Activation of the Sap-1a Transcription Factor by the c-Jun N-terminal Kinase (JNK) Mitogen-activated Protein Kinase*

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Ternary complex factors (TCFs) bind to the serum response element in the c-fos promoter and mediate its activation by many extracellular stimuli. Some of these stimuli activate the ERK subclass of mitogen-activated protein kinases (MAPKs) that target the TCF Sap-1a. We show that Sap-1a is also phosphorylated by the stress-activated JNK subclass of MAPKs leading to stimulation of both c-fos serum response element and E74-site-dependent transcription in RK13 cells. Several JNK-1 phosphorylation sites were mapped within Sap-1a, and mutation of these sites affected the transactivation mediated by Sap-1a and JNK-1. The impact of these phosphorylation sites varied at different promoters and was dependent on whether Sap-1a was stimulated by ERK-1 or JNK-1. Additionally, a comparison of Sap-1a with another TCF, Elk-1, revealed that these proteins behaved differently to stimulation by ERK-1 and JNK-1. Furthermore, activation of Sap-1a by JNK-1 was inhibited by the p38MAPK in RK13 cells, possibly by competition for a common upstream activator. Altogether, our data suggest that Sap-1a plays an important role in the nuclear response elicited by cellular stress.

However, the majority of the signals enters the c-fos promoter via the SRE, and the responses via the other elements are dependent on the presence of the SRE, which identifies the SRE as the pivotal control element within the c-fos promoter (5–7).

Before, during, and after induction of cells the c-fos SRE is occupied by a protein complex consisting of the serum response factor (SRF) and a ternary complex factor (TCF) (8), which belongs to the ETS transcription factor family (9). TCFs require protein-protein interactions with SRF in order to bind stably to the c-fos SRE, while the SRF protein can bind independently of TCF. However, TCFs also interact with and function in the absence of SRF at high affinity ETS binding sites (10).

Presently, three TCFs have been identified: Elk-1, Sap-1a, and Sap-2/Erp/Net (4). Phosphorylation of a TCF by the ERK subclass of mitogen-activated protein kinases (MAPKs) induces c-fos gene transcription in response to serum or growth factors (10–14). In addition, it has been shown that Elk-1 is phosphorylated by the stress-activated JNK subclass of MAPKs (15–18).

In this report, we demonstrate that the TCF Sap-1a is a target for JNK-1. Phosphorylation occurs at multiple sites, and most of them contribute to Sap-1a-mediated transactivation in a promoter-dependent manner. However, in comparison to Elk-1, Sap-1a is less potent activated by JNK-1, while the opposite appears to be true for activation by ERK-1 at the c-fos SRE. Furthermore, we show that JNK activation of Sap-1a can be negatively interfered with by the p38MAPK, which belongs to a third class of mammalian MAPKs (19).

EXPERIMENTAL PROCEDURES

Plasmids—Expression vectors for Sap-1a and point mutants thereof, Sap-1a-(1–267), Elk-1, and GAL4 fusions, have been described before (10, 12). GST (glutathione S-transferase) fusion protein expression vectors were constructed by isolating the EcoRI/BamHI inserts from the respective GS268–431 clones (10), ligating them into EcoRI/BamHI-cut pBlueScript KS+ (Stratagene), isolating the EcoRI/SacI inserts, and ligating them into pGEX2T-6His-PL2 (kindly provided by R. A. Hipskind), which was linearized with EcoRI and SacI. BXB and hemagglutinin (HA)-tagged ERK-1 mammalian expression vectors have been described in the literature (20, 21). The luciferase reporter plasmids were derived from the tk80-luc vector by cloning either two copies of the c-fos SRE or three copies of the E74 binding site in front of a herpes simplex virus thymidine kinase basal promoter and the firefly luciferase cDNA (12).

