Differential Regulation of the Dual-Specificity Protein-Tyrosine Phosphatases CL100, B23, and PAC1 in Mesangial Cells

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Abstract. The extracellular-signal-regulated kinase (ERK), the best described MAP kinase cascade, is a major signaling system by which cells transduce extracellular cues into intracellular responses. ERK is activated by phosphorylation both on tyrosine and threonine residues. Therefore, a new class of protein-tyrosine phosphatases (PTPases) that exhibit dual catalytic activity toward both regulatory sites on ERK is of special interest in the control of intracellular signaling. This study examined the expression and regulation of the dual-specificity PTPases CL100, B23, and PAC1. Findings included differential expression of these phosphatases in diverse cell lines and an expression of all three dual-specificity PTPases in human mesangial cells (HMC), thereby allowing investigation of their regulation in a single cell line. The MEK antagonist PD 098059 and selective extracellular agonists of ERK were used to demonstrate the induction of CL100, PAC1, and B23 in response to activation of the ERK cascade. In contrast, anisomycin, an agonist of the recently described MAP kinases stress-activated protein kinase (SAPK) and p38 MAP kinase, stimulated CL100 gene expression but had little effect on PAC1 and B23. This effect of anisomycin was partly inhibited in the presence of the p38 MAP kinase antagonist SB 203580. This study suggests a potential mechanism to regulate ERK activity through feedback inhibition by demonstrating the ERK cascade's induction of the dual-specificity PTPases CL100, PAC1, and B23. Moreover, this study suggests an ERK-independent induction of CL100 following stimulation of SAPK and p38 MAP kinase. This mode of induction of a phosphatase capable of inactivating ERK may play an important role in the cellular stress response. (J Am Soc Nephrol 8: 40-50, 1997)
mRNA because dual-specificity PTPases are regulated at the transcriptional level (17). Recently, we described the induction of MKP-1 (the mouse homologue of CL100) gene expression in murine fibroblast (NH3T3) cells by the stress-activated protein kinase (SAPK) cascade (18), a MAP kinase pathway thought to induce cell growth inhibition and apoptosis (19,20–22). In contrast, activation of ERK inhibited MKP-1 expression (18). In this study, we therefore evaluated the role of different MAP kinase cascades in the regulation of the dual-specificity PTPases CL100, PAC1, and B23 in a primary cell line. We found all three VH-I-like PTPases expressed in HMC, allowing us to investigate the regulation of these genes in the same cellular environment. Employing a selective antagonist of MEK, as well as extracellular stimuli that selectively activate the ERK or the SAPK cascade, we demonstrated the induction of CL100, PAC1, and B23 expression by activation of the MEK-ERK module, suggesting a feedback inhibition of ERK to control its activity. In addition, CL100 was inducible in response to activation of stress-response pathways like SAPK or p38 MAP kinase, a third mammalian MAP kinase (23) that, like SAPK, responds to extra- and intracellular stress stimuli. This mode of CL100 induction may play an important role in the stress response of mesangial cells following activation of the SAPK or p38 MAP kinase signaling pathways. Furthermore, this study demonstrates differential expression and regulation of CL100, PAC1, and B23 in diverse cell types pointing to unique roles for these dual-specificity PTPases in intracellular signaling in mesangial cells.

Materials and Methods

**Materials**

Endothelin-1 and leupeptin were purchased from Peptide Institute (Osaka, Japan). The ECL (enhanced chemiluminescence) system was obtained from Amersham (Arlington Heights, IL), RPMI 1640 and Dulbecco modified Eagle medium (DMEM) cell culture media from Gibco (Grand Island, NY). Cycloheximide was purchased from Calbiochem (La Jolla, CA) and [32P]dCTP from DuPont (NEN Research Products, Boston, MA). Phenylmethylsulfonylfluoride (PMSF), heparin, MCDB 105, phorbol 12-myristate 13-acetate (TPA), anisomycin and all other reagents were obtained from Sigma Chemical (St. Louis, MO). Endothelial cell growth supplement was purchased from Collaborative Research (Waltham, MA). Protein A-Sepharose was obtained from Pharmacia Biotech (Piscataway, NJ). GST-ATF2 (1–55) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Cell Culture**

