most likely composed by two C-terminally truncated SRF (SRF/SRF\(_c\)) bound to the SRE motif. Complex IV did not affect complex IV (Fig. 3B). Thus, complex III corresponds likely to a heterodimer composed of full-length SRF and C-terminally truncated SRF (SRF/SRF\(_c\)) bound to the SRE motif. Complex IV is most likely composed by two C-terminally truncated SRF fragments (SRF\(_\Delta\)/SRF\(_{\Delta\_c}\)) bound to the SRE motif since this complex that is recognized by the anti-N-terminal SRF antibody is not displaced by the anti-C-terminal SRF antibody.

To determine whether SRF was cleaved by caspases, we looked for SRF proteolysis in Jurkat T cells by Western blot. Although the anti-N-terminal SRF antibody failed to react with

**Cleavage of SRF during Induction of the Apoptotic Program—**The next series of supershift experiments was undertaken to identify the nature of complexes III and IV generated upon TRAIL or CH11 treatment of Jurkat T cells. Fig. 3A showed that an antibody directed against the N-terminal part of SRF displaced complexes I and II as well as the complexes III and IV. On the other hand, an anti-Elk1 antibody did not affect complex III and IV migration. These results demonstrate that complexes III and IV contain the SRF protein. Additionally, we performed supershift assays using a combination of antibodies directed against the N- or C-terminal part of SRF. Although the anti-N-terminal SRF antibody displaced complexes I and II, the anti-C-terminal SRF antibody displaced complexes I-III but did not affect complex IV (Fig. 3B). Thus, complex III corresponds likely to a heterodimer composed of full-length SRF and C-terminally truncated SRF (SRF/SRF\(_c\)) bound to the SRE motif. Complex IV is most likely composed by two C-terminally truncated SRF fragments (SRF\(_\Delta\)/SRF\(_{\Delta\_c}\)) bound to the SRE motif since this complex that is recognized by the anti-N-terminal SRF antibody is not displaced by the anti-C-terminal SRF antibody.

To determine whether SRF was cleaved by caspases, we looked for SRF proteolysis in Jurkat T cells by Western blot.

**Fig. 4. Cleavage of SRF correlates with activation of caspase 3.** A, 100 µg of proteins from control cells or cells exposed to 100 ng/ml TRAIL or CH11 for the indicated times were subjected to Western blot analysis using anti-C-terminal SRF (upper panel) or anti-caspase 3 (lower panel) antibodies. B, Jurkat T cells were preincubated for 24 h with 100 µM acetyl-DEVD-aldehyde and then treated for 6 h with 100 ng/ml TRAIL or CH11. 100 µg of proteins were subjected to Western blot analysis using anti-C-terminal SRF (upper panel) or anti-caspase 3 (lower panel) antibodies. Molecular masses, indicated on the left, are expressed in kilodaltons.

**Fig. 5. In vitro cleavage of SRF by recombinant caspase 3.** A, SRF was first transcribed and translated in vitro in the presence of \([\text{35S}]\)methionine. Then 2.5 µl of in vitro labeled SRF were incubated with 25 ng of recombinant caspase 3 for 6 h at 37 °C in the presence or absence of acetyl-DEVD-aldehyde. Reactions were then analyzed by electrophoresis and autoradiography. B and D, a part of the reactions carried out in A were used to perform band shift assays with the labeled SRE as a probe. When indicated 0.5 µl of anti-N-terminal SRF was added to the binding reaction. Arrows indicate the supershift of complexes III and IV. C, DNA binding activity of 10 µg cellular extracts from Jurkat T cells incubated 4 h with 100 ng/ml TRAIL.
denatured SRF and the anti-C-terminal SRF antibody did not recognize the cleavage products, we could observe a gradual disappearance of SRF as a function of time in the presence of TRAIL or CH11. After a 2-h incubation period most of the SRF protein was cleaved (Fig. 4A, upper panel). Disappearance of SRF immediately followed activation of caspase 3 as shown in Fig. 4A, lower panel. The cleavage of SRF is dependent on caspase activity since treatment with acetyl-DEVD-aldehyde was found to abrogate similarly activation of pro-caspase 3 and SRF cleavage (Fig. 4B, upper and lower panels).

