Mitogen-activated protein (MAP) kinases are important mediators of the cellular stress response. Here, we investigated the relationship between activation of the MAP kinase p38 and transcription factor NF-κB. Different forms of cellular stress were found to preferentially trigger either p38 or NF-κB. Arsenite or osmotic stress potently activated p38 but were ineffective in inducing NF-κB activation. Tumor necrosis factor-α and hydrogen peroxide, in contrast, led to NF-κB activation but only modestly stimulated p38. The activation of NF-κB was strongly abolished by antioxidants, while the activity of p38 and transcription factor AP-1 were increased. Inhibition of small GTPases including Rac and Cdc42 prevented p38 and AP-1 activation without interfering with NF-κB. In addition, inhibition of p38 by a pharmacological inhibitor or a dominant-negative mutant of MAP kinase kinase-6, an activator of the p38 pathway, interfered with NF-κB-dependent gene expression but not its DNA binding activity. Our results indicate that activation of p38 and NF-κB are mediated by separate pathways, which may converge further downstream in the cell nucleus. Different forms of cellular stress, however, initially trigger distinct signaling cascades involving either oxidative stress or GTPase-coupled pathways.

Gene induction by cellular stresses is mediated through biochemical processes that mostly involve the interplay of multiple signaling pathways. Depending on the nature of the stimulus, intricate protein kinases are activated that ultimately phosphorylate transcription factors and result in gene expression. Central mediators that propagate signals from the plasma membrane to the cell nucleus are protein kinases related to the mitogen-activated protein (MAP) kinase superfamily. To date, at least three different subtypes of MAP kinases are known (reviewed in Refs. 1–3). These are in turn activated by distinct upstream specificity kinases thus revealing the existence of protein kinase modules that can be independently and simultaneously activated. Whereas mitogens and growth factors lead to activation of protein kinase cascades resulting in activation of ERK family MAP kinases, many forms of cellular stress preferentially trigger two related signaling pathways (4–8). These center on two MAP kinase homologues called stress-activated protein kinases or Jun NH2-terminal kinases (JNKs), and p38, also termed reactivating kinase. Targets of stress-activated protein kinase and JNK include several transcription factors such as c-Jun, JunD, ATF-2, and Elk-1, which become activated after exposure to cellular stresses (9–12).

Mammalian p38 was originally identified in murine pre-B cells transfected with the lipopolysaccharide complex receptor CD14 and in macrophages where it is activated in response to lipopolysaccharide (13). The amino acid sequence of p38 is most similar to HOG-1, a MAP kinase homologue that lies in a signaling pathway that restores the osmotic gradient across the plasma membrane of Saccharomyces cerevisiae in response to increased external osmolarity (14). Like ERKs and JNKs, p38 requires phosphorylation of a closely spaced tyrosine and threonine for activation. However, the enzyme is distinguished by the sequence TGY in the activation domain, which differs from the TEF sequence found in ERKs, and the TPY sequence in the JNK and MAP kinase homologues.

In addition to hyperosmotic shock, p38 is activated by a wide spectrum of stimuli such as physicochemical stresses, lipopolysaccharide, and cytokines (13, 15–17). Despite rapid progress in the elucidation of structural elements of the p38 pathway, the physiological consequences of its stimulation by stress agents largely remain to be defined. One of the downstream targets of p38 is the MAPKAP kinase-2, which can activate the transcription factors CREB and ATF-1 (18) and phosphorylate heat-shock protein hsp27 (19, 20). It is assumed that hsp27 phosphorylation promotes the polymerization of actin and so counteracts the disruptive effects of stress on actin microfilaments (21). A number of evidences further suggest that p38 plays a key role in regulating apoptotic and inflammatory responses. Highly specific inhibitors of p38 such as pyridyl imidazoles potently block the synthesis of TNF and interleukin-1 (22).

Prevention of interleukin-1 and TNF synthesis was shown to be due to inhibition of translation initiation, which is regulated through the AUUUA motif in the 3′-untranslated regions of their transcripts (23). In addition, inhibition of p38 has been found to prevent interleukin-6 and granulocyte-macrophage colony-stimulating factor mRNA synthesis indicating that p38 may regulate transcriptional events (24). In this respect, p38 has been implicated in the activation of multiple transcriptional factors.
transcription factors, such as ATF-2 (17, 25), Elk-1 (25), c-Fos and c-Jun (26), CHOP (27), Max (28), and NF-κB (24).

