Regulation of the TAK1 Signaling Pathway by Protein Phosphatase 2C*

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Protein phosphatase 2C (PP2C) is implicated in the negative regulation of stress-activated protein kinase cascades in yeast and mammalian cells. In this study, we determined the role of PP2Cβ-1, a major isoform of mammalian PP2C, in the TAK1 signaling pathway, a stress-activated protein kinase cascade that is activated by interleukin-1, transforming growth factor-β, or stress. Ectopic expression of PP2Cβ-1 inhibited the TAK1-mediated mitogen-activated protein kinase kinase 4-e-Jun amino-terminal kinase and mitogen-activated protein kinase kinase 6-p38 signaling pathways. In vitro, PP2Cβ-1 dephosphorylated and inactivated TAK1. Coimmunoprecipitation experiments indicated that PP2Cβ-1 associates with the central region of TAK1. A phosphatase-negative mutant of PP2Cβ-1, PP2Cβ-1 (R/G), acted as a dominant negative mutant, inhibiting dephosphorylation of TAK1 by wild-type PP2Cβ-1 in vitro. In addition, ectopic expression of PP2Cβ-1 (R/G) enhanced interleukin-1-induced activation of an AP-1 reporter gene. Collectively, these results indicate that PP2Cβ negatively regulates the TAK1 signaling pathway by direct dephosphorylation of TAK1.

Stress-activated protein kinases (SAPKs) are a subfamily of the mitogen-activated protein kinase (MAPK) superfamily and are highly conserved from yeast to mammalian cells. SAPKs relay signals in response to various extracellular stimuli, including environmental stress and inflammatory cytokines. In mammalian cells, two distinct classes of SAPKs have been identified: the c-Jun amino-terminal kinases (JNKs) (JNK1, JNK2, and JNK3) and the p38 MAPKs (p38α, p38β, p38γ, and p38δ) (1, 2). Activation of SAPKs requires phosphorylation at conserved tyrosine and threonine residues in the catalytic domain. This phosphorylation is mediated by dual specificity protein kinases, which are the members of the MAPK kinase (MKK) family. Of these, MKK3 and MKK6 phosphorylate p38, MKK7 phosphorylates JNK, and MKK4 can phosphorylate either. These MKKs, in turn, are activated by phosphorylation of conserved serine and threonine residues (1, 2). Recently, several MKK-activating MKK kinases (MKKKs) have been identified. Some of these MKKKs are also known to be activated by phosphorylation, but the details are unclear at present.

In the absence of signaling, SAPK cascades return to their inactive, dephosphorylated state, suggesting a possible role for phosphatases in SAPK regulation. In yeast cells, molecular genetic analysis has indicated that two distinct protein phosphatase groups, protein tyrosine phosphatase and protein serine/threonine phosphatase type 2C (PP2C), act as negative regulators of SAPK pathways (3). In Schizosaccharomyces pombe, tyrosine phosphatase Pyp2 and the yeast homolog of PP2C (Ptc1 and Ptc3) have been shown to dephosphorylate and inactivate Spc1, the yeast homolog of SAPK (4, 5).

PP2C is one of four major protein serine/threonine phosphatases (PP1, PP2A, PP2B, and PP2C) in eukaryotes and is implicated in the regulation of various cellular functions. To date, at least six distinct PP2C gene products (2Ca, 2Cb, 2Cγ, 2Cδ, Wip1, and Ca2+/calmodulin-dependent protein kinase phosphatase) have been found in mammalian cells (6–12). In addition, two distinct isoforms of the human PP2Cα (α-1 and -2) and five isoforms of the mouse PP2Cβ (β-1, -2, -3, -4, and -5) have been identified (13–16). These isoforms are generated in each species as splicing variants of a single pre-mRNA. We have recently reported that ectopic expression of mouse PP2Cα or PP2Cβ-1 inhibited the stress-activated MKK3/6-p38 and MKK4/7-JNK pathways but not the mitogen-activated MKK1-ERK1 pathway. Thus, negative regulation by PP2Cα and PP2Cβ-1 is selective for different SAPK pathways (17). Essentially the same results were obtained in studies of human PP2Cα-1 and -2 in mammalian cells (14). Currently, the in vivo target molecule(s) of PP2C is unknown, although MKK4, MKK6, and p38 have been proposed as substrates of PP2Cα-2 (14).