These results demonstrate that execution of the apoptotic program is accompanied by the cleavage of SRF by caspases and resulted in the deletion of its C-terminal domain.

Cleavage of SRF by Caspase 3—To detect SRF cleavage products, we next examined whether SRF could serve as a substrate for caspases in vitro. In vitro transcribed and translated SRF was first incubated with recombinant caspase 3 in the presence or the absence of acetyl-DEVD-aldehyde. After 6 h at 37 °C, recombinant caspase 3 completely degraded the 67-kDa SRF generating a major fragment of approximately 32 kDa (Fig. 5A). In the presence of acetyl-DEVD-aldehyde, caspase 3 failed to cleave SRF (Fig. 5A). Furthermore, reactions carried out as described in Fig. 5A were used to perform gel shift assays (Fig. 5B). In vitro transcribed and translated SRF was found to bind labeled SRE. SRF processed in vitro by caspase 3, generated, in the band shift assay, two complexes migrating with faster electrophoretic mobility than the constitutive, homodimeric SRF-SRE complexes observed under control conditions or in the presence of acetyl-DEVD-aldehyde (Fig. 5B). Interestingly, complexes obtained in gel shift assay with in vitro processed SRF comigrated with those found in cellular extracts prepared from CH11 or TRAIL-stimulated Jurkat T cells (Fig. 5C). Moreover, addition of an anti-N-terminal SRF antibody to the binding reaction totally displaced complexes III and IV (Fig. 5D). Taken together these results demonstrate that, in vivo and in vitro, SRF is a target for caspase 3 during induction of apoptosis by TRAIL or CH11.

The Inhibition of SRF Cleavage by PMA Correlates with Cell Survival—In agreement with recent reports (42), we showed that internucleosomal DNA fragmentation observed upon exposure to TRAIL or CH11 was not detected when these death effectors were combined with PMA, demonstrating that PMA blocks TRAIL- and CH11-mediated apoptosis (Fig. 6A). As we observed a tight correlation between induction of apoptosis, cleavage of SRF, and inhibition of the c-FOS transcriptional activity, we expected that PMA treatment would block the apoptotic effect of TRAIL or CH11. Whereas TRAIL or CH11 decreased the basal transcriptional activity of the c-FOS promoter by about 50%, addition of PMA in the medium abrogated their effects and also maintained a full stimulation of the c-FOS promoter activity (Fig. 6B). Identical results were observed on the activity of the SRE-driven reporter plasmid (Fig. 6C). Interestingly, in the presence of PMA, we failed to detect any SRF cleavage upon TRAIL or CH11 stimulation as judged by band shift assays (Fig. 7A). In Western blot experiments, SRF expression was barely detectable after exposure to TRAIL or CH11, but in presence of PMA, TRAIL- or CH11-mediated cleavage of SRF was totally impaired (Fig. 7B, upper panel). Finally, when death effectors were combined with PMA, we failed to observe any activation of caspase 3 indicating that PMA is able to counteract the activation of procaspase 3 by death inducers (Fig. 7B, lower panel).