Glomerular mesangial cells from male Spraque-Dawley rats were isolated and characterized as reported previously (24). Human glomerular mesangial cell cultures were established from glomeruli isolated and characterized as previously reported (25). Mesangial cells were maintained in RPMI 1640 medium supplemented with 17% fetal bovine serum (FBS), 100 U/mL of penicillin, 100 µg/mL of streptomycin, 5 µg/mL each of insulin and transferrin, and 5 ng/mL of selenite at 37°C in a 5% CO2/95% air mixture. Mesangial cells in the passage 5–10 were employed in this study. NH3T3 cells were grown in DMEM medium supplemented with 10% calf serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. A7r5 cells, rat vascular smooth muscle cells, and A549 human lung epithelial carcinoma cells were cultured in DMEM-low glucose medium supplemented with 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Human Jurkat T-lymphocytes were grown in suspension in DMEM-low glucose medium. Human umbilical vein endothelial cells (HUVEC) were obtained by primary culture of cells harvested from umbilical cords using the technique of Lewis et al. (26). HUVEC were grown in fibronectin-coated tissue culture flasks (1 µg/cm²) in MCDB 107 medium supplemented with 20% FBS, 30 µg/mL of endothelial cell growth supplement, 90 µg/mL of heparin, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Cells were made quiescent for 24 hours in the respective serum-free media prior to stimulation with various agonists. The media used to induce quiescence in HUVEC contained 1% FBS without heparin or endothelial cell growth supplement. Prior to stimulation with ultraviolet light, the medium was removed and saved. Cells were washed once with phosphate-buffered saline (PBS), stimulated, and reincubated in the original medium for different periods of time.

**RNA Extraction and Northern Blot Analysis**

Total cellular RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform procedure (27). The quality of RNA was assessed by measuring the ratio of 28S:18S rRNA (2:1) in ethidium-bromide-stained denaturing agarose gels. For Northern analysis, RNA was denatured by heating for 15 min at 65°C in 0.02 M 3-[N-morpholino]propanesulfonic acid, 6.6% formaldehyde, and 50% formamide and fractionated by electrophoresis in a 1.2% agarose gel. The RNA was transferred to nitrocellulose and hybridized with CL100 cDNA (generous gift of S. M. Keyse, Dundee, UK), human PAC1 cDNA (10) (generous gift of K. Kelly, Bethesda, MD), or B23 cDNA (8) (generous gift of D.P. Bottaro) labeled by nick translation with [32P]dCTP. The hybridization buffer contained 50% formamide, 5 x salt sodium phosphate EDTA (SSPE), 0.5% sodium dodecyl sulfate (SDS), 10% dextran sulphate, 1 x Denhardt's, and 110 µg/mL salmon testes DNA. After hybridization overnight, the membranes were washed twice with 2 x SSPE-0.1% SDS at 20°C for 10 min and twice with 0.1 x SSPE-0.1% SDS at 42°C for 20 min (CL100 and PAC1) or twice with 0.025 x SSPE-0.1% SDS at 65°C for 20 min (B23). Cellulose membranes were exposed to Fuji RX films at -70°C with intensifying screens. After stripping, each blot was rehybridized with a nick-translated GAPDH cDNA probe. Autoradiograms are from representative experiments.

**Antibodies**

Monoclonal anti-PAC1 antibodies, P9D10 (generous gift of K. Kelly, Bethesda, MD) (12), and the polyclonal antibodies against ERK and SAPK were raised against the C-terminal peptide of either p42 ERK (28) or p46 SAPK (Santa Cruz Biotechnology, Santa Cruz, CA). The anti-CL100 antibody was produced by immunizing rabbits with a synthetic peptide, CALSYLQSPITTSP (the bold letters correspond to residues 353–366 in the carboxy-terminal segment of CL100). The cysteine at the amino-terminal end of the peptide was added for conjugation to ovalbumin.