TAK1 was originally identified as an MKKK that functions in the transforming growth factor-β signaling pathway (18). TAK1 can activate both the MKK4-JNK and MKK6-p38 pathways (18). Recent studies have indicated that TAK1 is also activated by various stimuli, including environmental stress and inflammatory cytokines, and that it plays critical roles in various cellular responses (19–22). Studies on the regulation of TAK1 activity have revealed that a TAK1-binding protein, TAB1, functions as an activator promoting TAK1 autophosphorylation (21, 23). However, the protein phosphatase(s) responsible for inactivation of TAK1 has not been identified. In this

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The abbreviations used are: SAPK, stress-activated protein kinase; MAPK, mitogen-activated protein kinase; JNK, c-Jun amino-terminal kinase; MKK, MAPK kinase; MKKK, MKK kinase; PF, protein serine/threonine phosphatase; Ah, antibody; HA, hemagglutinin; IL, interleukin; GST, glutathione S-transferase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; MEKK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
study, we provide evidence indicating that PP2Cβ-1 selectively associates with TAK1 and inhibits the TAK1 signaling pathway by direct dephosphorylation.

**EXPERIMENTAL PROCEDURES**

**Materials**—The restriction enzymes and other modifying enzymes used for DNA manipulation were obtained from Takara (Kyoto, Japan). Anti-6xHis, anti-Myc, and anti-TAK1 antibodies (Abs) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti- phospho-MKK4 and anti-phospho-MKK3/6 Abs were supplied by New England Biolabs (Beverly, MA). Anti-hemagglutinin (HA; 12CA5) and anti-Flag (M2) Abs were purchased from Roche Molecular Biochemicals and Kodak Scientific Imaging Systems, respectively. Anti-PP2Cβ Ab was raised in rabbit against an oligopeptide of mouse PP2Cβ (RILSAEINPNLPPGLAGK). Human interleukin-1β (IL-1β) was from Roche Molecular Biochemicals. All the other reagents used were from Wako Pure Chemical (Osaka, Japan).

**Construction of Expression Plasmids**—Expression plasmids were constructed by standard procedures. Plasmids that express PP2C, TAK1, TAB1, MAPKs, MKks, and MMKks in mammalian cells were constructed using cDNAs encoding these proteins (17, 21) under the control of the CMV promoter. Epitope tags were added to the constructs using synthesized oligonucleotides. Mutated cDNAs were generated by polymerase chain reaction. For bacterial expression of proteins, cDNAs encoding the proteins were subcloned into PBEX (Amerham Pharmacdia Biotech) to generate glutathione S-transferase (GST) fusion proteins or into pQ3E3 (Qiagen, Hilden, Germany) to generate hexahistidine-tagged protein and affinity-purified by standard procedures. Other expression plasmids were as described elsewhere (23, 24).

**Cell Culture and Transfection**—COS7, 293, and 293IL-1RI (25) cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal bovine serum. At 50–80% confluency the cells were transfected by the DEAE-dextran method or using LipofectAMINE (Life Technologies, Inc.). The total amount of DNA (0.5–2 μg per 35-mm dish) was kept constant by supplementing with empty vector. The cells were cultured for 24–48 h after transfection and then harvested.

**Kinase and Phosphatase Assays**—Immune complex kinase assays were performed as follows. The cells were lysed in a buffer containing 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% (v/v) Triton X-100, and 1 mM phenylmethylsulfonyl fluoride. The cell lysates were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal bovine serum. At 50–80% confluency the cells were transfected by the DEAE-dextran method or using LipofectAMINE (Life Technologies, Inc.). The total amount of DNA (0.5–2 μg per 35-mm dish) was kept constant by supplementing with empty vector. The cells were cultured for 24–48 h after transfection and then harvested.

