The lymphoblastoid CESS B cell line displays a CD19+, CD20−, CD44+, CD38−, CD77−, and IgGK+ surface phenotype (1), which suggests its origin from an antigen-selected, somatically hypermutated, and proliferating B lymphocyte, a stage ontogenetically close to that of memory B cells (2). Similar to memory B lymphocytes, CESS cells express both high affinity (p140Trk-A) and low affinity (p75NTR) NGF receptors. Autocrine production of NGF maintains the survival of CESS cells through the continuous deactivation of p38 MAPK, an enzyme able to induce Bcl-2 phosphorylation and subsequent cytochrome c release and caspase activation. In this paper, we show that NGF induces transcriptional activation and synthesis of MAPK phosphatase 1 (MKP-1), a dual specificity phosphatase that dephosphorylates p38 MAPK, thus preventing Bcl-2 phosphorylation. Furthermore, NGF increases MKP-1 protein stability by preventing its degradation during the proteasome pathway. Following NGF stimulation, MKP-1 protein mainly localizes on mitochondria, suggesting an interaction with p38 MAPK in this compartment. Incubation of CESS cells with MKP-1-specific antisense oligonucleotides induces cell death, which was not prevented by exogenous NGF. By contrast, overexpression of native MKP-1, but not of its catalytically impaired form, inhibits apoptosis induced by NGF neutralization in CESS cells. Thus, the molecular mechanisms underlying the survival function of NGF in CESS B cell line predominantly consist in maintaining elevated levels of MKP-1 protein, which controls p38 MAPK activation.

This paper is available on line at http://www.jbc.org

The abbreviations used are: NGF, nerve growth factor; MAPK, mitogen-activated protein kinase; MKP, mitogen-activated protein kinase phosphatase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular-signal-regulated kinase kinase; EGFP, enhanced green fluorescent protein; PE, phycocerythrin; PARP, poly(ADP-ribose) polymerase; NTR, neurotrophin receptor; hRNGF, human recombinant NGF; CMV, cytomegalovirus; dsPTPs, dual specificity phosphatases; TBS-T, Tris-buffered saline containing 0.05% Tween 20; PBS, phosphate-buffered saline; SAPK, stress-activated protein kinase.

G. De Chiara, M. E. Marcocci, P. Rosini, M. Torica, and F. Cozzolino, manuscript in preparation.
Survival Activity of NGF Is Mediated by Induction of MKP-1

Survival Activity of NGF Is Mediated by Induction of MKP-1.

EXPEDIENT PROCEDURES

Reagents—Human recombinant β-nerve growth factor, SB203580, p38 MAPK inhibitor, K252a-TRK-A inhibitor, and proteasome inhibitor lactacystin were purchased from Calbiochem. Neutralizing rat anti-human NGF monoclonal antibodies (clone dD11) were kindly donated by Dr. A. Cattaneo (International School for Advanced Studies, Trieste, Italy) and always were used at 10 μg/ml. Rabbit anti-MKP-1 antibody and rabbit anti-ubiquitin were from Sigma. Rabbit anti-poly-(ADP-ribose) polymerase (PARP, H-250) and goat anti-actin antibody were from Santa Cruz Biotechnology. Rabbit anti-p38 MAPK, anti-phospho-p38 MAPK, anti-phospho JNK, anti-JNK, anti-p44/42 MAPK, and anti-phospho-p44/42 MAPK were from New England Biolabs. Horseradish peroxidase-conjugated secondary antibodies were from Chemicon. Rabbit horseradish peroxidase-conjugated-anti-EGFP antibody was from Clontech. ECL Plus Western blotting detection system was purchased from Amersham Biosciences. MTT (3-[4,5-dimethylthia- zol-2-yl]-2,5-diphenyl-tetrazolium bromide) and actinomycin D were obtained from Sigma. All of the other materials were purchased from either Sigma or Merck-Eurolab.

Cell Culture—CESS cell line was obtained from American Type Culture Collection and grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 50 μM/mL penicillin, and 50 μg/ml streptomycin.

Reverse Transcription-PCR—Total RNA was isolated by using RNAFast reagent (Molecular Systems). Approximately 1 μg of RNA was reverse-transcribed in a total volume of 20 μl of specific buffer containing 50 ng of random hexamers, 1 mM dNTPs, 20 units of RNaseOUT (Invitrogen), and 20 units of avian myeloblastosis virus reverse transcriptase (Finnzymes) for 1 h at 37 °C. 2 μl of reverse transcription reaction was amplified by Dynazyme II DNA polymerase (Finnzymes) in the appropriate reaction buffer supplemented with 2 mM MgCl2 and 1 mM dNTPs using the following primers: 5′-GGG CAA GGT AGT GCC TGG AGT CCT TCC-3′ and 5′-GGA GAG TTC GTC AGA GTG AGG ACC-3′ for β-actin; and 5′-GGG CAA GGT AGT GCC TGG AGT CCT TCC-3′ and 5′-GGG CAA GGT AGT GCC TGG AGT CCT TCC-3′ for MKP-1, yielding a PCR products of 234 and 103 bp, respectively. PCR products were separated on 2% agarose gels with 100 bp Ladder marker (Invitrogen) and visualized with ethidium bromide staining.