Taken together, these results show that PMA prevents the cleavage of SRF, blocks the inhibitory effects of death effectors on c-FOS promoter activity, and finally protects Jurkat T cells from TRAIL- or CH11-induced apoptosis.
Cleavage of SRF during Induction of Apoptosis

One of the first and best characterized biological effects of growth factors is to induce a battery of genes, called immediate early genes, that plays a pivotal role in the control of cell growth and survival (28). In this report, focusing our attention on the immediate early gene c-FOS, we clearly demonstrated that induction of apoptosis by death effectors leads to a drastic decrease in the transcriptional activity of the c-FOS promoter. Since a similar inhibitory effect was observed on an SRE-dependent luciferase construct, the effect of death receptor ligands appears to be targeted to the SRE sequence of the c-FOS promoter. At the same time, TRAIL or CH11 did not impair the activity of a heterologous promoter, demonstrating that the effects of death effectors were specific of the c-FOS promoter and did not reflect a more general mechanism of DNA degradation in apoptotic cells. We showed that death factors induced a cleavage of SRF, likely through activation of caspase 3, suggesting that the inhibition of the c-FOS promoter activity, during induction of apoptosis, resulted from a cleavage of SRF. A role for SRF in the regulation of c-FOS expression has already been proposed. Indeed, in SRF<sup>−/−</sup> mice embryos, expression of c-FOS is severely impaired, and SRF-deficient mice do not develop to term (29). As c-FOS is tightly associated with cell growth control, extinction of the basal c-FOS expression may be a signal to engage the cell into a death pathway. By using a combination of antibodies directed against the N- or C-terminal part of SRF, we showed that the cleavage of SRF, during induction of apoptosis, leads to elimination of its C-terminal domain (SRF<sub>C</sub>). Additionally, we showed that SRF<sub>C</sub> still bound to the SRE sequence as demonstrated by the presence of the two fast electrophoretic mobility complexes III and IV. Our results are in agreement with other reports which previously showed that deletion of the SRF C-terminal domain did not impair its ability to dimerize and bind DNA (8, 9, 11, 30). In most cases, SRF functions in close association with a member of the TCF family that includes Elk1, Sap1, and Sap2/ERP/NET (31). TCF associates both with the dimerization domain of SRF and with the 5′ end of the SRE only if the SRE is already occupied by SRF (8). In our supershift experiments, we observed that an anti-Elk1 antibody displaced complex II, suggesting that in Jurkat T cells, Elk1 is likely the major TCF that associates with SRF. The shift evoked by anti-Elk1 antibody was less visible in TRAIL- or CH11-treated cells due to the cleavage of SRF. This observation agrees with earlier studies demonstrating the requirement of SRF for Elk1 binding to SRF motif (8). Moreover, since the anti-Elk1 antibody did not displace the fast mobility complexes (complexes III and IV), we conclude that Elk1 is probably not able to associate with the truncated form of SRF.

SRE sequences have been identified in the promoter of different immediate early genes such as EGR-1, EGR-2, β-actin, and JUN B that are also involved in cell growth and survival control (3). So, it is possible that the inhibitory effects evoked by death effectors on c-FOS promoter activity may also be observed on these SRF-dependent promoters as a consequence of SRF cleavage. Recently, an SRE-like motif was found in the promoter of MCL1, an anti-apoptotic member of the Bcl2 family (32). Proteins of the Bcl2 family include those that promote death (e.g. Bax, Bak, Bad, and Bid) and those that prevent death (e.g. Bcl2, BclxL, and MCL1) (33–35). In Jurkat T cells, apoptosis can be blocked by an overexpression of the anti-apoptotic members of the Bcl2 family that impairs caspase 3 activation (36). Interestingly, the expression of MCL1 appears to be controlled by SRF and TCF transcription factors (32). According to the crucial role of the Bcl2 family proteins in the regulation of apoptosis (37), the cleavage of SRF could also affect the expression of MCL1 thus favoring transmission of apoptotic signals. In this line, we hypothesize that SRF cleavage results in a decrease of SRF responsive gene expression and thereby would play a key role in the regulation of the apoptotic pathway. Numerous caspase substrates have been described so far, and some of them have also been involved in the control of cell growth and survival (38). Given the complexity of the molecular mechanisms involved in the regulation of programmed cell death, it has not been possible until now to demonstrate that the cleavage of one of these caspase target is absolutely required for cell death. Because of the presence of more than one cleavage site in SRF (Fig. 5A), we have not yet been able to generate a non-cleavable form of the protein. However, it would be interesting in the future to assess the effect of a non-cleavable form of SRF on TRAIL and CH11-induced apoptosis.