Production of GST Fusion Proteins—Escherichia coli BL21 bacteria, which were transformed with a GST fusion protein expression vector, were used to inoculate a 100-ml culture and were grown to an optical density of 0.8 measured at 600 nm. Then, bacteria were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside for 3 h and harvested by centrifugation. After a wash with phosphate-buffered saline, bacteria were suspended in 10 ml of 6 mM guanidine HCl, 0.1 mM sodium phosphate, pH 8, frozen for 10 min at −80 °C, and shaken for 1 h at room temperature. Debris was removed by centrifugation, and the supernatant was incubated with 0.5 ml of N.N'-nitrobiacrylic acid-agarose (Qiagen) for 3–12 h. The slurry was poured into a column, and after extensive washing with 6 mM guanidine HCl, 0.1 mM sodium phosphate, pH 8, bound proteins were eluted with 4 × 0.5 ml of 6 mM guanidine HCl, 0.1 mM sodium phosphate, pH 8, and 0.5 ml of 6 M guanidine HCl, 0.1 mM sodium phosphate, pH 8, and 0.5 ml of 6 M guanidine HCl, 0.1 mM sodium phosphate, pH 8.
phosphate pH 4. Proteins were renatured by stepwise dialysis against 25 mM Hepes, pH 7.5, 100 mM NaCl, 10% glycerol, 5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 8 M, 4 M, 2 M, 1 M, or no urea at 4°C. After the removal of precipitated material, renatured proteins were frozen in liquid nitrogen and stored at -70°C.

**In Vitro Kinase Assays—HA-tagged JNK-1 or HA-tagged ERK-1 was transiently produced in transfected rabbit kidney epithelial-like RK13 cells grown on 6-cm dishes. 36 h after transfection, RK13 cells were washed once with phosphate-buffered saline and then lysed with 600 μl of lysis buffer (10 mM Tris, 30 mM Na3PO4, pH 7.1, 50 mM NaCl, 50 mM NaF, 1% Triton X-100, 0.2 mM dithiothreitol, 20 μg/ml aprotinin, 20 μg/ml leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM NaVO4) at 4°C for 5 min. After scraping and transferring to a microcentrifuge tube, the lysate was briefly vortexed and tumbled for 20 min, and debris was removed by centrifugation. 1 μl of monoclonal 12CA5 antibody (Boehringer Mannheim) was added to the supernatant, and 15 μl of agarose beads with coupled protein A (RepliGlen) after 1 h of incubation. After one more hour, the beads were pelleted by centrifugation for 20 s, washed three times with 500 μl of the above described lysis buffer, and then twice with 500 μl of kinase buffer (15 mM Mops, pH 7.2, 12 mM MgCl2, 15 mM β-glycerophosphate, 0.5 mM EGTA, 0.2 mM dithiothreitol, 0.5 mM Na3VO4, 10 μM ATP). Finally, the beads were suspended in 50 μl of kinase buffer, and a typical in vitro kinase reaction was set up with 10 μl of this slurry plus 1 μg of purified protein (in a volume of approximately 1 μl) plus 0.65 μM [γ-32P]ATP (3000 Ci/mmol). The reaction was allowed to proceed for 30 min at 30°C and then stopped by boiling in SDS-PAGE sample buffer. Phosphorylated proteins were resolved on a 10% SDS-PAGE gel, and the gel was, after drying, exposed to an x-ray film.