Plasmid Construction and Cell Transfection—The human full-length MKP-1 cDNA sequence is amplified by PCR with primers DUSPI-FW (5′-GCT AGC AGA TCA TCC TCT GCA ACC-3′) and DUSPI-RV (5′-GCT AGC AGA TCA TCC TCT GCA ACC-3′) and DUSPI-FW (5′-GCT AGC AGA TCA TCC TCT GCA ACC-3′) and DUSPI-RV (5′-GCT AGC AGA TCA TCC TCT GCA ACC-3′) using total cDNA of CESS cell line as template. The PCR product was subcloned into pCR2.1 vector (Invitrogen), yielding the p14-DUSPI plasmid. MPE10R plasmid coding for MKP-1-EGFP fusion protein under the CMV promoter was constructed by ligating in pEGFP-C1 (Clontech) in-frame to the N terminus of EGFP, the 1.1-kb Hnt fragment from p14DUSPI containing the MKP-1 sequence. Transiently transfected cells were obtained using the LipofectAMINE reagent (Invitrogen) by following manufacturer's instructions. Catalytically impaired form (Cys258 to Ser) of MKP-1 was generated by using the QuickChange site-directed mutagenesis kit (Stratagene) with primers 5′-AGG GTG GTT GTC CAC AGC GAG GCA GCC TCC-3′ and 5′-GGA GAT GCC TGC TGT GCT GAG AAC CAC CTC-3′ and the pMEC10R plasmid as cDNA template. All of the products were sequenced by BigDye Terminator kit (Applied Biosystem).

Immunoprecipitation Analysis—For immunoprecipitation studies, 106 cells cultured in the presence of 10 μM lactacystin with or without 10 hr hrNGF were lysed in 10 mM Hepes, 142.5 mM KCl, 1 mM MgCl2, 1 mM EDTA, 50 μg/ml leupeptin, 30 μg/ml aprotinin, 1 mM phosphomethionylsulfonamide fluoride, 0.25% Triton X-100, and 200 μg/ml of actin (asayed to Bradford assay) were preclarified with protein A-Sepharose. Immunoprecipitated with 2 μg/ml rabbit anti-MKP-1 or control IgG followed by protein A-Sepharose. The immunoprecipitates were washed twice with PBS, boiled in Lmnaami sample buffer, run on 10% SDS-PAGE, blotted onto nitrocellulose filter, and stained with anti-MKP-1 antibody or anti-ubiquitin antibody. Reaction was detected with ECL.

Purification of Nuclear, Mitochondrial, and Cytosolic Fraction—106 cells were suspended in 5 mM Tris, pH 7.4, with 5 mM KCl, 1.5 mM MgCl2, and 0.1 mM EDTA, pH 8.0, containing 1 mM dithiothreitol, 0.2 mM phosphomethionylsulfonamide fluoride, 5 μg/ml leupeptin, 5 μg/ml aprotinin, and 0.7 μg/ml pepstatin for 30 min on ice, Dounce-homogenized, and centrifuged at 750 × g at 4 °C to obtain the nuclear fraction. The supernatant that was further centrifuged at 10,000 × g for 30 min at 4 °C to obtain the mitochondrial fraction (pellet) and the cytosolic fraction (supernatant). For Western blot analysis, the nuclear and mitochondrial fractions were directly lysed in sample buffer, whereas the cytosolic fraction was vacuum-concentrated and subsequently subjected in sample buffer. The purity of each fraction was assessed by staining aliquots with Abs to HSP60 for mitochondria, to actin for cytosol, and to PARP for nuclei.

Immunoblot Analysis—106 cells were lysed on ice in 20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 30 μg/ml pepstatin, 1 mM phosphomethionylsulfonamide fluoride, phosphatase inhibitor mixture (Sigma), and 0.25% Triton X-100, pH 7.4. Lysates were centrifuged at 50,000 × g for 10 min and stored at −80 °C for further analyses, and protein concentration was determined by Bradford assay. Equivalent amounts of proteins were diluted in Laemmli sample buffer, heated at 90 °C for 3 min, loaded on 8% (for PARP analysis) or 12% (for MKP-1 and p38 MAPK analysis) SDS-polyacrylamide gels, and transferred onto nitrocellulose membranes (Amersham Biosciences). Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) and incubated with 1 μg/ml of primary antibodies diluted in TBS-T containing 5% nonfat dry milk for 1 h at room temperature. After washing with TBS-T, membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody diluted 1:1000 in TBS-T containing 5% nonfat dry milk for 1 h at room temperature, rinsed with TBS-T, and developed in ECL reagent. When necessary, membranes were stripped by heating at 56 °C in 62.5 mM Tris-HCl, pH 6.7, with 100 mM 2-mercaptoethanol and 2% SDS.

