Multiple Members of the Mitogen-activated Protein Kinase Family Are Necessary for PED/PEA-15 Anti-apoptotic Function*

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293 kidney embryonic cells feature very low levels of the anti-apoptotic protein PED. In these cells, expression of PED to levels comparable with those occurring in normal adult cells inhibits apoptosis induced by growth factor deprivation and by exposure to H2O2 or anisomycin. In PED-expressing 293 cells (293PED), inhibition of apoptosis upon growth factor deprivation was paralleled by decreased phosphorylation of JNK1/2. In 293PED cells, decreased apoptosis induced by anisomycin and H2O2 was also accompanied by block of JNK1/2 and p38 phosphorylations, respectively. Impaired activity of these stress kinases by PED correlated with inhibition of stress-induced Cdc-42, MKK4, and MKK6 activation. At variance with JNK1/2 and p38, PED expression increased basal and growth factor-stimulated Ras-Raf-1 co-precipitation and MAPK phosphorylation and activity. Treatment of 293PED cells with the MEK inhibitor PD98059 blocked ERK1/2 phosphorylations without an effect on inhibition of JNK1/2 and p38 activities. Complete rescue of JNK and p38 functions in 293PED cells by over-expressing JNK1 or p38, respectively, enabled only partial recovery of apoptotic response to growth factor deprivation and anisomycin. However, simultaneous rescue of JNK and p38 activities accompanied by block of ERK1/2 fully restored these responses. Thus, PED controls activity of the ERK, JNK, and p38 subfamilies of MAPKs. PED anti-apoptotic function in the 293 cells requires PED simultaneous activation of ERK1/2 and inhibition of the JNK/p38 signaling systems by PED.

PED is a 15-kDa protein with almost ubiquitous expression (1, 2). cDNA sequencing led to the identification of a canonical death effector domain (DED) at PED N terminus (3, 4). More recently, we and others (3, 5) demonstrated that the DED of PED binds the DED of both FAS-associated death domain (FADD) and caspase 8 (FLICE). Through this mechanism, PED inhibits FLICE activation by tumor necrosis factor-α and FasL, thereby blocking the apoptotic effects of these cytokines. It has subsequently been shown that PED potentiates phosphorylation of the ERKs through a Ras-dependent mechanism (6). PED also anchors the ERKs in the cytoplasm, preventing phosphorylation of their nuclear substrates and restraining cell proliferation (7). These findings suggest that PED may feature a broad function in control of cell survival. However, the molecular mechanisms involved in PED action have only partially been elucidated.

Mitogen-activated protein kinases (MAPKs) play a major role in mediating cellular responses to a variety of extracellular stimuli, including death and survival signals (8, 9). MAPKs include at least three main subgroups: the extracellular signal-regulated kinases (ERK1/2 or p42/44MAPK), the c-Jun N-terminal kinases (p46/54JNK), and p38 MAPK. While structurally related, MAPK families undergo activation in response to extracellular stimuli through distinct upstream dual specificity kinases, thereby functioning in separate MAPK cascades (10, 11). The Raf/ERK kinase1/2/ERK1/2 cascade is stimulated by mitogenic and survival stimuli, largely through the Ras-Raf-1-dependent pathway (12, 13). At variance, p46/54JNK and p38MAPK are primarily activated by cellular stresses including oxidative agents, UV irradiation, hypoxia, proinflammatory cytokines, and anisomycin (14, 15). Exposure to these agents often results in cell apoptosis (16). Dual specificity kinases activating p46/54JNK and p38MAPK are markedly activated by cellular stresses including oxidative agents, UV irradiation, hypoxia, proinflammatory cytokines, and anisomycin (14, 15). Exposure to these agents often results in cell apoptosis (16). Dual specificity kinases activating p46/54JNK are MAPK kinases 4 and 7 (MKK4 and MKK7), while MKK3 and MKK6 were proved to activate p38MAPK. MAPKs phosphorylate and activate a variety of cytoplasmic and nuclear substrates, including transcription factors.