**Western Blot Analysis**—Proteins in the cell lysates and immunoprecipitated or immunoprecipitated with appropriate Abs for 1 h at 4 °C. The resulting immune complexes were recovered with protein G-Sepharose (Amersham Pharmacia Biotech), concentrated washed twice with Tris-buffered saline (20 mM Tris-HCl, pH 7.5, 150 mM NaCl), twice with 20 mM Tris-HCl, pH 7.5, and then incubated with or without appropriate substrates in 25 μl of kinase buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, and 1 mM dithiothreitol) containing 0.5–3 μl of (γ-32P)ATP (NEG-002A, PerkinElmer Life Sciences) at 30 °C for 10–30 min. The reactions were stopped by adding SDS-sample buffer and boiled for 2 min. Protein phosphatase assays were carried out as follows. COS7 cells seeded onto 10-cm dishes were cotransfected with Flag-TAK1 and Myc-TAB1 expression plasmids. The Flag-TAK1-Myc-TAB1 complex was immunoprecipitated from cell extracts with anti-Flag Ab, and phosphorylation was carried out in kinase buffer containing (γ-32P)ATP at 30 °C for 30 min. After washing three times with 20 mM Tris-HCl, pH 7.5, the immune complex was then incubated with or without recombinant GST-PP2Cβ in kinase buffer at 30 °C for the indicated times. Phosphorylated proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and the radioactivities incorporated into the proteins were detected with a BAS 2000 image analyzer (Fuji, Tokyo, Japan).

Western Blot Analysis—Proteins in the cell lysates and immunoprecipitated or immunoprecipitated with appropriate Abs for 1 h at 4 °C and then incubated with horseradish peroxidase-conjugated secondary Ab at 25 °C for 1 h. The chemiluminescence of each blot was detected with an enhanced chemiluminescence system (Amersham Pharmacia Biotech).

** Reporter Assay—**Cells were transfected with the AP-1-luciferase reporter plasmid (26). After the transfection, cells were treated with IL-1β and submitted to Western blot analysis.

**RESULTS**

**PP2Cβ Inhibits TAK1-induced Signal Transduction**—We have previously reported that two mouse PP2C isoforms, PP2Cα and PP2Cβ-1, selectively inhibit stress-activated MKks (MKK5, MKK3, MKK4, MKK6, and MKK7) (17). However, the target molecule(s) of PP2Cβ has not been identified. Because both the MKK4-JNK and MKK6-p38 signaling pathways are activated by TAK1 (18), we examined whether expression of PP2Cβ-1 affects TAK1-induced phosphorylation of MKK4 and MKK6 at their serine or threonine residues. Coexpression of TAK1 and TAB1 enhanced phosphorylation of MKK4 or MKK6 when expressed together in COS7 cells (Fig. 1, A and B). However, concomitant expression of PP2Cβ-1 markedly inhibited TAK1-

![Fig. 1. PP2Cβ inhibits TAK1-induced signaling pathways. A and B, expression plasmids for HA-TAK1, HA-TAB1, and His-MKK4 (A) or His-MKK6 (B) were cotransfected with the indicated amounts of Myc-PP2Cβ-1 expression plasmid into COS7 cells. Aliquots of the lysates were immunoblotted with anti-phospho-MKK4 (A) or anti-phospho-MKK3/6 (B) Abs (upper panels) or anti-His Ab (lower panels). C and D, expression plasmids for Flag-TAK1 and Myc-JNK1 (C) or Myc-p38α (D) were cotransfected with the indicated amounts of expression plasmids for HA-PP2Cβ-1 or HA-PP2Cβ-1(ΔRg) into COS7 cells. Myc-JNK1(ΔC) or Myc-p38α(ΔD) was immunoprecipitated from each cell extract, and immunoprecipitates were subjected to in vitro kinase assays with bacterially expressed GST-c-Jun (C) or GST-ATF2 (D), respectively, as substrate (upper panels). The amounts of Myc-JNK1 or Myc-p38α in aliquots of the lysates were determined by immunoblotting with anti-Myc Abs (lower panels).](http://www.jbc.org/content/283/16/5754/F1.large.jpg)
induced phosphorylation of MK4 and MKK6.