Antisense Assays—CESS cells were plated in 6-well plates in serum-free medium at the concentration of 106 cells/well. Phosphorothioate oligonucleotides (MWG Biotech) were used for all of the antisense transfection experiments. Equivalent amounts of the oligonucleotides were diluted in 200 μl of serum-free medium. 5 μl of LipofectAMINE were added to each tube and incubated at room temperature for 30 min to allow LipofectAMINE reagent-DNA complex formation. The transfection mixtures were added to the cells and incubated for 5 h in serum-free medium. At this point, the cells were rinsed with TBS-T, and developed in ECL reagent for different times (24-72 h). The cultures were washed in PBS and lysed as described above. The phosphorothioate oligonucleotides used were as follows: p38 MAPK antisense, 5′-ctg TTG TTC AGC TCC tgg-3′; p38 MAPK sense, 5′-gca GGA GCT GTA CAA ggc-3′; p38 MAPK scrambled, 5′-tgc TTA GTC GTC Cgg-3′; MKP-1 antisense, 5′-cct ACT CTC ATG GAA-3′; MKP-1 sense, 5′-GTT CTA GGG Tgg ggc-3′; and MKP-1 scrambled, 5′-gca GGA GCT GTA CAA ggc-3′.

Survival Assay—Approximately 5 × 105 cells/well were seeded in triplicate onto 96-well plates in RPMI 1640 medium and incubated for 4 h with 10 μM phosphorothioate anti-MKP-1 or control oligonucleotides. NGF then was added at the final concentration of 10 nM, and cells were maintained for an additional 48 h. Detection was performed as described previously (29). MTI was added to a final concentration of 0.5 mg/ml for 4 h. 100 μl of lysis buffer (20% SDS, 50% N,N-dimethylformamide, pH 7.4) and 100 μl of isopropl alcohol were added to each well, and colorimetric reaction was measured by spectro-
CESS cells were transiently transfected with pEMC10R or vector alone in serum-free medium with 100 nM K252a. PARP cleavage induced by K252a was inhibited by SB203580. Results from one experiment of three performed are shown. CESS cells were transfected with 30 μM antisense oligonucleotides and incubated with 100 nM K252a as NGF-neutralizing agent for 6 h. The cells were then lysed and blotted with anti-PARP antibodies as a measure of caspase activation. Fig. 1, panel C, shows that antisense oligonucleotides to p38 MAPK completely abolished PARP cleavage induced by K252a incubation, whereas sense or scrambled oligonucleotides did not. The above data confirm that p38 MAPK activation is necessary in cell apoptosis induced by NGF neutralization and support the contention that endogenous NGF exerts its survival factor function through a pathway involving p38 MAPK deactivation.

NGF Induces p38 MAPK Dephosphorylation and MKP-1 Expression—Stimulation of B lymphocytes with NGF induced tyrosine phosphorylation and activation of the two isoforms, p42 and p44, of Erk (32) as well as p38 MAPK dephosphorylation (4). To study the effect of NGF on phosphorylation status of MAPK, we cultured CESS cells in serum-free conditions for 4 h and stimulated them with 10 nM hrNGF for different times (from 10 min to 1 h). The phosphorylation status of ERK, p38 MAPK, and JNK was then studied by immunoblot analysis with antibodies specific for the phosphorylated enzymes. Fig. 2 shows that, under these experimental conditions, the activated forms of p38 MAPK and ERK were detected in unstimulated cultures. Activated JNK was never detected (data not shown). NGF stimulation had opposite effects on p38 and ERK, because it promptly induced p38 MAPK dephosphorylation, whereas ERK was instead strongly activated. 1 h after NGF stimulation, p38 MAPK continued to be dephosphorylated, whereas ERK phosphorylation kept returning to the base-line level. These results suggest the action of specific MAPK phosphatases with different activity toward ERK and p38 MAPK and are consistent with data generated using memory B lymphocytes (4).
Among MAPK phosphatase family members known to deac-tivate p38 MAPK (33), MKP-1 and MKP-3 were reported to be induced by NGF in embryonic sympathetic neurons and in PC12 cells (34). Whereas MKP-3 interacts preferentially with ERK MAPK (35), it has been reported that JNK and p38 MAPKs were preferentially inactivated by MKP-1 with the following order of affinity, p38 MAPK > JNK > ERK (21). Furthermore, MKP-1 is encoded by an immediate early gene and is rapidly induced by many of the stimuli that activate MAPKs (20). For these reasons, we focused our attention on MKP-1.