**Western Blot Analysis**

Confluent HMC were washed with ice-cold PBS and lysed in 400 µl Triton X-100 lysis buffer of 50 mM N-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) pH 7.5, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 1 µg/mL aprotinin, 1 µg/mL leupeptin, and 1 mM PMSF at 4°C. After 5 min, cells were scraped with a cell lifter and centrifuged at 4°C for 15 min at 10,000 g. The soluble cell lysates were mixed 1:4 with 5 x Laemmli buffer and heated for 5 min at 95°C. Soluble mesangial cell lysates (80 µg) were loaded per lane and separated by SDS-PAGE using 4% and
10% acrylamide for stacking and resolving gels, respectively. Protein was transferred to nitrocellulose (pore size: 0.45 μm, Schleicher and Schuell, Keene, NH) and probed with monoclonal anti-PAC1 antibody, P9D10 (12), or with polyclonal antibodies against the C-terminal peptide of either p42 ERK (28), p46 SAPK (Santa Cruz Biotechnology, Santa Cruz, CA), or CL100 (see above). The primary antibodies (diluted 1/1000) were detected using horseradish peroxidase-conjugated rabbit anti-mouse IgG visualized by the Amersham ECL system after intensive washing of the membranes.

**Immunoprecipitation**

Confluent HMC or 7.5 × 10^6 Jurkat cells were washed with ice cold PBS and lysed in Triton X-100 lysis buffer (as described above) for 5 min at 4°C. Insoluble material was removed by centrifugation at 10,000 g for 15 min at 4°C. HMC lysates (500 μg protein) or Jurkat cell lysates were incubated for 2 h with 1 μl monoclonal anti-PAC1 antibody (P9D10) (12), 2 μl polyclonal anti-p42 ERK antibody (28), or 2 μl polyclonal anti-CL100 antibody (as described above). Immunocomplexes were adsorbed to protein G-Sepharose and washed three times with lysis buffer. Proteins were resolublized by addition of an equal volume of 2X Laemmli buffer and were detected by Western blot analysis as described above.

**Kinase Activity Assays**

500 μg of soluble cell lysates (as described above) were incubated for 90 min with 2 μl of polyclonal antibody recognizing p42 ERK (28) or 2 μg of polyclonal antibody recognizing p46 SAPK (Santa Cruz). Immunocomplexes were adsorbed to protein A-Sepharose, washed twice with lysis buffer and twice with kinase buffer (10 mM MgCl₂, 20 mM HEPES, pH 7.4, containing 200 μM Na-orthovanadate), and resuspended in 60 μl of kinase buffer containing 50 μM adenosine triphosphate (ATP) and 5 μCi [γ-32P]ATP. The final reaction buffer also contained 15 μg of myelin basic protein (MBP) or 4 μg of glutathione-S-transferase activating transcription factor 2 fusion protein (GST-ATF2) for ERK or SAPK activity assay, respectively. The reaction was initiated by incubation at 30°C for 15 min. Thereafter, 20 μl of 4X Laemmli buffer was added to terminate the reaction, and samples were subjected to SDS-PAGE.