We then tested whether PP2Cβ-1 expression affects TAK1-induced activation of JNK1 and p38. Both the JNK1 and p38 kinases expressed in COS7 cells were activated by the exogenous TAK1. However, these kinase activities were inhibited when PP2Cβ-1 was coexpressed (Fig. 1, C and D). In contrast, expression of PP2Cβ-1(R/G), a phosphatase-defective mutant containing an Arg-179 to Gly mutation, had no inhibitory effect on TAK1-induced activation of JNK1 or p38. These results suggest that PP2Cβ-1 inhibits the TAK1 signaling pathway at TAK1 or downstream of TAK1, e.g. MKKs and MAPKs.

PP2Cβ Acts upon TAK1—We have previously shown that TAK1, when coexpressed with TAB1, is activated by autophosphorylation (23). TAK1 autophosphorylation can be monitored by decreased mobility on SDS-PAGE, and this mobility shift was cancelled when Ser-192 of TAK1, which is the site of autophosphorylation, was mutated to alanine (Ref. 23; also shown in Fig. 2).

To determine whether PP2Cβ-1 affects the phosphorylation state of TAK1, we coexpressed TAK1, TAB1, and PP2Cβ-1 in COS7 cells. As shown in Fig. 2, expression of wild-type PP2Cβ-1, but not PP2Cβ-1(R/G), caused a substantial decrease in the levels of TAK1 phosphorylation. This result suggests that PP2Cβ-1 acts upon TAK1 directly.

To investigate whether TAK1 is a substrate of PP2C, we examined the phosphorylation and kinase activity of TAK1 incubated with PP2C in vitro. Flag-TAK1 and TAB1 were coexpressed in COS7 cells, and Flag-TAK1 was immunoprecipitated from cell extracts with anti-Flag Ab. When the immunopurified TAK1 complex was incubated with [γ-32P]ATP, TAK1 became autophosphorylated. This reaction mixture was next incubated with bacterially produced GST-PP2Cβ-1 or GST-PP2Cβ-1(R/G). TAK1 was found to be dephosphorylated by GST-PP2Cβ-1, but not by GST-PP2Cβ-1(R/G), in a dose-dependent manner (Fig. 3A). The PP2Cβ-1-mediated dephosphorylation reaction was dependent on the presence of Mg2+ (Fig. 3B).

We then determined whether dephosphorylation of TAK1 by PP2Cβ-1 reduces TAK1 activity. Flag-TAK1 immunoprecipitates were treated with GST-PP2Cβ-1 and measured for TAK1 activity in vitro. The presence of PP2Cβ-1 decreased the ability of TAK1 to phosphorylate itself and MKK6 (Fig. 3C). Thus, PP2Cβ-1 dephosphorylates and inactivates TAK1 in vitro. This supports the possibility that PP2Cβ-1 negatively regulates the TAK1 signaling pathway by dephosphorylating TAK1.

PP2Cβ Does Not Dephosphorylate MKK6 in Vitro—Recent studies have indicated that one of the human PP2C isoforms, PP2Cα-2, dephosphorylates and inactivates MK4, MKK6, and p38 in vitro (14). Therefore, we tested whether PP2Cβ-1 could also dephosphorylate and inactive MKK6 in vitro. Bacterially produced MKK6 is activated by autophosphorylation and is able to phosphorylate p38 in vitro (27). We used this system to determine the effect of recombinant PP2Cβ-1 on MKK6 activity. We found that PP2Cβ-1 treatment did not affect MKK6 kinase activity under conditions where it inactivates TAK1 (Fig. 3, A and C versus Fig. 4A).