To investigate whether NGF is able to activate MKP-1 gene expression, CESS cells were starved from serum for 2 h and cultured in the presence or absence of 10 nM hrNGF for different times. mRNA was extracted, and MKP-1 gene expression was analyzed by PCR with specific oligonucleotides. Fig. 3, panel A shows that MKP-1 gene was rapidly induced in CESS cells after 30 min of incubation with exogenous NGF, reached a maximum expression after 1 h, and returned to the prestimulation levels after 6 h. Pretreatment of cells with 3 μg/ml actinomycin D completely blocked the induction of MKP-1 mRNA in the presence of NGF (Fig. 3, panel B), thus indicating a transcriptional regulation, rather than mRNA stabilization mechanism. We next examined the time-dependent effect of NGF on MKP-1 protein levels. CESS cells were cultured in serum-free conditions in the presence or absence of 10 nM hrNGF for different times, lysed, and analyzed by Western blot analysis with specific antibodies. Fig. 3, panel C, shows that MKP-1 protein was constitutively expressed by CESS cells but NGF was able to increase expression up to 1.5-fold as early as 30 min after stimulation, reaching a peak (>3-fold increase) after 2 h. The increased levels of protein were stable up to 6 h after NGF stimulation. These data suggest that, in basal conditions, the production of endogenous NGF contributes to continuously sustain MKP-1 gene expression and protein synthesis.

NGF Decreases Degradation of MKP-1 through Proteasome Pathway—The early detection and the sustained levels of NGF-induced MKP-1 protein suggests that, in addition to the transcriptional regulation of MKP-1 gene expression, NGF can stabilize MKP-1 protein by inhibiting its degradation. Recently, it has been demonstrated that MKP-1 is degraded via the proteasome pathway (36). To investigate the effect of NGF on MKP-1 degradation, we culture CESS cells in the presence of the proteasome inhibitor lactacystin with or without 10 nM hrNGF for different times (1–6 h). Cells were lysed and immunoprecipitated with rabbit anti-ubiquitin antibodies or with rabbit anti-MKP-1 or rabbit IgG as control. Anti-ubiquitin-immunoprecipitated proteins were blotted and stained with anti-MKP-1 antibodies. Anti-MKP-1-immunoprecipitated proteins were blotted and stained with anti-ubiquitin antibodies. Western blot analysis was also performed with the same anti-MKP-1 antibodies to detect the level of intact MKP-1. Fig. 4 shows that ubiquitinated MKP-1 molecules were increased in untreated cultures in comparison with NGF-stimulated cultures. These results indicate that, with its combined action on mRNA as well as protein levels, endogenous NGF contributes to maintain adequate amounts of active MKP-1 in CESS cells.

Modulation of MKP-1 Protein Levels Changes the Phosphorylation Status of p38 MAPK—Because exogenous NGF increases MKP-1 synthesis and stability, we wanted to investigate whether NGF-neutralizing agents are able to modulate MKP-1 expression and whether the latter modulation is temporally related to the phosphorylation status of p38 MAPK. CESS cells were cultured in the presence of neutralizing antibodies to NGF for 12 h or with 100 nM K252 for 4 h, lysed, and blotted with anti-MKP-1 antibodies. Fig. 5, panel A, shows that incubation of cells with the above NGF-neutralizing agents inhibited the constitutive expression of MKP-1. In these experimental conditions, phosphorylation of p38 MAPK increased, whereas ERK phosphorylation decreased. Transfecting CESS cells with MKP-1-specific antisense oligonucleotides (37) yielded similar results. Fig. 5, panel B, shows that although p38 MAPK activation was increased under MKP-1 antisense oligonucleotides treatment, ERK activation was slightly down-modulated, suggesting that basal ERK activation escapes MKP-1 regulation and that another phosphatase (PP-2a) is possibly responsible for its dephosphorylation (38). However, these results clearly show that MKP-1 is involved in p38 MAPK deactivation in CESS cells and suggest its relevant role in NGF-dependent cell survival.

MKP-1 Is Involved in NGF-mediated Survival Activity—To investigate the role of MKP-1 in the survival-promoting function of NGF, we performed two different experiments. In the first series of experiments, we studied whether forced expression of MKP-1 could prevent apoptosis induced by NGF withdrawal in CESS cell line. MKP-1/EGFP and a catalytically inactive form of MKP-1/EGFP (23) were cloned in pMEC10R plasmid under the CMV minimal promoter, and CESS cells were transiently transfected with these constructs or with
EGFP alone as control. After 48 h, cells were treated with 100 nM K252a for 6 h and apoptosis of transfected fluorescent cells was recorded as percentage of PE-annexin V-positive cells by cytofluorimetric analysis. Fig. 6 shows that cells expressing the MKP-1/EGFP fusion protein did not undergo apoptosis following K252a incubation, although control-inactive MKP-1/EGFP- or EGFP-expressing cells did, indicating that forced expression of MKP-1 is able to prevent the effect of NGF neutralization on apoptosis.