Despite the dichotomy in the main action of ERKs and stress kinases, current evidence indicates that ERKs and p46/54JNK-p38MAPK cooperate in inducing cells to survive or to die. Activation of the stress kinase cascade, alone, may not be sufficient to induce apoptosis under all circumstances (17). Also, concomitant inactivation of survival signals appears to be necessary for enabling p46/54JNK and p38MAPK induction to cause cell death (17–21). It appears therefore that the balance between the ERK and the p46/54JNK-p38MAPK signaling systems is criti-
ical for inducing the cell to die or survive. However, the molecular mechanisms responsible for tuning the ratio of activities in these cascades are largely elusive. For instance, there is little information on whether checkpoint proteins exist in the cell that foster survival signal transduction through the ERK pathway while inhibiting p46/54JNK-p38MAPK signaling or vice versa. Answering this question will greatly help in the understanding how the cells control their own survival.

In the present report we show that PED inhibits p46/54JNK and p38MAPK signaling and protects cells from stress-induced apoptosis. More importantly, we report that full PED antiapoptotic action requires both ERK activation and inhibition of p46/54JNK-p38MAPK function, indicating that PED represents a major MAPK regulatory protein, which gathers antia apoptotic signals toward the cell machinery.

EXPERIMENTAL PROCEDURES

Materials—Sera, sera, antibiotics for cell culture, and the LipofectAMINE reagent were from Invitrogen. Rabbit polyclonal antibodies toward ERK1/2, p38, and JNK/SAPK were purchased from Santa Cruz, Inc. (Santa Cruz, CA). All phosphokinase antibodies were from New England Biolabs Inc. (Beverly, MA), and PED antibodies have been described previously (2). The HA-tagged JNK-1 and GST-tagged MKK6 cDNA plasmids were generously donated by Dr. S. Gutkind (National Institutes of Health, Bethesda, MD) and the GST-CRIB cDNA by Dr. P. Di Fiore (Istituto Europeo di Oncologia, Milan, Italy). The c-Jun cDNA has been reported previously (22). SDS-PAGE reagents were purchased from Bio-Rad, and radiochemicals, Western blotting, and ECL reagents were from Amersham Biosciences. All other chemicals were from Sigma.

Cell Culture and Transfection and Cell Death Assays—The 293 human kidney embryonic cells were grown in DMEM supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 IU/ml streptomycin, and 2% l-glutamine in a humidified CO₂ incubator. A pcdNA3 expression plasmid containing the Myc-tagged PED cDNA was generated by first amplifying PED wild-type cDNA with the following two primers: PEDS' (5'-GGCCGCTACGCCAGCGCCGCGGCGGAGGTG-3') and 3'MycPEDB (5'-GCCGGATCTCAGGTATCTTGGGGCGGGGAAAACTTGC-3'), carrying the KpnI and BamHI restriction sites, respectively. The amplification product was then cloned in the pcDNA3 vector and sequence verified using the T7 Sequencing Kit by Amersham Biosciences. The construct was stably transfected in 293 cells using the lipofectamine method as in Ref. 2. Selection was accomplished using G418 at the effective dose of 0.8 mg/ml. Transient transfections of JNK-1, MKK6, and c-Jun cDNAs were accomplished by the LipofectAMINE method according to the manufacturer's instructions. Briefly, cells were cultured in 96-dish plates up to 80% confluence and incubated for 24 h with 3 μl of cDNA and 15 μl of LipofectAMINE in serum-free DMEM. An equal volume of DMEM supplemented with 20% fetal calf serum was added for 5 h. The medium was then replaced with DMEM supplemented with 10% serum and cells further incubated for 24 additional hours before being assayed.