Next, we determined the effect of PP2Cβ-1 on stress-induced phosphorylation of MKK6. COS7 cells were transfected with Flag-MKK6 and subjected to hyperosmotic stress, and Flag-MKK6 was immunoprecipitated from the cell lysates with anti-Flag Ab. The immunoprecipitates were then incubated with GST-PP2Cβ-1. Increasing concentrations of GST-PP2Cβ-1 had no effect on the phosphorylation level of MKK6 (Fig. 4B). Taken together, these results indicate that PP2Cβ-1 does not act upon MKK6.

PP2Cβ Associates with TAK1 in Mammalian Cells—To determine whether PP2Cβ-1 associates with TAK1, we coexpressed Myc-TAK1 and HA-PP2Cβ-1 or HA-PP2Cβ-1(R/G) into COS7 cells. Aliquots of the lysates were immunoblotted with anti-HA Ab (upper panel) or anti-Myc Ab (lower panel).
PP2Cβ-1 with TAK1 was substantially weaker than that of PP2Cβ-1(R/G). This interaction is specific for PP2Cβ-1, because HA-PP2Cα, another major mouse PP2C isoform (28), did not coimmunoprecipitate with Myc-TAK1 under the same conditions (Fig. 5B). Therefore, the association of PP2Cβ-1 with TAK1 is not caused by a nonspecific protein interaction.

The observation that the catalytically inactive PP2Cβ has a higher affinity for TAK1 than that of wild-type PP2Cβ suggested that PP2Cβ might preferentially bind phosphorylated TAK1. The TAK1(S/A) mutant, in which Ser-192 is replaced by Ala, is defective in both phosphorylation and activation (23). PP2Cβ, similar to wild-type PP2Cβ, is capable of dephosphorylating the catalytic inactive MKK6(S-A) (23, 29), which mimics the phosphorylation state of TAK1(S/A) (Fig. 5C). Thus, PP2Cβ might preferentially bind phosphorylated TAK1 that is serine-192 phosphorylated, which is a characteristic feature of TAK1 activation.

The Region of TAK1 Required for Association with PP2Cβ—To determine which region of TAK1 is required for its association with PP2Cβ, we generated three Myc-tagged, truncated proteins, Myc-TAK1(N400), Myc-TAK1(C366), and Myc-TAK1(C176), containing the amino-terminal 400, carboxyl-terminal 366, and carboxyl-terminal 176 amino acids of TAK1, respectively (Fig. 6A). We coexpressed each deletion mutant along with Flag-MKK6 in 293 cells and immunoprecipitated Flag-PP2Cβ-1(R/G) from cell extracts with anti-Flag Ab. Subsequent immunoblot analysis using anti-Myc Ab revealed that Flag-PP2Cβ-1 was associated with Myc-TAK1(N400) and Myc-TAK1(C366) but not with Myc-TAK1(C176) (Fig. 6B). This result indicates that the central region of TAK1 is responsible for its association with PP2Cβ-1.

**PP2Cβ Does Not Associate with MEKK3, MEKK4, MKK6, JNK, or p38**—To evaluate the specificity of the association of PP2Cβ-1 with TAK1, we examined whether PP2Cβ-1 could associate with other SAPK signaling pathway components. Flag-PP2Cβ-1 was coexpressed with Myc-TAK1, Myc-MEKK3, Myc-MEKK4, Myc-MKK6, Myc-JNK1, or Myc-p38α in 293 cells. Flag-PP2Cβ-1 was immunoprecipitated from cell extracts with anti-Flag Ab, and the immune complexes were subjected to immunoblotting with anti-Myc Ab. None of these proteins, except for Myc-TAK1, coimmunoprecipitated with PP2Cβ-1 (Fig. 7). Thus, PP2Cβ-1 specifically interacts with TAK1.