In the second series of experiments, we used antisense oligonucleotides to inhibit MKP-1 gene expression and evaluated the survival of transfected cells. CESS cells were incubated in serum-free medium with antisense oligonucleotides to MKP-1 or sense or scrambled oligonucleotides as control for 72 h in the presence or absence of 10 nM hrNGF. At the end of incubation, the amount of surviving cells was quantitated by MTT uptake and reduction by spectrophotometric analysis using a 570-nm filter as described previously (29). Table I shows that NGF is able to increase the survival of untransfected CESS cells in serum-free conditions. Antisense oligonucleotides to MKP-1 strongly decreased cell survival, but the addition of NGF to antisense-transfected cells could not prevent cell death. These data strongly suggest that the transcriptional activation of MKP-1 gene is crucial in maintaining the survival of CESS cells, being part of the metabolic pathway triggered by NGF.

NGF Induces Mitochondrial Localization of MKP-1 Protein—
Mitochondrial localization of activated p38 MAPK and JNK/
SAPK, induced by appropriate stimuli, was described in memory B lymphocytes and human U937 monocytic cell line, respectively (4, 39). In this compartment, the activated enzymes bind and phosphorylate Bcl-2 or Bcl-X<sub>L</sub> protein, an event that triggers the apoptotic process. Because both p38 MAPK and JNK/SAPK represent the most relevant substrates for MKP-1 action, we asked whether MKP-1 could localize also in the mitochondrial compartment in CESS cell line. Mitochondrial, nuclear, and cytosolic fractions from 10<sup>6</sup> CESS cells, cultured in the presence or absence of 10 nM hrNGF for 30 min, were purified and blotted with anti-MKP-1 antibodies. To evaluate the purity of subcellular fractionation, the above fractions were also stained with anti-actin (cytosolic), anti-HSP60 (mitochondria), and anti-PARP (nuclear) antibodies. Fig. 7 panel A shows that, besides the nuclear compartment, MKP-1 is detected also in the mitochondrial compartment in CESS cell line, even in the absence of stimuli. However, NGF increased the amount of mitochondrial MKP-1.

To confirm these data, we transiently transfected CESS cells with a plasmid coding for a MKP-1/EGFP fusion protein or EGFP alone as control. 48 h after transfection, cells were cultured in the presence or absence of NGF for 30 min and labeled with Mitotracker (a rhodamine-like mitochondrial dye). After washing, the cells were attached on poly-L-lysine-sensitized glass slides and stained with a nuclear dye. Transfected (green fluorescent) cells were analyzed by confocal laser-scanning microscopy. Mitochondrial localization of MKP-1/EGFP fusion protein defined by yellow spots indicating overlap of fluorescein and rhodamine was strongly increased in NGF-treated cultures.

### DISCUSSION

NGF is a classical survival factor, essential for a large number of cell types, including neurons, keratinocytes, and memory B lymphocytes. In all of these cells, discontinuation of the NGF signal causes apoptotic death that critically involves mitochondria with alterations of proteins of the Bcl-2 family (3, 40–42). In memory B lymphocytes and in the lymphoblastoid CESS cell line, we described that, upon neutralization of endogenous NGF, p38 MAPK in its active form rapidly translocates onto mitochondria, specifically interacts with Bcl-2, and phosphorylates it in the Ser<sup>37</sup> and Thr<sup>38</sup> residues, an event that decreases the anti-apoptotic potential of Bcl-2, thus allowing cytochrome <em>c</em> release and apoptotic cell death (4). Bcl-2 phosphorylation by activated p38 MAPK is a crucial event in apoptosis induced by NGF neutralization because cells expressing the loop-deletional mutant cDNA construct, Bcl-2<sup>Δ40–91</sup>, were completely resistant to apoptosis induced by NGF withdrawal (1). The role of activated p38 MAPK in Bcl-2 phosphorylation and apoptosis caused by NGF neutralization was further demonstrated by its ability to induce cytochrome <em>c</em> release in isolated mitochondria and by inhibition of cell death operated by p38 MAPK inhibitors or p38 MAPK-specific antisense oligonucleotides (data reported in Fig. 1) (4).

Conversely, when NGF is added to factor-starved cells, p38 MAPK is promptly deactivated and, hence, Bcl-2 phosphorylation is inhibited (4). In this paper, we characterize the NGF-induced phosphatase MKP-1, which is responsible for active p38 MAPK dephosphorylation in CESS cell line and which plays a relevant role in NGF-mediated autocrine circuit of survival. This NGF-induced phosphatase is a dual specificity phosphatase that selectively dephosphorylates tyrosine and threonine residues on MAPKs, being particularly active toward...
p38 MAPK and JNK/SAPK substrates (21). In fact, the addition of NGF to serum-starved cell cultures induces a rapid dephosphorylation of p38 MAPK, which is maintained up to 1 h after NGF stimulation. Conversely, ERK phosphorylation, induced by upstream kinases activated by Trk-A signaling, starts to decrease 1 h after NGF stimulation. We interpreted the above data as the result of an NGF-induced MAPK phosphatase with greater affinity toward p38 MAPK than ERK and hypothesized that MKP-1 was the most probable candidate.