For detecting apoptosis, the cells were kept in the presence or the absence of serum or incubated with 50 μM anisomycin (18 h) or 500 nm H₂O₂ (1 h) or 100 μM PD98059 (18 h), as indicated in the description of the individual experiments. Apoptosis was then assayed by DNA ladder detection according to Ref. 23 or quantitated using the Apoptosis ELISA Plus kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions.

Western Blot Analysis—For these assays, the cells were solubilized in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 4 mM EDTA, 10 mM Na₃PO₄, 2 mM Na₂VO₄, 100 mM NaF, 1% glycerol, 1% Triton, 1 mM phenylmethylsulfonyl fluoride, 100 μg/ml aprotinin, 1 mM leupeptin) for 30 min at 4 °C. Lysates were centrifuged at 5,000 × g for 15 min. Solubilized proteins were separated by SDS-PAGE and transferred on 0.45 μm Immobilon-P membranes (Millipore, Bedford, MA). Upon incubation with the primary and secondary antibodies, immunoreactive bands were detected by ECL according to the manufacturer's instructions.

MAPK Activity and Cdc-42 GTP Loading—MAPK activity was assayed in vitro as reported previously (24). Briefly, cells were solubilized in lysis buffer and lysates clarified by centrifugation at 5,000 × g for 20 min. Equal aliquots of the lysates (200 μg) were immunoprecipitated with MAPK antibodies, and phosphorylation reactions were initiated by adding 2 μg of the myelin basic protein substrate, the phosphorylation mixture (20 mM HEPES, pH 7.2, 10 mM MgCl₂, 10 mM MnCl₂, 1 mM diithiothreitol, 5 mM ATP, 0.2 mM EGTA, 1 mM P2 KIA, final concentrations) and 10 μCi/reaction (γ-³²P)ATP. Phosphorylation reactions were prolonged for 30 min at 22 °C, stopped by rapid cooling on ice, and spotted on phosphocellulose disc papers. Discs were washed twice with 1% H₃PO₄, followed by additional washes in water, and the disc-bound radioactivity was quantified by liquid scintillation counting.

For estimating GTP loading of Cdc-42, cells were incubated in the presence or the absence of cytotoxic necrotizing factor 1 (CNF1) (30 ng/ml) for 6 h and solubilized in 1% Nonidet P-40, 25 mM Tris, pH 7.5, 5 mM CaCl₂, 100 mM NaCl, 5% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 2 mM Na₂VO₄. Protein lysates (1 mg) were then incubated for 20 min at 4 °C in the presence of 10 μg of agarose-bound GST CRIB in 2 volumes of binding buffer (0.5% Nonidet P-40, 25 mM Tris, pH 7.5, 30 mM MgCl₂, 40 mM NaCl, 1 mM diithiothreitol). Complexes were washed four times with lysis buffer and then resuspended in Laemmli buffer followed by boiling for 4 min and centrifugation at 25,000 × g for 3 min. Supernatants were analyzed by SDS-PAGE and blotting with Cdc-42 antibodies.

RESULTS

Reduced Apoptosis in PED-overexpressing 293 Cells—293 human embryonic kidney cells were stably transfected with a Myc-tagged PED cDNA. Several clones of PED-overexpressing cells were obtained and three of them, termed 293PEDCl1, 293PEDCl2, and 293PEDCl3, were studied in detail. Immunoblotting with either Myc or PED antibodies indicated that the expression of PED in these transfected cells was increased by >100-fold compared with control cells, either those transfected with the resistance plasmid alone (293pc) or the 293 untransfected cells (293wt) (Fig. 1A). The 293wt cells feature very low levels of endogenous PED. On a per milligram protein bases, the levels of PED expression achieved in the transfectants were comparable with those in mouse brain and fat tissues (data not shown). Based on DNA laddering, serum deprivation of parental cells induced a time-dependent increase in DNA fragmentation (Fig. 1B). In these cells, fragmentation was well detectable upon 24 h of serum deprivation, further increasing upon 48 and 72 h. At variance, no DNA fragmentation was observed in PED-transfected cells up to 48 h after serum deprivation. In these cells, apoptosis became detectable only by 72 h. The same delay in the onset of DNA laddering in response to serum starvation was observed in the two other transfected clones (data not shown). To further investigate PED apoptosis-protective action, we compared the effects of the stress kinase activators anisomycin and hydrogen peroxide in 293PEDCl1 and control cells. As shown in Fig. 1C, treatment with anisomycin for 18 h increased apoptosis by >3-fold in parental 293wt and 293pc cells, same as 48-h serum deprivation. In these same cells, 1-h incubation with hydrogen peroxide induced a 5-fold increase in apoptosis. In 293PEDCl1 cells there was a >2-fold inhibition in the apoptotic responses to all of these agents, compared with the control cells (p < 0.001). Almost identical inhibition of apoptosis was observed in the other two clones of PED-overexpressing cells.