**Results**

We examined the mRNA expression of CL100, PAC1, and B23 by Northern blot analysis in several cell lines to find a cell line that allows a direct comparison of the regulation of multiple VH-1-like phosphatases in the same cellular environment (Figure 1). We investigated the mRNA expression of these VH-1-like PTPases in Jurkat T-lymphocytes because PAC1 has been shown as the dual-specificity PTPase of the hematopoietic tissue (10,29). We found PAC1 mRNA (1.8 kilobases in length) expressed after activation of quiescent Jurkat cells with phytotohemagglutinin (1 μg/mL) and PMA (20 ng/mL). In addition, B23 mRNA (2.6 kilobases in length) was expressed in activated T-lymphocytes, whereas CL100 mRNA was not detectable (Figure 1). Furthermore, we examined the expression of dual-specificity PTPases in nonhematopoietic cell lines and detected PAC1 mRNA in HMC and HUVEC after stimulation of quiescent cells with 17% FBS (Figure 1). As shown in Figure 1 and summarized in Table 1, B23 mRNA was expressed in all examined cell lines, whereas CL100 mRNA (2.2 kilobases in length) was detectable in all nonhematopoietic cell lines. These data provide evidence for a cell line-specific and tissue-specific expression of VH-1-like PTPases and raised the question as to whether multiple dual-specificity PTPases found in one cell line exhibit distinct functions.

The expression of three different VH-1-like phosphatases in HMC enabled us to compare the pattern of expression of these genes in one cell line. We observed moderate but significant differences in the time responses of mRNA expression after stimulation of quiescent mesangial cells with 17% FBS, sug-
ggesting that different regulatory pathways are involved in the regulation of these genes. As shown in Figure 2A and 2B, CL100 is the earliest expressed dual-specificity PTPase in HMC. PAC1 exhibits the most transient expression, whereas B23 showed a more prolonged expression. In addition, all three phosphatases behave like immediate early response genes, since their expression is not inhibited in the presence of cycloheximide (20 μg/mL) (Figure 2A). Moreover, CL100, PAC1, and B23 were superinducible in the presence of cycloheximide (Figure 2A). Direct effects of cycloheximide on intracellular signaling pathways like the ERK (30,31, Figure 5) cascade rather than the ERK cascade induces MKP-1/CL100 antagonists of either MEK or of p38 MAP kinase to evaluate the role of MAP kinase signaling pathways in the regulation of three different dual-specificity PTPases in a primary cell line and to specify the differences of expression of CL100, B23, and PAC1 after induction with serum, we stimulated HMC with extracellular stimuli that activate either the ERK or the SAPK pathway. Because of a very low transfection efficiency and loss of the characteristics of HMC within early passages, we were not able to perform transfection studies in this primary cell culture. Therefore, in addition we used recently described synthetic antagonist of either MEK or of p38 MAP kinase to evaluate the role of MAP kinase cascades in the regulation of CL100, PAC1, and B23 in HMC.

Addition of TPA induced a rapid and strong activation of ERK, detected by the electrophoretic retardation that indicated phosphorylated protein forms and a sixfold increase of MBP phosphorylation after 10 and 30 min compared with unstimulated cells (Figure 6A). In contrast, anisomycin induced only a weak and transient activation of ERK (1.3 fold increase of MBP phosphorylation at 30 min compared with unstimulated cells) (Figure 6A). Anisomycin potently activated SAPK (8.4 fold increase of ATF2 phosphorylation after 30 min), whereas TPA was only a weak stimulator of SAPK (Figure 6B). TPA, like FBS, potently induced the expression of all PTPases examined (Figure 6C), suggesting that the ERK cascade induces the expression of CL100, PAC1, and B23 in HMC. In accordance with this finding, platelet-derived growth factor and epidermal growth factor, agonists known to activate the ERK pathway (17,28,35), also stimulated the expression of CL100, B23, and PAC1 effectively (data not shown). Anisomycin potently induced the expression of CL100 but had only a weak stimulatory effect on the gene expression of PAC1 and B23 (Figure 6C). These data suggest that the expression of different dual-specificity phosphatases is inducible by distinct