**Phosphopeptide Analysis—** GST-Sap proteins were phosphorylated in vitro as described above with the following modifications: the ATP concentration was 1 μM in the kinase buffer, and the amount of radiolabel was increased to 0.5 μM [γ-32P]ATP (3000 Ci/mmol). After SDS-PAGE, a gel slice with the protein of interest was cut out of the dried gel, rehydrated with 500 μl of freshly made 50 mM NH4HCO3, and then ground up. Another 500 μl of 50 mM NH4HCO3, 10 μl of β-mercaptoethanol, and SDS to a final concentration of 0.1% were added, and the slurry was boiled for 3 min. After 5 h of extraction with such a volume of 50 mM NH4HCO3, was performed so that the combined supernatants had a volume of 1300 μl. After a final clear spin, 20 μl of boiled RNase A and 300 μl of ice-cold trichloroacetic acid were added, and proteins were precipitated for 1 h at 4°C. After centrifugation, the supernatant was lyophilized and the pellet was dissolved in pH 1.9 buffer, bovine serum albumin, and triton-X-100, dried, and taken up in 100 μl of 50 mM NH4HCO3 supplemented with 5 μg of chymotrypsin. Proteolytic digestion was performed for a total of 6 h with another 5 μg of chymotrypsin added after 3 h. 500 μl of water were added, and the sample was lyophilized, reconstituted after addition of 300 μl of water, and finally dissolved in 400 μl of pH 1.9 buffer (88% (w/v) formic acid/glacial acetic acid/water (50:156:1794)). After centrifugation, the supernatant was lyophilized and the pellet was dissolved in pH 1.9 buffer in a volume adequate for two-dimensional phosphopeptide mapping. This was performed by spotting up to 5 μl of the phosphopeptide mixture onto cellulose thin-layer plates (Merck Darmstadt, No. 5716), electrophoresis in pH 1.9 buffer (1.3 kV; 25 min), and, as descending chromatography in 1-butanol/pyridine-glacial acetic acid/water (75:50:15:60) in the second dimension (22).

**Reporter Gene Assays—** RK13 cells were transiently transfected by the calcium phosphate coprecipitation method with indicated luciferase reporter gene constructs and protein expression plasmids (12). Luciferase activity was determined 36 h after transfection and normalized to transfection efficiency with the help of a cotransfected β-galactosidase expression vector (10). Typically, the results are given as the mean (± S.E.) of three experiments.

**RESULTS**

**Phosphorylation of Sap-1a by JNK-1**—To investigate whether the ETS family transcription factor Sap-1a can be phosphorylated by JNK-1, HA-tagged JNK-1 was transiently expressed in RK13 cells, immunoprecipitated with an anti-HA antibody, and then phosphorylated in an in vitro kinase assay with a GST fusion protein encompassing the C-terminal transactivation domain of Sap-1a. As shown in Fig. 1A (upper left panel, lane 7), JNK-1 alone was unable to elicit a significant degree of phosphorylation of GST-Sap. However, coexpression of an upstream activator of JNK-1, a constitutively activated form of a MAPK kinase kinase (MEKKc), resulted in considerable phosphorylation of GST-Sap (lane 6). As a control, the GST moiety itself was not phosphorylated by JNK-1 (Fig. 1A, lower left panel). We then compared the phosphorylation of GST-Sap by JNK-1 and by immunoprecipitated HA-tagged ERK-1. Already ERK-1 expression on its own led to some phosphorylation of GST-Sap, and coexpression of the ERK-1 upstream activator BXB, which is a constitutively active Raf-1 kinase (20), potentiated this phosphorylation (Fig. 1A, upper left panel, lanes 2 and 3). Since the amounts of HA-tagged JNK-1 present in the immunoprecipitates were even slightly higher than those for HA-tagged ERK-1 as judged by Western blotting utilizing an anti-HA antibody (Fig. 1B, lower right panel), although this difference was not as pronounced as with GST-Sap.

Since we wanted to employ BXB and MEKKc as selective activators of ERK-1 and JNK-1, respectively, we had to demonstrate that BXB does not activate JNK-1 and, conversely, that ERK-1 was not activated by MEKKc. Therefore, BXB and JNK-1 or alternatively MEKKc and ERK-1 were coexpressed, and the ability of JNK-1 and ERK-1 to phosphorylate GST-Sap, GST-Elk, and histidine-tagged Jun in vitro was assessed.
activation of JNK-1 by BXB or of ERK-1 by MEKKc was observed (Fig. 1A); rather, as can be best seen with the in vitro phosphorylation of GST-Elk, a small inhibitory effect of BXB on JNK-1 (compare lanes 7 and 8) and of MEKKc on ERK-1 (compare lanes 3 and 4) was noticeable. This is not due to an effect of BXB or MEKKc on the level of JNK-1 or ERK-1 expression, since protein levels were fairly comparable under these conditions (Fig. 1B). Thus, cross-activation between the BXB-activated ERK pathway and the MEKKc-activated JNK pathway does not occur in RK13 cells under our experimental conditions.