JNK and p38 Function in PED-overexpressing 293 Cells—In different cell types, the MAPK-related enzymes JNK and p38 play a major role in signaling apoptosis and undergo phosphorylation and activation in responses to growth factor withdrawal, anisomycin, and oxidative stress (19–21). In control 293 cells, anisomycin also induced a 10-fold increase in phosphorylation of the key activation sites of JNK (Thr183 and Tyr185, Fig. 2A). Anisomycin-induced phosphorylation was reduced by 5-fold in PED-transfected compared with the control cells with no change in JNK expression levels. Similar to anisomycin, serum deprivation increased JNK phosphorylation in 293wt cells. This effect peaked at 10–16 h after serum starvation and was 2.5-fold less evident in the 293PEDCl1 cells (Fig. 2B). The decrease in JNK phosphorylation upon anisomycin treatment
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FIG.1. PED action on stress-induced apoptosis in 293 cells. A, 293 human embryonic kidney cells were stably transfected with a Myc-tagged PED cDNA as indicated. PED-overexpressing cell clones (293PEDCl1, 293PEDCl2, 293PEDCl3) and control cells (untransfected cells, 293WT; cells transfected with the empty plasmid, 293pc) were solubilized and immunoblotted with Myc, PED, or actin antibodies, as indicated. Blotted proteins were revealed by ECL and autoradiography. B, alternatively, the cells were incubated in the absence or the presence of 10% serum for the indicated times. Cytoplasmic DNA was extracted and analyzed by 1% agarose gel electrophoresis and ethidium bromide staining. Representative experiments are shown in A and B. C, the cells were incubated in the absence or the presence of 10% serum (48 h), 50 μM anisomycin (18 h), or 500 μM H2O2 (1 h), as indicated. Apoptosis was quantitated by the ELISA Plus detection kit as described under "Experimental Procedures." Bars represent the mean ± S.D. of four independent experiments in duplicate.

FIG.2. PED action on JNK activity in 293 cells. A, 293PEDCl1 and control cells were incubated with 50 μM anisomycin for 30 min. The cells were solubilized, and 50 μg of cell proteins were blotted with phospho-JNK (pJNK1/2) antibodies as reported under "Experimental Procedures." Filters were revealed by ECL according to the manufacturer's instructions. Alternatively (B), the cells were incubated in the presence or the absence of serum for the indicated times, solubilized, and blotted with pJNK1/2 antibodies as above. C, to test JNK activity, cells were first transfected with c-Jun cDNA as reported under "Experimental Procedures." The cells were then exposed to anisomycin for 30 min. Phospho-c-Jun was identified by Western blotting equal amounts of cell lysates with phospho-c-Jun antibodies. The autoradiograms shown are representative of four (A) and three (B) representative experiments.

C). Further increase in ERK phosphorylation and activity in response to serum were reduced in these cells, with no significant change in ERK1/2 expression.