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<th>Table 1. Summary of the differential gene expression of dual specificity PTPases CL100, PAC1, and B23 as shown in Figure 1</th>
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We (18) and others (11) showed previously that the down-regulation of ERK in NIH3T3 cells is dependent on the production of new protein, presumably of the expression of transcriptionally regulated dual-specificity PTPases like CL100, PAC1, and B23. However, this may not be the case in all cell systems. The inactivation of ERK following mitogenic stimulation of chromaffin cells (PC12), adipose cells (3T3-L1), or endothelial cells occurs normally when protein synthesis is inhibited (33,34). To investigate the relevance of inducible genes in the downregulation of ERK in mesangial cells, we examined the time curve of ERK activity after mitogenic stimulation in the presence and absence of the protein synthesis inhibitor, cycloheximide. As shown in Figure 5, FBS induced a rapid activation of ERK followed by a slow inactivation over 6 h, whereas in the presence of cycloheximide, FBS induced a sustained activation of ERK. The extent of MBP phosphorylation 240 and 360 min after stimulation was increased by 32% and 75%, respectively, in the presence of cycloheximide. Thus, the synthesis of new protein, presumably of a dual-specificity PTPase, is required to dephosphorylate ERK in HMC.

Recently, we have shown in NIH3T3 cells that the SAPK cascade rather than the ERK cascade induces MKP-1/CL100 expression in NIH3T3 cells (23). To examine the role of MAP kinase signaling pathways in the regulation of three different dual-specificity PTPases in a primary cell line and to specify the differences of expression of CL100, B23, and PAC1 after induction with serum, we stimulated HMC with extracellular stimuli that activate either the ERK or the SAPK pathway. Because of a very low transfection efficiency and loss of the characteristics of HMC within early passages, we were not able to perform transfection studies in this primary cell culture. Therefore, in addition we used recently described synthetic antagonist of either MEK or of p38 MAP kinase to evaluate the role of MAP kinase cascades in the regulation of CL100, PAC1, and B23 in HMC.
Figure 2. Time course of CL100, B23, and PAC1 mRNA expression after stimulation in HMC and effect of cycloheximide on mRNA levels. (A) HMC brought to quiescence by serum deprivation were stimulated to reenter the G1 phase by 17% serum. Total RNA was isolated at indicated times after stimulation and examined by Northern blot analysis. The blots were sequentially probed with CL100, B23, and PAC1 cDNA. As a loading control, the same filters were stripped and rehybridized with the GAPDH probe. One representative Northern blot analysis of three independent experiments is shown. Incubation of the cells for 1 h with cycloheximide (20 μg/mL) prior to stimulation with serum did not inhibit the gene expression of CL100, B23, or PAC1. The same results were obtained in three independent experiments. (B) Hybridization signals shown in Figure 2A were quantified by scanning densitometry using National Institute of Health IMAGE software and normalized to the glyceraldehyde phosphate dehydrogenase (GAPDH) signal.

Figure 3. PAC1 protein expression. (A) Jurkat cells and HMC were stimulated for 90 min with phytohaemagglutinin (1 μg/mL) and PMA (10 ng/mL) or 17% FBS, respectively. Protein was immunoprecipitated (IP) using the monoclonal anti-PAC1 antibody P9D10 (11). Alternatively, the cell lysate was analyzed by SDS-PAGE (L). The whole blot was immunoblotted with the same monoclonal antibody. The band correlating to the expected size of 32 kDa of the PAC1 protein is indicated by the arrow. (B) Time course of PAC1 protein (indicated by the arrow) expression after stimulation of quiescent HMC with 17% FBS. Whole cell lysates were analyzed by gel-electrophoresis and Western blot using anti-PAC1 antibody P9D10 and the ECL detection system.
intracellular signaling pathways, thus providing more evidence for unique roles of CL100, B23, or PAC1 in intracellular signaling. Because anisomycin is known to activate p38 MAP kinase (36), we examined the effect of the p38 MAP kinase antagonist, SB 203580 (generous gift from Dr. J.C. Lee) (37), on the gene expression of CL100, PAC1, and B23 to define the role of p38 MAP kinase in anisomycin induced CL100 induction. The antagonist alone did not affect the expression of either one of these PTPases and also did not affect the FBS-induced gene expression of CL100, PAC1, and B23, suggesting that the FBS-induced expression of these PTPases results from an intracellular pathway distinct from the p38 MAP kinase cascade (Figure 6C). However, the anisomycin-induced expression of CL100 was inhibited after preincubation with SB 203580, indicating that the anisomycin-induced expression of CL100 is in part the result of p38 MAP kinase activation.