Interestingly, we observed that the protective effect of PMA on TRAIL- and CH11-mediated cleavage of SRF and c-FOS promoter inhibition correlated with a protection against apoptosis. We also established that the specific MAPK/extracellular signal-regulated kinase inhibitor, PD 98059, partially inhibited the protective effects of PMA, whereas a
complete inhibition was achieved with the PKC inhibitor GF109203X. These results demonstrate that the protective effects of PMA depend on both the p42/44 MAPK signaling pathway and PKC activity. This observation is in accordance with a recent study indicating that neuronal cell survival was promoted by activation of the Raf MAPK signaling pathway and involved the MAPK-activated p90 ribosomal S6 kinase (pp90RSK) family (39). In a recent report, it has also been shown that pp90RSK blocked bad-mediated cell death via a PKC-dependent pathway (40). Importantly, pp90RSK has also shown to phosphorylate SRF on serine 103, an event that facilitates formation of an active transcription complex at the SRE (41).

Taken together, we demonstrated in this report that the transcription factor SRF was cleaved during induction of apoptosis. This observation was correlated with a decrease of the c-FOS promoter transcriptional activity. As SRF and c-FOS have been involved in the control of cell survival, we hypothesized that the cleavage of SRF and the inhibition of c-FOS expression represent an important step in the execution of the apoptotic program in Jurkat T cells.