In both the PED-transfected and the control cells, inhibition of the ERK upstream kinase MEK with PD98059 blocked basal and serum-stimulated ERK activities (Fig. 5A). Interestingly, however, PD98059 had no effect on the impaired activation of JNK by anisomycin in 293PEDCl1 cells (Fig. 5B). Similarly, PD98059 treatment did not allow any recovery of p38 phosphorylation by hydrogen peroxide in PED-overexpressing as compared with wild-type cells (Fig. 5C) and to cells transfected with the empty plasmid (not shown). Thus, ERK activation, alone, did not account for the inhibition of JNK and p38 functions in PED-overexpressing cells.

Cdc-42 Signaling in PED-overexpressing 293 Cells—JNK and p38 have been reported to be induced by the Cdc-42 signaling cascade in response to different stress-inducing agents (25). In 293 cells, CNF1 (26) increased the pull-down of the CRIB interaction domain of PKA-1 kinase with Cdc-42 by 4-fold (Fig. 6A). These effects did not occur in PED-expressing 293 cells, however. Phosphorylation of the key activation sites on PKA-1 downstream kinases MKK4 and MKK6 (Thr223 and Ser189/Ser207, respectively) were also increased by 8- and 2.5-fold by anisomycin and hydrogen peroxide (Fig. 6B). As was the case for the CRIB-Cdc-42 interaction, these effects were abolished in the 293PEDCl1 cells, suggesting that PED may inhibit JNK and p38 activation by blocking signaling through the

or serum starvation was paralleled by a 2.5-fold decreased phosphorylation of the JNK substrate c-Jun in the PED-overexpressing compared with control cells (Fig. 2C). Based on Western blotting with specific phospho-p38 antibodies, hydrogen peroxide and serum deprivation treatments of control cells also increased phosphorylation of p38 key activation sites (Thr180, Thr182) by 6- and 2.5-fold, respectively (Fig. 3, A and B). Phosphorylation of p38 by hydrogen peroxide and serum deprivation were 2- and 4-fold inhibited in PED-transfected cells. Control experiments with the 293PEDCl2 and 293PEDCl3 cells revealed very similar results (data not shown).

In Chinese hamster ovary cells, PED overexpression induces increased Raf-1 co-precipitation with active Ras (Fig. 4A). In addition, the 293 PED-transfected cells showed a constitutive increase in ERK1/2 phosphorylation and activity (Fig. 4, B and C). These effects did not occur in PED-expressing 293 cells, however. Phosphorylation of the key activation sites on PKA-1 downstream kinases MKK4 and MKK6 (Thr223 and Ser189/Ser207, respectively) were also increased by 8- and 2.5-fold by anisomycin and hydrogen peroxide (Fig. 6B). As was the case for the CRIB-Cdc-42 interaction, these effects were abolished in the 293PEDCl1 cells, suggesting that PED may inhibit JNK and p38 activation by blocking signaling through the

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that in the 293WT cells. This finding suggested that the inhibition in these cells, apoptosis remained significantly lower than 0.01 level). Thus, despite full activation of JNK and p38 function of JNK and p38 by PED overexpression in 293 cells does not fully account for PED anti-apoptotic effect. solubilized, and 50 μg of cell proteins were blotted with phospho-p38 (P-p38) or with p38 antibodies as indicated. Alternatively (B), the cells were incubated in the presence or the absence of serum for the indicated times, and cell proteins were immunoblotted with P-p38 antibodies as outlined above. Filters were revealed by ECL and autoradiography. The autoradiographs shown are representative of four (A) and three (B) independent experiments.

Cdc-42/PAK-1 pathway at an upstream step. There were no changes in the expression levels of Cdc-42, MKK4, and MKK6 (Fig. 6, A and B) in PED-overexpressing compared with the control cells.