To examine the role of ERK cascade in the regulation of the dual-specificity phosphatases CL100, PAC1, and B23 in more detail, we performed experiments employing the MEK antagonist PD 098059 (generous gift from Dr. A.R. Saltiel) (38). PD 098059 inhibited the FBS-induced ERK activation in HMC by 50%, as detected by the reduction of the FBS-induced band shift of ERK and the inhibition of ERK activity assayed in an immunocomplex kinase assay (Figure 7A). As shown in Figure 7B, inhibition of ERK activation by PD 098059 significantly reduced the FBS-induced gene expression of CL100, PAC1, and B23 in HMC. Therefore, based on the effects of the MEK
inhibitor and extracellular stimuli that activate the ERK signaling pathway rather than the SAPK cascade, our data suggest that the ERK cascade induces the expression of CL100, PAC1, and B23 in HMC. Because all three dual-specificity PTPases were shown to dephosphorylate and inactivate ERK, this mode of regulation illustrates a potential mechanism to maintain balanced cell growth through feedback inhibition.

**Discussion**

The specific roles of dual-specificity kinases (4,20,39–41), the selective upstream kinases of different MAP kinases, leads to speculation that working in parallel, distinct members of the group of dual-specificity phosphatases also serve unique roles in intracellular signal transduction. Possible mechanisms of maintaining a specific role of dual-specificity PTPases include differential and tissue-specific expression. In accordance with this hypothesis, PAC1 was described as a dual-specificity PTPase of the hematopoietic tissue (10,16,29). Furthermore, we found in this study a differential expression of CL100, B23, and PAC1 in distinct cell lines, indicating tissue-specific expression of dual-specificity PTPases. However, we detected at least two dual-specificity PTPases in each cell line examined. In correlation with our data, Kwak and Dixon (16) recently described a coexpression of VHR and B23 in liver cell lines. We also detected PAC1 mRNA and protein expression in two nonhematopoietic cell lines, HMC, and HUVEC.

An alternative mechanism for control of distinct dual-specificity PTPases could be through differential regulation. The expression of PAC1, CL100, and B23 in HMC allowed us a direct comparison of the regulation of these genes in a single cell line without influence of cell line-specific differences. We detected distinct kinetics of PAC1, CL100, and B23 gene expression after stimulation of quiescent HMC with serum, suggesting that different intracellular signaling pathways regulate the gene expression of dual-specificity PTPases. Recently, in PC12 cells the duration of ERK activation by extracellular stimuli was shown to be critical for cell signaling outcomes because transient activation of MAP kinase induced mitogenesis, whereas sustained activation of MAP kinase induced cell differentiation (42,43). Furthermore, only potent growth factors seem capable of inducing a sustained phase of ERK activation in mesangial cells and fibroblasts (28,44). These data emphasize the importance of mechanisms to terminate the activity of ERK.

We demonstrate in this study a decline of ERK activity following stimulation with FBS over a time period of 6 h, indicating the sustained requirement of phosphatase activity toward ERK. Therefore, it is unlikely that a single, transiently expressed dual-specificity PTPase is sufficient for ERK down-regulation. Moreover, we show in this study a prolonged ERK activation in the presence of the protein synthesis inhibitor cycloheximide, emphasizing the requirement of transcriptionally regulated phosphatases, presumably the dual-specificity PTPases CL100, PAC1, and B23, for ERK inactivation in HMC.