The up-regulation of MKP-1 gene expression induced by NGF was reported also in other cellular systems, such as dissociated embryonic sympathetic neurons or fibroblasts transfected with the high affinity NGF receptor Trk-A (34). It is believed that activation of Trk-A is crucial for MKP-1 gene expression, independently of the cellular origin or type on which the Trk-A receptor is active (34). NGF increases MKP-1 protein levels through both an active transcriptional mechanism and a decrease of protein degradation. The induction of MKP-1 mRNA by NGF was rapid (within 30 min) and completely blocked by actinomycin, consistent with data reported in other experimental systems (37, 43). In fact, MKP-1 is considered an early response gene (20) whose transcriptional activity is controlled via a strong block of elongation in the exon I of the gene (44). Epidermal growth factor is able to remove this block in neuroendocrine cells (44), and activation of the p42/p44 MAPK cascade as well as that of protein kinase Ce is involved in the induction of MKP-1 gene (27, 44). In B lymphocytes and in lymphoblastoid cell lines, the autophosphorylation of Trk-A induced by NGF strongly activates the p42/p44 MAPK cascade (our data and Ref. 45), delineating a pathway for MKP-1 gene induction. Consistently, a specific Trk-A inhibitor is able to block MKP-1 gene expression (Fig. 5).

Although MKP-1 mRNA reached a peak after 1 h and returned to basal level after 2 h of stimulation with NGF, the increased levels of MKP-1 protein, evident already 30 min after stimulation, were stable for several hours. It is known that MKP-1 is degraded through the classical proteasome pathway (36, 46) and the reported half-life of MKP-1 is not longer than 45 min (36). The stable (up to 6 h) levels of MKP-1 protein induced by NGF stimulation are related to a reduced ubiquitin binding and degradation of MKP-1 protein, probably because of its phosphorylation, again possibly operated by ERK, as suggested by Brondello et al. (36) and Chen et al. (47). This latter point is actually under investigation in our laboratory.

Previous reports show the importance of MKP-1 in the regulation of apoptosis in various cells (48). For example, spontaneous or conditional expression of MKP-1 in cancer cell models is paralleled by their resistance to apoptosis (48–50). MKP-1 was also involved in the anti-apoptotic effect of retinoids in mesangial cells incubated with H2O2 (43) as well as in the case of MKP-1, namely ERK, JNK, and p38 MAPK, were found in the mitochondrial compartment (4, 39, 52). The mitochondrial carriers of these kinases are not completely defined. An adapter protein, Sab (SH3BP5), was described as able to address JNK to mitochondria (53), but it can be hypothesized the involvement of Grb proteins (52), possibly shuttling between plasma membrane receptors and the apoptosis-operating machinery whose activity is modulated through phosphorylation/dephosphorylation of proteins involved in maintaining outer membrane mitochondrial permeability. However, we cannot rule out the involvement of other adapter proteins, such as HSP, able to transport kinases/phosphatases to mitochondria (53). Recently, the role of JNK and p38 MAPK on mitochondria has been defined in more detail and, besides Bcl-2/Bcl-XL phosphorylation, activation of other pro-apoptotic members of Bcl-2 family (Bim, Bmp) has been reported previously (54) as well as phosphorylation of other ill-defined mitochondrial proteins (55).

The final result of JNK and p38 MAPK mitochondrial localization is the release of cytochrome c and SMAC/DIABLO (4, 39, 56). By contrast, ERK localization on mitochondria was instead reported as related to Bad phosphorylation through interaction with PKCe, resulting in a mitochondrial activity likely to be an inhibition of cell apoptosis (52). Although we do not know how ERK is transported to mitochondria (see above), it is conceivable that this compartmentalization increases in those conditions such as NGF stimulation, leading to strong ERK activation. MKP-1 translocation, in turn, can be driven by high concentrations of its substrate ERK. Post-translational modifications probably influence the interaction of MKP-1 with different mitochondrial proteins as suggested by preliminary results obtained in our laboratory, indicating that NGF induces a down-modulation of MKP-1 tyrosine phosphorylation. However, MKP-1 mitochondrial localization in NGF-stimulated cultures supports the hypothesis that the enzyme is ideally suited for dephosphorylation of p38 MAPK molecules activated following stimuli not strictly related to apoptosis induction. In other words, mitochondrial MKP-1 can play a “sentinel” role toward potentially dangerous activated p38 MAPK or JNK molecules.