The transcriptional activator NF-κB is a key component controlling the synthesis of cytokines and many other immunoregulatory gene products (reviewed in Refs. 29 and 30). The factor is triggered by a great variety of proinflammatory or pathogenic stimuli including inflammatory cytokines, phorbol ester, T cell mitogens, and lipopolysaccharide, as well as ionizing and UV irradiation. In unstimulated cells, NF-κB resides in the cytoplasm as an inactive heterodimeric complex bound to a third inhibitory subunit called IκB. Upon stimulation of cells, NF-κB is rapidly activated by the dissociation of IκB. This exposes nuclear localization sequences in the remaining NF-κB heterodimer, leading to nuclear translocation and subsequent binding of NF-κB to DNA-regulatory elements within target genes. It has been found that the dissociation of IκB is preceded by its phosphorylation, an event which subsequently leads to the proteolytic degradation of IκB (31). A number of evidences further suggest that reactive oxygen intermediates (ROIs) are the essential second messengers involved in the course of NF-κB activation (30). This is supported by the findings that (i) NF-κB is readily activated in many cell types exposed to hydrogen peroxide (32, 33), and (ii) activation in response to all stimuli tested so far is blocked by a variety of antioxidants (32, 34). Therefore, it is currently assumed that the intracellular production of ROIs, which is stimulated by stress inducers, activates a redox-sensitive kinase that in turn phosphorylates IκB.

As many of the stimuli leading to NF-κB and p38 activation are similar, we wished to establish the relationship between p38 and NF-κB-mediated transcription. A potential role of p38 in NF-κB activation has recently been implicated by the finding that p38-specific inhibitors potently attenuate NF-κB activation (24). We report here that although some stress inducers such as TNF activate both p38 and NF-κB, other stimuli including sodium arsenite and hyperosmotic shock selectively trigger p38. In addition, the antioxidants that prevent NF-κB activation did not inhibit but even increased p38 activity. However, blockade of p38 activity by a specific p38 inhibitor or a dominant-negative MAP kinase kinase-6 (MKK6) mutant inhibited NF-κB-dependent gene expression without interfering with NF-κB DNA binding activity. These data indicate that activation of NF-κB and p38 lie on primarily separate stress-responsive pathways but may converge downstream at the level of NF-κB-mediated transactivation.

MATERIALS AND METHODS

Cells and Reagents—The human embryonic kidney cell line 293 and HeLa cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal calf serum, 100 units of penicillin/milliliter, and 0.1 mg of streptomycin/milliliter. All reagents were obtained from Sigma unless otherwise specified. Recombinant human TNF-α was a gift from Dr. F. Hofmann and K. Aktories (Freiburg, FRG). The plasmid pMAG was kindly provided by Dr. L. E. Zon. GST-ATF-2 (amino acids 1–109), which served as a positive control. A CMV-driven eukaryotic ATF-2 expression plasmid has been described (9). A dominant-negative expression plasmid of MKK6 (K82A) (25) was originally obtained from Dr. R. J. Davis. Transfections of cells were performed by the calcium phosphate coprecipitation method (37). Briefly, 1.5 × 106 cells for kinase assays and 2 × 106 cells for luciferase assays were seeded on 10-cm and 6-cm plates, respectively, and transfected after overnight incubation. Eighteen h later, cells were either left untreated or stimulated with the indicated reagents. After 1 or 6 h cells were harvested and assayed for p38 kinase or luciferase activity, respectively.

Luciferase Assay—Cells were harvested 24 h post-transfection, lysed in 25 mM glycylglycine, pH 7.8, 1% Triton X-100, 15 mM MgSO4, 4 mM EGTA, 1 mM DTT, and centrifuged at 13,000 × g at 4 °C for 5 min. Fifty microliters of supernatant was resuspended in 100 μl of assay buffer (25 mM glycylglycine, 15 mM MgSO4, 4 mM EGTA, 15 mM potassium P, pH 7.8, 1 mM DTT, 1 mM ATP) using a LB 96 P luminometer (Berthold, Bad Wildbad, FRG). Following injection of 100 μl of luciferin (0.3 mg/ml) light emission was measured in 30-s intervals. Results are given in relative light units.