Previously, we have shown that in NIH3T3 cells, activation of the SAPK cascade rather than the ERK cascade induces the expression of the murine homologue of CL100 (18). Because CL100 is known to inactivate ERK, this cross-talk of MAP kinase signaling cascades may contribute to the inhibition of cell growth following activation of SAPK (19–22). In this study, we examined the role of three distinct MAP kinase signaling cascades in the induction of multiple dual-specificity PTPases in a primary cell line. Our data strongly suggest that activation of the ERK cascade, in contrast to NIH3T3 cells, induces the expression of CL100, PAC1, and B23 in HMC, thereby providing a potential mechanism of feedback inhibition because CL100, PAC1, and B23 were shown to dephosphorylate and inactivate ERK (8,11–14). In addition, our data

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**Figure 5.** Time curve of ERK activation after stimulation of quiescent HMC with 17% FBS alone or together with 20 μg/mL cycloheximide (Cx). (A) upper panel shows a Western blot analysis detecting both ERK isoforms, p42 and p44, in whole cell lysate. Activation is identifiable by the appearance of bands with delayed mobility indicating phosphorylated protein forms (starred). The lower panel shows ERK activity assayed by the ability of immunoprecipitated ERK to phosphorylate MBP. These results were obtained in two independent experiments. (B) MBP phosphorylation shown in Figure 5A was quantified by scintillation counting.
suggest CL100 to be the predominantly expressed dual-specificity PTPase in response to cellular stress in HMC. This induction of CL100 is likely to be mediated by the SAPK and the p38 MAP kinase pathways. These data correlate with findings that CL100 was cloned from a cDNA library from human skin fibroblasts treated with hydrogen peroxide and that CL100 is inducible by oxidative stress (45). CL100 is known to effectively dephosphorylate ERK (13,46). It is, therefore, reasonable to speculate that in mesangial cells, activation of the SAPK/p38 MAP kinase pathways in response to cellular stress induces the expression of the dual-specificity PTPase CL100, thereby inhibiting the ERK pathway. This cross-talk between these independent signal transduction pathways would be a logical cellular response to agonists activating the SAPK/p38 MAP kinase pathways because activation of SAPK and p38 MAP kinase inhibits cell growth and induces apoptosis (21,22). Therefore, inhibition of the growth stimulatory and antiapoptotic ERK pathway (22) would contribute to the effects of activated SAPK/p38 MAP kinase pathways. However, although anisomycin had only a weak stimulatory effect on the gene expression of PAC1 and B23, we cannot exclude the possibility that these genes may also play a role in the cellular stress response.

Recently, MKP-1, the murine homologue of CL100, was shown to inhibit the activation of SAPK and p38 MAP kinase in HeLa and NIH3T3 cells (46,47). The induction of CL100 in
Figure 7. Effect of the MEK antagonist PD 098059 on the gene expression of the dual-specificity PTPases CL100, B23, and PAC1 in HMC. (A) Quiescent cells were preincubated with PD 098059 for 20 min (+) or untreated (-) prior to stimulation with FBS (17%) for the indicated periods of time. The upper panel shows a Western blot analysis detecting p42 and p44 ERK as described above (phosphorylated protein forms starred) and the lower panel shows ERK activity assayed by the ability of immunoprecipitated ERK to phosphorylate MBP. (B) Quiescent cells were preincubated with PD 098059 for 20 min (+) or untreated (-) prior to stimulation with FBS (17%) for 1 h. Total RNA was isolated and examined by Northern blot analysis. The blots were sequentially probed with CL100, B23, and PAC1 cDNA. As a loading control, the same filters were stripped and rehybridized with the GAPDH probe. One representative Northern blot analysis of three independent experiments is shown.

response to cellular stress may, therefore, be of importance for the deactivation of SAPK and p38 MAP kinase as a negative feedback mechanism. Interestingly, PAC1 failed to inactivate SAPK in HeLa and NIH3T3 cells, despite its activity towards ERK and p38 MAP kinase (46). Therefore, it would not be surprising if other dual-specificity PTPases like B23 or the recently cloned MKP-2 (48,49) and MKP-3 (50,51) will be shown to exhibit selective activities toward distinct MAP kinases, thereby introducing further tiers of control of the regulatory networks.
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References