This hypothesis is strengthened by the occurrence of NGF-induced MKP-1 mitochondrial localization as well as in other cellular systems in which NGF performs an autocrine survival function, such as keratinocytes.

REFERENCES

1. Rosini, P., De Chiara, G., Lucibello, M., Garaci, E., Cozzolino, F., and Torcia, M. (2000) Biochem. Biophys. Res. Commun. 278, 753–759
2. Ridderstad, A., and Tarlinton, D. M. (1998) J. Immunol. 160, 4688–4695
3. Torcia, M., Bracci-Laquis, L., Lucibello, M., Nencioni, L., Labardi, D., Roderelli, A., Cozzolino, F., Aloe, L., and Garaci, F. (1999) Cell 88, 345–356
4. Torcia, M., De Chiara, G., Nencioni, L., Ammendola, S., Labardi, D., Lucibello, M., Rosini, P., Marlier, L. N., Bonini, P., Dello, S. P., Palmera, A. T., Zambrano, N., Russo, T., Garaci, E., and Cozzolino, F. (2001) J. Biol. Chem. 276, 39097–39106
5. Bilagkoslonny, M. V., Giannakakou, P., el Deiry, W. S., Kingston, D. G., Higgs, P. I., Neckers, L., and Pojo, T. (1997) Cancer Res. 57, 130–135
6. Chen, C. Y., and Fuller, D. V. (1996) J. Biol. Chem. 271, 2376–2379

3 P. Rosini, G. De Chiara, P. Bonini, M. Lucibello, F. Cozzolino, and M. Torcia, manuscript in preparation.
Survival Activity of NGF Is Mediated by Induction of MKP-1