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REFERENCES

1. Hill, C. S., and Treisman, R. (1995) EMBO J. 14, 5037–5047
2. Karin, M., and Hunter, T. (1995) Curr. Biol. 5, 747–757
3. Treisman, R. (1995) EMBO J. 14, 4905–4913
4. Shaw, P. E., Schroeter, H., and Nordheim, A. (1994) Cell 78, 563–572
5. Treisman, R. (1992) Trends Biochem. Sci. 17, 423–426
6. Treisman, R. (1994) Curr. Opin. Genet. & Dev. 4, 96–101
7. Shore, P., and Sharrocks, A. D. (1995) Eur. J. Biochem. 229, 1–13
8. Hipshkind, R. A., Rao, V. N., Mueller, C. G. F., Reddy, R. S. P., and Nordheim, A. (1991) Nature 354, 531–534
9. Dalton, S., and Treisman, R. (1992) Cell 68, 597–612
10. Giovanne, A., Piuntas, A., Maira, S. M., Schissczuk, P., and Wasylyk, B. (1994) Genes Dev. 8, 1502–1513
11. Shore, P., and Sharrocks, A. D. (1994) Mol. Cell. Biol. 14, 3283–3291
12. Gille, H., Kortenjann, M., Thomae, O., Moosau, C., Slaughter, C., Cobb, M. H., and Shaw, P. E. (1995) EMBO J. 14, 951–962
13. Janknecht, R., Ernst, W. H., Pingoud, V., and Nordheim, A. (1993) EMBO J. 12, 5097–5104
14. Janknecht, R., and Hunter, T. (1997) EMBO J. 16, 1620–1627
15. Whitmarsh, A. J., Shore, P., Sharrocks, A. D., and Davis, R. J. (1995) Science 269, 403–407
16. Whitmarsh, A. J., Yang, S. H., Su, M. S., Sharrocks, A. D., and Davis, R. J. (1997) Mol. Cell. Biol. 17, 2360–2371
17. Thompson, C. B. (1995) Science 267, 1456–1462
18. Böse, R., Verheij, M., Haimovitz-Friedman, A., Scotto, A., Fuchs, Z., and Kolesnick, R. N. (1995) Cell 82, 405–414
19. Hasegawa, J., Kamada, S., Kamyk, W., Shimizu, S., Imazu, T., Matsuda, H., and Tsujimoto, M. (1996) Cancer Res. 56, 1713–1718
20. Le-Niculescu, H., Bonfoco, E., Kasuya, Y., Claret, F. X., Green, D. R., and Karin, M. (1999) Mol. Cell. Biol. 19, 751–763
21. Walczak, H., Debi-Esposti, M. A., Johnson, R. S., Smolak, P. J., Waugh, J. Y., Besiani, N., Timour, M. S., Gerhart, M. J., Smith, C. A., Goodwin, R. G., and Rauch, C. T. (1997) EMBO J. 16, 5386–5397
22. Dubrez, L., Savoy, I., Hamman, A., and Solary, E. (1996) EMBO J. 15, 5504–5512
23. Enari, M., Hug, H., and Nagata, S. (1995) Nature 375, 78–80
24. Enari, M., Talanian, R. V., Wong, W. W., and Nagata, S. (1996) Nature 380, 723–726
25. Thome, M., Schneider, P., Hofmann, K., Finkenscher, H., Meim, E., Neipel, F., Mattmann, C., Burns, K., Bodmer, J. L., Schröter, M., Scaffidi, C., Krammer, P. H., Peter, M. E., and Tschopp, J. (1997) Nature 386, 517–521
26. Ricci, J. E., Maulon, L., Luciano, F., Guerin, S., Livolsi, A., Mari, B., Breitmayr, J. P., Feyron, J. F., and Aubberger, P. (1999) Oncogene 18, 3963–3969
27. Cursio, R., Gegenhuber, J., Ricci, J. E., Crenesse, D., Rostagno, P., Maulon, L., Saint-Paul, M. C., Ferri, E., and Aubberger, P. (1999) FASEB J. 13, 253–261
28. Hershman, H. R. (1991) Annu. Rev. Biochem. 60, 281–319
29. Arsenian, S., Weinhold, B., Oelgeschlager, M., Ruther, U., and Nordheim, A. (1998) EMBO J. 17, 6289–6299
30. Treisman, R., Maas, R., and Wulle, J. (1992) EMBO J. 11, 4631–4640
31. Price, M. A., Rogers, A. E., and Treisman, R. (1998) EMBO J. 17, 2589–2601
32. Townsend, K. J., Zhou, P., Qian, J., Bieszczad, C. K., Lowrey, C. H., Yen, A., Cheng, J., and Eriksson, J. E. (1998) J. Immunol. 160, 2626–2636
33. Adams, J. M., and Cory, S. (1998) Science 281, 1322–1326
34. Kroemer, G. (1997) Nat. Med. 3, 614–624
35. Reed, J. C. (1994) J. Cell Biol. 124, 1–6
36. Scaffidi, C., Fulda, S., Srinivasan, A., Friescu, C., Li, J., Tomasselli, K. J., Debatin, K. M., Krammer, P. H., and Peter, M. E. (1998) EMBO J. 17, 1675–1687
37. Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., and Greenberg, M. E. (1997) Cell 91, 231–241
38. Widmann, C., Gibson, S., and Johnson, G. L. (1998) J. Biol. Chem. 273, 7141–7147
39. Bonni, A., Brunet, A., West, A. E., Datta, S. R., Takasu, M. A., and Greenberg, M. E. (1999) Science 286, 1358–1362
40. Tan, Y., Ruan, H., Demeter, M. R., and Comb, M. J. (1999) J. Biol. Chem. 274, 34859–34867
41. Rivera, V. M., Missa, R. P., Ginty, D. D., Chen, R. H., Blenis, J., and Greenberg, M. E. (1999) Mol. Cell. Biol. 19, 8260–8273
42. Holmstrom, T. H., Chow, S. C., Ebo, I., Cofley, E. T., Orrenius, S., Sistonen, L., and Eriksson, J. E. (1998) J. Immunol. 160, 2626–2636
2 C. Bertolotto, personal communication.
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