**Effect of PP2Cβ on IL-1-stimulated AP-1 Activation**—Because PP2Cβ-1(R/G) appeared to have a higher affinity for TAK1 than did wild-type PP2Cβ-1 (Fig. 5A), we asked whether
PP2Cβ-1(R/G) could act as a dominant negative mutant. To test this possibility, we examined the effect of PP2Cβ-1(R/G) on PP2Cβ-1-mediated TAK1 dephosphorylation in vitro. We found that PP2Cβ-1(R/G) inhibited the dephosphorylation of TAK1 by PP2Cβ-1 in a dose-dependent manner (Fig. 8A).

It has recently been reported that IL-1 treatment of cells activates the JNK signaling pathway through activation of TAK1 (21). Therefore, we examined the ability of PP2Cβ-1 to affect activation of TAK1 and AP-1 following IL-1 stimulation. We transfected 293IL-1RI cells with PP2Cβ-1 and TAK1 and determined the effect of PP2Cβ-1 expression on IL-1-induced mobility shift on SDS-PAGE and activation of TAK1. IL-1 treatment caused a slight mobility shift of TAK1 on SDS-PAGE and activation of TAK1. IL-1 with PP2C

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**DISCUSSION**

MAPK cascades are intracellular signaling modules composed of three tiers of sequentially activating protein kinases: MKKK, MKK, and MAPK (1, 2). Because phosphorylation of these components is essential for the activation of the MAPK cascades, protein phosphatases may be expected to play important roles in the regulation of these cascades. Indeed, we recently demonstrated that two major protein serine/threonine phosphatases, PP2Ca and PP2Cβ, inactivate the stress-activated JNK and p38 MAPK pathways (17). Furthermore, Takekawa et al. (14) showed that PP2Cβ inhibits the JNK and p38 cascades by phosphorylating MKK4, MKK6, and p38. TAK1 is a member of the MKKK family and activates the JNK and p38 pathways. In this study we elucidated the role of PP2Cβ in TAK1-mediated signaling pathways.

We present several lines of evidence suggesting that PP2Cβ negatively regulates the TAK1 pathways by dephosphorylating and inactivating TAK1. First, ectopic expression of PP2Cβ inhibits the MKK4-JNK and MKK6-p38 pathways activated by TAK1 (Fig. 1). Second, it is known that the TAK1-binding protein TAB1 activates TAK1 by promoting its autophosphorylation (23). We found that PP2Cβ overexpression decreased TAB1-induced TAK1 autophosphorylation in vitro (Fig. 2). Third, PP2Cβ dephosphorylates and inactivates TAK1 in vitro (Fig. 3) but fails to dephosphorylate MKK6 (Fig. 4). Finally, PP2Cβ interacts with TAK1 but not with MKK3, MKK4, MKK6, JNK, or p38 (Figs. 5 and 7). Collectively, these data are consistent with the idea that PP2Cβ suppresses TAK1-mediated signaling by associating with and dephosphorylating TAK1. Because TAK1 functions in various biological responses, including acting as a positive regulator of transforming growth factor-β and IL-1-induced signal transduction (18, 21) and as a negative regulator in Wnt-induced signal transduction (22), it would be interesting to examine whether PP2Cβ contributes to the control of these physiological responses.

Ectoexpression of TAK1/TAB1 with PP2Cβ-1 did not result in complete dephosphorylation of TAK1, as judged by the fact that the mobility of TAK1 is still slower than that of TAK1 ex-
TAK1 associates with PP2Cβ but not with PP2Coα (Fig. 5). Thus, the interaction of TAK1 with PP2Cβ is rather specific. TAK1 is activated via autophosphorylation of Ser-192 in the activation loop between kinase domains VII and VIII. Mutation of TAK1 Ser-192 to Ala to create TAK1(S/A) abolishes both phosphorylation and activation of TAK1 (23). TAK1(S/A) has an affinity for PP2Cβ similar to that of wild-type TAK1 (Fig. 5C), indicating that phosphorylation of TAK1 is not required for its association with PP2Cβ. This suggests that the association of TAK1 with PP2Cβ does not occur simply through affinity of the enzyme (PP2Cβ) for its substrate (phosphorylated TAK1), but rather that PP2Cβ and TAK1 are stably associated. This may ensure appropriate localization of PP2Cβ and facilitate the specific and rapid deactivation of TAK1.