Solid-phase Kinase Assay—p38 activity was determined by a solid-phase kinase assay using GST-ATF-2 as substrate. Following transfection with the GST-p38 construct, 293 cells (1.5 × 106) were extracted in 100 μl of lysis buffer containing 20 mM HEPES, pH 7.5, 300 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM ATP, 10 mM NaF, 1 mM Na3VO4, 2 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100. After centrifugation to remove cellular debris, 300 μl of equilibration buffer (20 mM HEPES, pH 7.5, 2.5 mM MgCl2, 1 mM EDTA, and 0.05% Triton X-100) were added. p38 was adsorbed by the addition of 30 μl of a 50% slurry of glutathione-coupled Sepharose beads (Pharmacia Biotech Inc.). After incubation at 4 °C for 30 min, the beads were washed twice with washing buffer (20 mM HEPES, pH 7.5, 50 mM NaCl, 2.5 mM MgCl2, 1 mM EDTA, 0.1 mM Na3VO4, 0.05% Triton X-100), twice in LiCl buffer (20 mM Tris-HCl, pH 7.8, 500 mM LiCl, 0.1 mM Na3VO4), and twice in washing buffer. The beads were then equilibrated by two washing steps in kinase buffer (20 mM HEPES, pH 7.5, 2.5 mM MgCl2, 40 μM ATP, 2 mM DTT, 0.1 mM Na3VO4). The kinase reaction was performed by adding 25 μl of kinase buffer containing 5 μCi of [γ-32P]ATP and 5 μg of GST-ATF-2 as substrate. After incubation for 30 min at 30 °C, the reaction was terminated by the addition of 4 × Laemmli sample buffer. Phosphorylated proteins were resolved on a 15% SDS-polyacrylamide gel electrophoresis gel, visualized by autoradiography, and quantitated by PhosphorImager analysis.

Electrophoretic Mobility Shift Assay (EMSA)—For detection of NF-κB and AP-1 DNA binding activities, cells were essentially processed as described (39). Binding reactions were performed in a 20-μl volume containing 3–4 μg of extract, 4 μl of 5 × binding buffer (20 mM HEPES, pH 7.5, 50 mM KCl, 2.5 mM MgCl2, 20% Ficoll), 2 μg of poly(dI-dC) as nonspecific competitor DNA, 2 μg of bovine serum albumin, and 10,000–15,000 cpm Cerenkov radiation of the labeled oligonucleotide. After a 30-min binding reaction at room temperature, samples were loaded on a 4% non-denaturing polyacrylamide gel and run in 0.5 × TBE buffer (89 mM Tris, 89 mM boric acid, 1 mM EDTA, pH 8.3). The sequences of the oligonucleotides used to detect the DNA binding activity of NF-κB and AP-1 were as follows (binding sites are underlined): NF-κB, 5′-AGCTTCAAGGGGATTTCCGAGAGG-3′; AP-1, 5′-CGGCTTGATGAGTCAGCCGGAA-3′. The oligonucleotides were annealed with their complementary strands and labeled using T4 polynucleotide kinase (Boehringer Mannheim) and [γ-32P]ATP. The labeled probes were purified from free nucleotides on push columns (Stratagene, Heidelberg, FRG).

RESULTS

Multiple Stress Inducers Activate p38 MAP Kinase without Concomitant NF-κB Activation—p38 MAP kinase is known to be activated by a variety of cellular stresses. To measure kinase activity we expressed GST-tagged p38 in 293 cells and determined p38 activity in a solid-phase assay with ATP-2 as substrate. The classical stress inducers such as the sulfhydryl agent sodium arsenite and hyperosmotic concentrations of sorbitol stimulated p38 activity to as much as 11- and more than 20-fold of the control, respectively (Fig. 1, A and C). p38 activity was also strongly induced by the phorbol ester PMA and to a lesser extent by TNF. The combined incubation with both TNF and PMA resulted in additive stimulatory effects. Treatment of cells with hydrogen peroxide caused only a moderate increase in p38 activity, and in some experiments stimulation by hydro-

2 K. von Schack, unpublished data.
gen peroxide was only slightly above basal activity. In parallel experiments we measured the activation of tran-
scription factor NF-κB, which is activated by various stimuli
many of those resembling p38 activators. In contrast to p38,
NF-κB was virtually not activated by sorbitol and sodium ar-
senite (Fig. 1, B and C). Maximum activation of NF-κB was
observed after treatment with TNF. Phorbol ester did not ac-
tivate NF-κB in 293 cells, although in other cell types activa-
tion by PMA was obtained. As reported earlier (32, 33), NF-
κB was also activated in response to hydrogen peroxide. We fur-
ther analyzed the effects of okadaic acid (OA) and thapsigargin (TG) on the activation of p38 (top panel) and NF-κB (bottom panel). 293 cells were treated for 1 h with the indicated concentrations of each drug. Only sections of the fluorograms are shown.