Stimulation of Sap-1a Activity by JNK-1—Previously, Sap-1a was shown to stimulate transcription upon phosphorylation by ERKs (10, 14). This was reproduced here with a c-fos SRE-driven luciferase construct (Fig. 2A). Expression of ERK-1 alone had little effect on c-fos SRE-driven luciferase activity, indicating that the basal activity of ERK-1 as observed in our in vitro kinase assay (see Fig. 1A) was insufficient to trigger gene transcription. However, the joint expression of BXB and ERK-1 stimulated transcription by nearly 30-fold, while BXB alone was 4 times less stimulatory presumably due to the levels of endogenous ERKs being insufficient. As a control, the Sap-1a-(1–267) molecule was employed, which lacks the C-terminal activation domain but is still capable of binding to DNA and interacting with the SRF protein (10). No activation of transcription was observable with Sap-1a-(1–267) (Fig. 2A), proving once more that the C terminus of Sap-1a is required for transactivation. Also, Sap-1a was unable to elicit an activation of a reporter construct devoid of a c-fos SRE (Fig. 2C), indicating that the observed effects were binding site-specific.

Expression of JNK-1 had no effect on Sap-1a-mediated c-fos SRE stimulation, but expression of MEKKc led to 5-fold activation of luciferase activity (Fig. 2A). This degree of activation could be enhanced to 16-fold by coexpression of JNK-1, indicating that JNK-1 and MEKKc synergize to activate Sap-1a. Again, Sap-1a-(1–267) was unresponsive to stimulation, and the effects were binding site-specific (see Fig. 2C). Consistent with the in vitro phosphorylation data, MEKKc was unable to cooperate with ERK-1 nor was BXB able to do so with JNK-1, confirming that the ERK and JNK pathways are not stimulated by the same upstream activator in RK13 cells.

Sap-1a is not only capable of forming a ternary complex with the SRF protein at the c-fos SRE but can also, independently of SRF, activate transcription from an ETS binding site, such as the E74 site (10). Thus, we tested whether Sap-1a was able to mediate activation of transcription via the E74 site upon expression of MEKKc and JNK-1. As shown in Fig. 2B, Sap-1a-dependent transcription was stimulated by both MEKKc/JNK-1 and BXB/ERK-1. Thus, Sap-1a is not only a target for JNK-1 at the c-fos promoter but also at ETS binding sites without a juxtaposed SRF binding site.

Next, we investigated whether the C-terminal transactivation domain of Sap-1a is targeted by JNK-1. The DNA binding domain of GAL4 alone or fused to the C-terminal activation domain of either Sap-1a or Elk-1 was assessed for its transactivation potential with a GAL4 binding site-driven luciferase reporter construct in transiently transfected RK13 cells. Where indicated, MEKKc/JNK-1 or BXB/ERK-1 were coexpressed.

FIG. 2. Sap-1a-mediated transactivation upon JNK-1 stimulation. Sap-1a or the C-terminal truncation Sap-1a-(1–267) were cotransfected into RK13 cells with the indicated protein kinase expression vectors and a luciferase reporter gene driven by either a c-fos SRE (A), an E74 site (B), or the basal tk80 promoter (C). Relative luciferase activity measured with solely Sap-1a was set to 1.

FIG. 3. Comparison of Sap-1a and Elk-1. Either Sap-1a or Elk-1 was transiently transfected into RK13 cells with a c-fos SRE-driven (left panel) or an E74 site-driven (right panel) luciferase reporter construct. Fold induction of luciferase activity due to BXB/ERK-1 or MEKKc/JNK-1 coexpression is given.