**ERK and JNK/p38 Function in PED Anti-apoptotic Effect**—To further investigate the hypothesis that abrogation of JNK and p38 activation is responsible for PED anti-apoptotic effect, we sought to force JNK and p38 function in PED-expressing 293 cells. We transfected the cells with the HA-tagged JNK-1, GST-tagged MKK6, or with both cDNAs. Western blot analysis with phospho-JNK and phospho-p38 antibodies revealed that these overexpressations activated JNK and p38 kinases to almost identical extents in 293PEDCl1 and in control cells (Fig. 7A), indicating that kinase overexpression succeeded in overcoming their inhibition by PED. Consistently, co-transfection of JNK-1 with the c-Jun substrate cDNA resulted in a 4-fold increase in c-Jun phosphorylation compared with the cells transfected with c-Jun alone, both in 293PEDCl1 and in control cells (Fig. 7B). In the control cells, overexpression of JNK-1 or MKK6 increased apoptosis in response to serum deprivation by 2.2-fold (Fig. 7C). Simultaneous overexpression of these two kinases showed no additive effect. In the PED-transfected cells, overexpression of JNK-1, MKK6, or both cDNAs caused a 70% increase in cell apoptosis, bypassing PED inhibition (difference with the 293WT cells significant at the p < 0.01 level). Thus, despite full activation of JNK and p38 function in these cells, apoptosis remained significantly lower than that in the 293WT cells. This finding suggested that the inhibition of JNK and p38 by PED overexpression in 293 cells does not fully account for PED anti-apoptotic effect.

We therefore tested whether induction of ERK1/2 in PED-overexpressing cells may also contribute to protection from apoptosis, while not accounting for the inhibition of JNK and p38. To this end, we blocked ERK activity with the PD98059 inhibitor. Treatment of serum-deprived cells with PD98059, alone, caused no change in apoptosis, either in control or in PED-expressing cells. In control cells overexpressing JNK-1 and/or MKK6, however, PD98059 enhanced apoptotic response by 50%. Interestingly, in PED-transfected cells overexpressing JNK-1 and/or MKK6, PD98059 treatment increased apoptosis to levels identical to the control cells. Similar data were obtained when cell apoptosis was induced by actinomycin rather than serum depletion. Thus, PED protection from stress-induced apoptosis in 293 cells appeared to depend on the simultaneous induction of ERK activity and inhibition of JNK and p38 function.

**DISCUSSION**

We have recently identified PED as a DED-containing protein, which blocks FasL- and tumor necrosis factor-α-induced apoptosis through its DED. In the present report, we show that PED features a broader biological function, forwarding mutu-
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**Effect of MAPK inhibition on JNK and p38 activation.** A, 293\_PEDCl1 and control cells were preincubated in serum-free medium for 18 h, exposed to 50 \( \mu \text{M} \) PD98059 for further 45 min, and then to 10% serum for 10 min as indicated. Cells were solubilized, and lysates (50 \( \mu \text{g} \) of protein) were Western blotted with phospho-ERK antibodies (P-ERK1/2). B, the cells were preincubated in serum-free culture medium for 18 h in the presence or the absence of PD98059 and further incubated for 30 min with either 50 \( \mu \text{M} \) anisomycin or 500 \( \mu \text{M} \) H\(_2\)O\(_2\), as indicated. Cells were then solubilized and cell protein (50 \( \mu \text{g} \)) immuno-blotted with either phospho-JNK (P-JNK) or phospho-p38 (P-p38) antibodies. Filters were revealed by ECL and autoradiography. The autoradiographs shown are representative of three (A and C) and four (B) independent experiments.