The central region of TAK1 is required for its association with PP2Cβ (Fig. 6). A similar region of TAK1 is involved in its association with TAB1 (20), which suggests that PP2Cβ might prevent the association of TAK1 with TAB1. However, this possibility is unlikely, because we did not observe any competition between TAB1 and PP2Cβ in their association with TAK1. Consistent with this, endogenous TAK1 constitutively associates with TAB1 in the absence of ligand stimulation (23). Therefore, the minimum regions of TAK1 required for association with PP2Cβ and TAB1 must be different. It is still not clear whether PP2Cβ associates with TAK1 directly or indirectly. However, the observation that PP2Cβ fails to interact with TAB1 argues against the possibility that TAB1 mediates the association between PP2Cβ and TAK1.

To understand what role PP2C may play in regulating SAPK signaling pathways, it is important to determine how cellular PP2C activity is affected by extracellular stimuli. In fission yeast cells, the expression of Ptc1 is enhanced by hyperosmotic stress (29). In contrast, expression levels of PP2Coα and PP2Coβ are not altered following stress treatment of cells (17). PP2Coα has been shown to preferentially bind to the phosphorylated form of p38 and may function in the adaptive phase of the stimulation cycle to restore p38 to the inactive state following stimulation by stress (14). PP2Cβ may play an analogous role in maintaining TAK1 signaling. TAK1 mediates IL-1-induced JNK signaling (21), and ectopic expression of PP2Cβ blocks IL-1-induced AP-1 activation. PP2Cβ(R/G), a catalytically inactive mutant, has a higher affinity for TAK1 than does wild-type PP2Cβ and acts as a dominant negative factor, antagonizing the inhibitory effect of wild-type PP2Cβ on IL-1-induced AP-1 activation. Furthermore, ectopic expression of PP2Cβ(R/G) enhances IL-1-stimulated AP-1 activation but does not cause constitutive activation of AP-1. These results raise the possibility that PP2Cβ may down-regulate TAK1 activity after ligand stimulation. Because endogenous PP2Cβ constitutively associates with TAK1 (Fig. 5D), and ligand stimulation does not affect this association, it is tempting to speculate that regulation of PP2Cβ enzymatic activity is involved in regulation of TAK1 signaling. Alternatively, PP2Cβ activity may be constitutive and serve to restore TAK1 to the inactive state following stimulation. Therefore it is important to determine whether the phosphatase activity of PP2Cβ is enhanced when cells are subjected to stress or treated with pro-inflammatory cytokines.

Takekawa et al. (14) recently reported that PP2Coα dephosphorylates MKK4, MKK6, and p38 in vitro. In this study, we show that PP2Cβ dephosphorylates and inactivates TAK1. Thus, in mammalian cells, SAPK pathways are negatively regulated by multiple PP2C isoforms at different levels; PP2Cβ
inhibits the pathways at the TAK1 MKKK level, and PP2Cα acts at the MKK and MAPK levels. In addition, two distinct groups of protein phosphatases other than PP2C also participate in the regulation of the SAPK pathways. The first group consists of the dual specificity phosphatases (also known as MAPK phosphatases) that inactivate MAPKs by dephosphorylating both tyrosine and threonine residues in the catalytic domains. Of the nine isolated MAPK phosphatases, M3/6 and MAPK phosphatase-5 have been shown to selectively dephosphorylate and inactivate p38 and JNK (30, 31). The second group includes PP2A, which inactivates partially purified p38 kinase (32). Cells treated with the PP2A inhibitor okadaic acid show enhanced MKK6 activity in epithelial cells (27). These results suggest that PP2A may also negatively regulate SAPK pathways and raise the possibility that several different groups of protein phosphatases each negatively regulate distinct targets in SAPK pathways.

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