FIG. 4. Comparison of Sap-1a and Elk-1. Either Sap-1a or Elk-1 was transiently transfected into RK13 cells with a c-fos SRE-driven (left panel) or an E74 site-driven (right panel) luciferase reporter construct. Fold induction of luciferase activity due to BXB/ERK-1 or MEKKc/JNK-1 coexpression is given.
the activation of transcription mediated by the GAL4 fusion proteins is due to the C-terminal activation domains of Sap-1a and Elk-1.

Finally, we compared the two TCFs Sap-1a and Elk-1 under conditions where both factors are expressed to an equivalent level (10). Sap-1a was more sensitive to activation by ERK-1 than by JNK-1 (Fig. 4), and while activation levels at the c-fos SRE and the E74 site were comparable upon ERK-1 activation, the E74 site was only stimulated about half as well as the c-fos SRE upon JNK-1 activation. On the contrary, Elk-1 activated c-fos SRE-dependent transcription more upon JNK-1 than upon ERK-1 stimulation, while the opposite held true for E74 site-dependent transcription (Fig. 4). Also, Elk-1 was more active at the E74 site than at the c-fos SRE. Furthermore, while Elk-1 was more active than Sap-1a on the E74 site, Sap-1a was 2.6-fold more inducible at the c-fos SRE upon ERK-1 stimulation but ~35% less inducible than Elk-1 upon JNK-1 stimulation. Altogether, Sap-1a and Elk-1 do not behave equivalently in response to activation of the ERK and JNK MAPK pathways.

Identification of Phosphorylation Sites—To identify JNK-1 phosphorylation sites within Sap-1a, GST fusion proteins of wild-type Sap-1a and mutations thereof at potential MAPK sites were phosphorylated in vitro by JNK-1 and digested with chymotrypsin, and the resulting phosphopeptides were separated in two dimensions on cellulose thin-layer plates (Fig. 5). Phosphopeptides 3–5 vanished upon mutation of serines 381 and 387 to alanines, and phosphopeptides d–f vanished upon mutation of threonine 420 and serine 425, indicating that these sites are targeted by JNK-1. In addition, mutation of threonines 361, 366, and 420 and serines 381, 387, and 425 led to the disappearance of phosphopeptides (3–5) and c–f, indicating that phosphopeptide c is due to phosphorylation at threonines 361 and 366. However, more JNK-1 phosphorylation sites exist in Sap-1a, since the phosphopeptides a and b were still observable with the latter mutant. In conclusion, Sap-1a is phosphorylated by JNK-1 at several sites, including residues 361, 366, 381, 387, 420, and 425.

We next assessed the importance of these phosphorylation sites for the activity of Sap-1a. To that end, alanine mutations of Sap-1a were compared with the wild-type molecule for their ability to activate c-fos SRE and E74 site-dependent transcription (Fig. 6A). The alanine double mutant T361A/T366A displayed a reduced transactivation potential, behaved similarly in response to BXB/ERK-1 and MEKKc/JNK-1 stimulation, and was less active on the c-fos SRE than at the E74 site. The single alanine mutants S381A and S387A were only 50% as active as wild-type Sap-1a upon MEKKc/JNK-1 stimulation, but they had nearly wild-type activity upon BXB/ERK-1 stimulation on the c-fos SRE. Consistently, the S381A/S387A double mutant reduced transactivation to a level of ~10% of wild-type Sap-1a at the c-fos SRE upon MEKKc/JNK-1 stimulation compared with only 25% upon BXB/ERK-1 stimulation. No such difference was observable at the E74 site where the response to both ERK-1 and JNK-1 was reduced equally. The T420A/S425A mutant was approximately 30% less active than the wild-type with the exception of BXB/ERK-1 stimulation at the c-fos SRE where it was ~40% more active than wild-type Sap-1a. Finally, mutation at all six aforementioned sites resulted in a protein that was only ~5% as active as the wild-type, indicating that phosphorylation at several sites, especially at threonines 361 and 366 and serines 381 and 387, cooperatively stimulates Sap-1a-mediated transactivation.