10. Pathan, N., Aime-Stempel, C., Kitamura, S., Haldar, S., and Reed, J. C. (2001) Neoplasia 3, 70–79
11. Srivastava, R. K., Mi, Q. S., Hardwick, J. M., and Longo, D. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3775–3780
12. Thomas, A., Giesler, T., and White, E. (2000) Oncogene 19, 5259–5269
13. Ojala, P. M., Yamamoto, K., Castanos-Velez, E., Biberfeld, P., Korsmeyer, S. J., and Malek, T. P. (2000) Nat. Cell Biol. 2, 819–825
14. Yamamoto, K., Ichijo, H., and Korsmeyer, S. J. (1999) Mol. Cell. Biol. 19, 8469–8478
15. Blagosklonny, M. V. (2001) Leukemia 15, 869–874
16. Whitmarsh, A. J., and Davis, R. J. (1999) Science’s STKE 1, RE1–RE6
17. Hunter, T. (1995) Cell 80, 225–236
18. Sun, H., and Tonks, N. K. (2000) Trends Biochem. Sci. 25, 2538–2542
19. Liu, Y., Gorospe, M., Yang, C., and Holbrook, N. J. (1995) J. Biol. Chem. 270, 8377–8380
20. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993) Cell 75, 487–493
21. Muda, M., Boschert, U., Dickinson, R., Martinou, J. C., Martinou, I., Camps, M., Schlegel, W., and Arkinastll, S. (1996) J. Biol. Chem. 271, 4319–4326
22. Korsmeyer, S. J., Antonsson, B., Magnenat, E., Camps, M., Muda, M., Chabert, C., Gillieron, C., Boschert, U., Vial-Knecht, E., Martinou, J. C., and Arkinastll, S. (1997) J. Biol. Chem. 272, 25238–25242
23. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993) Curr. Opin. Cell Biol. 5, 256–267
24. Peinado-Ramon, P., Wallen, A., and Hallbo, F. (1998) Brain Res. Mol. Brain Res. 56, 256–267
25. Keyse, S. M., and Emslie, E. A. (1992) Nature 359, 644–647
26. Rohan, P. J., Davis, P., Moskaluk, C. A., Kearns, M., Krutzsch, H., Siebenlist, U., and Kelly, K. (1993) Science 259, 1763–1766
27. Brondello, J. M., Brunet, A., Pouyssegur, J., and McKenzie, F. R. (1997) J. Biol. Chem. 272, 1368–1376
28. Kiemer, A. K., Weber, N. C., Furst, R., Bildner, N., Kulhanek-Heinze, S., and Vollmar, A. M. (2002) Circ. Res. 90, 874–881
29. Shearman, R. L., and Iversen, L. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1470–1474
30. Knusel, B., and Hefti, F. (1992) J. Neurochem. 59, 1987–1996
31. Lee, J. C., Kassis, S., Kumar, S., Badger, A., and Adams, J. L. (1999) Pharmacol. Ther. 82, 389–397
32. Frankin, R. A., Bredie, C., Melamed, I., Terada, N., Lucas, J. J., and Gelfand, E. W. (1995) J. Immunol. 154, 4965–4972
33. Keyse, S. M. (2000) Curr. Opin. Cell Biol. 12, 186–192
34. Peinado-Ramon, P., Wallen, A., and Hallbo, F. (1998) Brain Res. Mol. Brain Res. 56, 256–267
35. Camps, M., Nichole, A., Gillieron, C., Antonsson, B., Muda, M., Chabert, C., Boschert, U., and Arkinastll, S. (1998) Science 280, 1262–1265
36. Brondello, J. M., Pouyssegur, J., and McKenzie, F. R. (1999) Science 286, 2514–2517
37. Duff, J. J., Monia, B. P., and Berk, B. C. (1995) J. Biol. Chem. 270, 7161–7166
38. Jacob, A., Molkentin, J. D., Smolenski, A., Lohmann, S. M., and Begum, N. (2002) Am. J. Physiol. 283, C704–C713
39. Kharbanda, S., Saxena, S., Yoshida, K., Pandey, P., Kaneki, M., Wang, Q., Cheng, K., Chen, Y. N., Campbell, A., Sudha, T., Yuan, Z. M., Narula, J., Weichselbaum, R., Nalin, C., and Kufe, D. (2000) J. Biol. Chem. 275, 322–327
40. Greenlund, L. J., Korsmeyer, S. J., and Johnson, E. M., Jr. (1995) Neuron 15, 649–661
41. Kato, S., Mitsu, Y., Kitani, K., and Suzuki, T. (1996) Biochem. Biophys. Res. Commun. 229, 653–657
42. Pincelli, C., and Marceni, A. (2000) J. Dermatol. Sci. 22, 71–79
43. Xu, Q., Kenta, T., Furuse, A., Nakayama, K., Lucio-Cazana, J., Fine, L. G., and Kitamura, M. (2002) J. Biol. Chem. 277, 41683–41700
44. Ryser, S., Tortola, S., van Haasteren, G., Muda, M., Li, S., and Schlegel, W. (2001) J. Biol. Chem. 276, 33319–33327
45. Franklin, R. A., Bredie, C., Melamed, I., Terada, N., Lucas, J. J., and Gelfand, E. W. (1995) J. Immunol. 154, 4965–4972
46. Kassel, O., Sancono, A., Krtzschmar, J., Kref, B., Stassen, M., and Cato, A. C. (2001) EMBO J. 20, 7108–7116
47. Chen, P., Li, J., Barnes, J., Kokkonen, G. C., Lee, J. C., and Liu, Y. (2002) J. Immunol. 168, 6468–6416
48. Magi-Galluzzi, C., Mishra, R., Fiorentino, M., Montironi, R., Yao, H., Capodici, P., Wishnow, K., Kaplan, I., Stork, P. J., and Loda, M. (1997) Lab. Invest. 76, 37–51
49. Srikantan, S., Franklin, C. C., Duke, R. C., and Kraft, R. S. (1999) Mol. Cell Biochem. 199, 169–178
50. Franklin, C. C., Srikantan, S., and Kraft, A. S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3014–3019
51. Guo, Y. L., Kang, B., and Williamson, J. R. (1998) J. Biol. Chem. 273, 10362–10366
52. Baines, C. P., Zhang, J., Wang, G. W., Zheng, Y. T., Xu, J. X., Cardwell, E. M., Bolli, R., and Png, P. (2002) Circ. Res. 90, 390–397
53. Wiltshire, C., Matsushita, M., Tsukada, S., Gillespie, D. A., and May, G. H. (2000) J. Biol. Chem. 275, 2437–2440
54. Lei, K., and Davis, R. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2432–2437
55. Wiltshire, C., Matsushita, M., Tsukada, S., Gillespie, D. A., and May, G. H. (2002) J. Biol. Chem. 277, 577–585
56. Lei, K., and Davis, R. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2432–2437
57. Schroeter, H., Boyd, C. S., Ahmed, R., Spencer, J. P., Duncan, R. F., Rice-Evans, C., and Cadenas, E. (2003) Biochem. J. 372, 359–369
58. Chauhan, D., Li, G., Hideshima, T., Fodar, K., Mitsiades, C., Mitsiades, N., Munshi, N., Kharbanda, S., and Anderson, K. C. (2003) J. Biol. Chem. 278, 17593–17596

Downloaded from http://www.jbc.org/ by guest on December 29, 2019
Nerve Growth Factor-dependent Survival of CESS B Cell Line Is Mediated by Increased Expression and Decreased Degradation of MAPK Phosphatase 1
Paolo Rosini, Giovanna De Chiara, Paolo Bonini, Maria Lucibello, Maria Elena Marcocci, Enrico Garaci, Federico Cozzolino and Maria Torcia

J. Biol. Chem. 2004, 279:14016-14023.
doi: 10.1074/jbc.M305356200 originally published online January 14, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M305356200

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 55 references, 31 of which can be accessed free at http://www.jbc.org/content/279/14/14016.full.html#ref-list-1