We found that PED overexpression in 293 kidney cells strongly inhibits apoptosis caused by serum deprivation, oxidative stress, and anisomycin treatment. Simultaneously, PED blocks phosphorylation and activation of JNK and p38 by these agents, indicating an important role of decreased signaling through the JNK-p38 system in PED protection from stress-induced apoptosis. Consistent with this mechanism, forced expression of JNK and p38 activities rescues the sensitivity to stress-induced apoptosis in PED-overexpressing 293 cells. In parallel with the block of stress-induced activation of JNK and p38, their upstream activating kinases MKK4 and MKK6 were also blocked in PED-overexpressing cells. In addition, in these cells, PED inhibits Cdc-42 co-precipitation with the crib interaction domain of PAK-1, suggesting that high levels of PED may inhibit Cdc-42 signaling. Collectively, these data indicate that PED may prevent induction of JNK and p38 stress kinases by acting at a very early step in their activation pathways. It is possible that PED binds PAK-1 and inhibits its interaction with Cdc-42. However, we could not detect co-precipitation of PED with PAK-1 (data not shown). Alternatively, PED may block Cdc-42 activation, either by interacting with Cdc-42 or with a protein upstream Cdc-42 in the signaling cascade. Understanding the mechanism of PED activation of JNK and p38 stress kinases will require the identification of PED-binding proteins, which is presently in progress in our laboratory.

Studies by Ramos et al. (6) showed that, in Chinese hamster ovary cells, PED activates ERK1/2 in a Ras-dependent manner. ERK activation by PED also occurs in 293 cells, accompanied by that of early mechanisms in the Ras signaling cascade. Activation of the ERKs was not responsible for the inhibition of JNK and p38 function in cells expressing high levels of PED. Hence, block of ERK activity with the PD98059 inhibitor caused no change in JNK-p38 inactivation in the 293\_PED cells. PD98059 treatment does not affect apoptosis caused by serum deprivation either in wild-type 293 cells (expressing almost no endogenous PED) or in PED-overexpressing cells. Thus, ERK activity, alone, is unable to prevent stress-induced apoptosis in these cells. Rescue of JNK-p38 activities in PED-overexpressing 293 cells is not sufficient either, since it did not allow complete recovery of sensitivity to stress apoptosis. Interestingly, the full apoptotic effect of serum deprivation and anisomycin in PED-overexpressing 293 cells was restored by simultaneous block of ERK activation and reintegration of JNK-p38 function. This finding led us to propose that (i) in 293 cells, PED operates as a major survival protein, which simultaneously controls the major mitogen-activated protein kinases; and (ii) in these cells, stress-induced activation of apoptotic programs depends on the concomitant inhibition of ERK survival pathway and on activation of death signaling through the JNK-p38 routes.

Triggering of stress-activated kinases in concomitance with the inhibition of the ERK pathway has been observed in a number of cell systems undergoing programmed cell death (17–21). Activation of the stress kinase cascade may not always be sufficient to induce apoptosis in the cell (17). In fact, concomitant inactivation of survival signals has been proposed to be a prerequisite for the JNK-p38 kinases to induce cell death (17–21). Thus, the balance between the ERK and the JNK-p38 signaling systems seems critical for enabling the cell to die or survive. How the cells control this balance has been less extensively investigated. The pathways leading to activation of...
ERKs and of JNK-p38 consist of multiple signaling components, most of which are regulated by protein phosphorylation. Cross-talk may occur between these pathways and contribute to regulate ERK/JNK-p38 activity balance. Consistent with this possibility, simple inhibition of ERK basal activity in HeLa cells has been recently reported to be sufficient to trigger p38 activation in a caspase-dependent manner (27). It has also been proposed that ERK and JNK-p38 pathways may down-regulate activation in a caspase-dependent manner (27). It has also been shown). Thus, PED is a multifunctional protein. Increasing its level may allow the cells to become quiescent and resistant to different kinds of apoptotic stimuli.

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Most recently, PED has been shown to possess a nuclear export sequence (7). Through this sequence, PED was reported to restrict ERKs to the cytoplasm in cultured astrocytes (7). The cytoplasmic anchoring of ERKs does not affect phosphorylation of their cytosolic substrates, but inhibits ERK-dependent c-Fos transcription and cell proliferation. Inhibition of cell proliferation also occurs in 293 cells overexpressing PED (data not shown). Thus, PED is a multifunctional protein. Increasing its level may allow the cells to become quiescent and resistant to different kinds of apoptotic stimuli.