Sap-1a and Elk-1 are very similar to each other at the C terminus and possess homologous potential MAPK phosphorylation sites (Fig. 6B). Thus, we tested whether alanine mutants of Elk-1 would display a phenotype comparable with the homologous Sap-1a mutants. The Elk-1 S383A mutation caused a drastic phenotype, especially with regard to activation of the E74 site upon BXB/ERK-1 stimulation, but surprisingly had much less effect on MEKKc/JNK-1 stimulation at the E74 site, a behavior clearly distinct from the homologous S381A mutant of Sap-1a. Mutation of serine 389 in Elk-1 resulted in a protein that responded differently to BXB/ERK-1 and MEKKc/JNK-1 on the E74 site; either ~50% less active than the wild-type or ~30% more active, respectively. Again, this behavior contrasts that of the homologous Sap-1a mutant S387A. Finally, while the T420A/S425A mutant of Sap-1a, as well as the single T420A and S425A mutants (data not shown), behaved similarly to the wild-type, mutation of the homologous sites in Elk-1 at positions 417 or 422 reduced transactivation at least 3-fold under all conditions tested. Thus, the homologous phosphorylation sites in Elk-1 and Sap-1a affect the function of these two TCFs differently.

p38MAPK Blocks JNK-1 Activation—Having established that two of the MAPK subclasses target Sap-1a, we tried to detect an activation of Sap-1a by the p38 subclass of MAPKs in RK13 cells but failed to do so, although it can occur in other cell types.2 Rather, we observed that application of the specific p38MAPK inhibitor SB202190 (23) even promoted slightly the Sap-1a/JNK-1-mediated activation of the c-fos SRE-driven luciferase reporter (Fig. 7A), which may indicate that endogenous p38MAPK inhibits JNK-1. Indeed, exogenous p38MAPK was able to reduce MEKKc/JNK-1-mediated transactivation of Sap-1a (Fig. 7A).

2 Janknecht, R., and Hunter, T. (1997) EMBO J., in press.
These results prompted us to investigate whether p38MAPK could inhibit JNK-1 activation. To that end, increasing amounts of p38MAPK were coexpressed with JNK-1/MEKKc, and JNK-1 activity was measured in an in vitro kinase assay after immunoprecipitation. As shown in Fig. 7B, p38MAPK drastically reduced the activity of JNK-1 in vitro without significantly affecting the protein levels. Less inhibition was observable when the cells had been incubated with the SB202190 compound (data not shown). As a control, ERK-1 activation by BXB was not inhibited by p38MAPK. One possible reason for the inhibitory effect of p38MAPK on JNK-1 could be the sequestration of a common upstream activator. Indeed, coexpression of JNK kinase (JNKK), which can activate both JNKs and p38MAPK (24), reduced the inhibitory effect of p38MAPK on JNK-1 activation (Fig. 7B). Altogether, these data suggest that p38MAPK can interfere with the activation of JNK-1 in RK13 cells.

DISCUSSION

In this report, we have shown that the transcription factor Sap-1a is phosphorylated by JNK-1 at multiple sites and thus activated to stimulate transcription from either the c-fos SRE or an ETS binding site. Phosphorylation at the different serine and threonine residues in the C-terminal activation domain appears to cooperate in enhancing the transactivation potential of Sap-1a, since the more these amino acids were mutated to alanine, the less active Sap-1a was. The phosphorylation sites in Sap-1a that are recognized by JNK-1 are also targeted by ERKs (10, 14), but we observed that they may have different importance for signaling via ERK-1 and JNK-1. For instance, alanine instead of serine at position 387 reduced Sap-1a activation of the c-fos SRE by one-half upon JNK-1 stimulation, but had no impact on stimulation by ERK-1. This hints at the fact that the stoichiometry of phosphorylation at a particular site is different upon ERK-1 and JNK-1 phosphorylation, and ERK-1 and JNK-1 indeed appear to have a different site preference in the Sap-1a molecule.3

Interestingly, alanine mutants of Sap-1a had a different quantitative impact on Sap-1a-mediated transactivation as measured with a c-fos SRE-driven luciferase reporter construct. Since the employed reporter plasmids were identical except for the SRE or E74 binding site, this is not an effect due to different core promoters. Rather, one possible explanation is that the surface of Sap-1a at these binding sites is different; at the c-fos SRE, but not at the E74 site, Sap-1a is interacting with SRF, which may alter its conformation. Thus, phosphorylation sites would be exposed differently at the c-fos SRE and the E74 site and could therefore contribute differently to the

3 R. Janknecht and T. Hunter, unpublished observation.
interaction with its coactivator CBP (25, 26) or components of the basal transcription machinery. Our results highlight the fact that alterations of protein conformation by either inter- or intramolecular protein-protein interactions may lead to different effects of phosphorylation at a particular site with regard to the activity of a protein. Since intramolecular interactions between N- and C-terminal regions of TCFs cannot be excluded (27), this may cast doubts on studies using fusions of the C terminus to a heterologous DNA binding domain such as that from the yeast transcription factor GAL4 to study the impact of phosphorylation. However, our study proves that full-length Elk-1 is also targeted and activated by JNK-1, as shown previously solely with fusion proteins (15–17).

A comparison of Sap-1a with Elk-1 revealed that these two TCFs respond differently to ERK and JNK activation depending on the promoter context. It appears that Elk-1 is in general more responsive to JNK-1 stimulation, while ERK-1 activates Sap-1a more than Elk-1 at the c-fos SRE and vice versa at the E74 site. Since TCFs are expressed in a cell-type-specific manner (13), the molar ratio of Elk-1 to Sap-1a could determine the strength of gene activation in response to ERKs and JNKs.

However, only p46 JNK-1 was employed in this study, and at least 10 different JNK isoforms encoded by three genes exist (28). These isoforms appear to have preferences in the targeting of different transcription factors (28), which leaves the possibility that one or more of them phosphorylates and thus activates Sap-1a more than Elk-1 in contrast to p46 JNK-1. Thus, in addition to the molar ratio of TCFs expressed in a certain cell type, the spectrum of JNKs present would also determine the degree of activation of a TCF-regulated gene.

Surprisingly, we found that p38MAPK can inhibit the stimulation of JNK-1 in RK13 cells. Since expression of the common upstream activator JNKK (24) alleviated this repression, this effect is, at least in part, due to the competition for a limiting upstream activator. Thus, cells with a high load of p38MAPK may not be able to activate the JNK pathway efficiently. Interestingly, application of the p38MAPK inhibitor SB202190 led to a smaller degree of JNK-1 inhibition. This may be due to a lower affinity of SB202190-complexed p38MAPK for JNKK or may indicate that enzymatic activity of p38MAPK is also contributing to repression of JNK-1 activity, for instance by phosphorylation and activation of JNK phosphatases or by an inhibitory phosphorylation of JNK kinases.

Extracellular stimuli leading to the activation of JNKs may take different routes within the cell. Some of these stress signals, such as ultraviolet light or heat, may lead to the activation of the Rac and Cdc42 small G-proteins (29), while tumor necrosis factor α or changes in osmolarity lead to the activation of the Ca\(^{2+}\)-induced Pyk2 protein kinase (30). But finally, these different routes converge on the JNKs, and our study has identified Sap-1a as a novel effector of this subclass of MAPKs that may therefore play an important role in the nuclear response to stress. Often, stress induces apoptosis of cells, and it has recently been shown that JNK activation is required for ceramide-initiated apoptosis (31). This suggests that Sap-1a may also be involved in programmed cell death, consistent with the fact that c-fos is induced prior to apoptosis in vivo (32).

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