FIG. 1. Differential activation of p38 MAP kinase and NF-κB in response to stress-inducing agents. A, 293 cells were transiently transfected by calcium phosphate precipitation with an expression vector encoding GST-tagged p38 MAP kinase or the empty vector pEBG (Mock). After 18 h, cells were stimulated for 30 min with PMA (50 ng/ml), TNF (10 ng/ml), sodium arsenite (0.5 mM), sorbitol (400 mM), H2O2 (200 μM), or left untreated (Medium). p38 activity was measured in a solid-phase assay with [γ-32P]ATP and GST-ATF-2-(1–109) as substrate. Phospho-
rulyated ATF-2 was detected by autoradiography after 15% SDS-polyacrylamide gel electrophoresis. B, NF-κB activation was measured by EMSA using a radiolabeled oligonucleotide encompassing the NF-κB consensus motif from the human immunodeficiency virus, type 1 long terminal repeat. Specificity of NF-κB DNA binding was verified by competition analysis with an excess of unlabeled wild-type and mutated κB-specific oligonucleotides as well as by supershift analysis with antibodies against NF-κB subunits (data not shown, see Ref. 37). C, the rate of p38 and NF-κB activation was quantitated by PhosphorImager analysis and is presented as activity relative to the medium control. The experiments were repeated twice with essentially similar results. D, effects of okadaic acid (OA) and thapsigargin (TG) on the activation of p38 (top panel) and NF-κB (bottom panel). 293 cells were treated for 1 h with the indicated concentrations of each drug. Only sections of the fluorograms are shown.
the subunit composition or requirement of de novo protein synthesis in response to each individual stress stimulus. Based on previous reports it can be presumed that activation of AP-1 by PMA, TNF, and other cellular stressors involves different types of MAP kinases targeting distinct AP-1 subunits. Thus, physicochemical stresses preferentially lead to ATF-2 activation, whereas phorbol ester and TNF mainly activate c-Jun (41, 42). The experiments demonstrate that, in contrast to NF-κB, a larger variety of extracellular stimuli converge at the level of AP-1.

**p38 MAP Kinase and NF-κB Activation Involve Distinct Signaling Pathways**—The activation of both NF-κB and AP-1 is controlled by redox-sensitive signaling pathways (30). NF-κB is a prooxidant-inducible factor whose activation is inhibited by a variety of antioxidants. In contrast, antioxidants have been shown to increase the activation of AP-1 by inducing c-fos and c-jun transcription (43, 44). To investigate the involvement of ROIs in p38 activation we used the antioxidant pyrrolidine dithiocarbamate (PDTC), an iron chelator and glutathione precursor. Fig. 4A demonstrates that preincubation of 293 cells with PDTC considerably increased p38 kinase activity in response to all stimuli analyzed. Consistent with this and previous reports, AP-1 activation was also enhanced by PDTC. In contrast, NF-κB activation in response to TNF, when measured in identical extracts, was completely abolished (Fig. 4A). The data demonstrate that p38 and NF-κB are oppositely regulated by redox-dependent processes. Similar to AP-1, p38 activity is enhanced by antioxidants.

To investigate whether antioxidants have a direct effect on p38 kinase activity, we incubated p38 extracted from PMA-treated cells with PDTC. Virtually no change in kinase activity was observed following treatment of p38 with different concentrations of PDTC (Fig. 4B) or glutathione (data not shown) in vitro. This shows that antioxidants could potentiate p38 activity only in intact cells.

We further sought to dissect the signaling pathways by studying the involvement of GTPase proteins. The small GTPases Rac and Cdc42 have recently been identified as important intermediates to the JNK and p38 activation cascades (4, 5). The activity of Rac and Cdc42 but not of Ras is specifically inhibited by enterotoxin toxin A from *C. difficile* (45). Pretreatment of 293 cells with toxin A caused a strong inhibition of p38 activation indicating the involvement of Rac and Cdc42 in p38 kinase activation (Fig. 4A). In line with the previous results, NF-κB activation was also inhibited by toxin A (Fig. 4A) in response to all stimuli. Virtually no effect was observed for NF-κB suggesting that signals leading to NF-κB activation are not transduced through Rho-related GTPases.

**Overexpression of p38 and ATF-2 Does Not Cooperate with NF-κB Transactivating Activity**—In the previous experiments it could not be excluded that additional components lying in the p38 kinase pathway cooperate with NF-κB-mediated transactivation. We therefore overexpressed p38 and ATF-2 in 293 and HeLa cells and investigated the effects on expression of a NF-κB-controlled reporter gene. Transfections with different concentrations of the p38 encoding plasmid did not markedly change NF-κB activity in unstimulated or stimulated cells (Fig. 5). Likewise, overexpression of increasing amounts of ATF-2, either alone or in combination with p38, was ineffective in enhancing expression of the NF-κB-controlled luciferase construct (Fig. 6). In contrast, transfection of a NF-κB p65-encoding construct, which served as a positive control, strongly increased NF-κB-mediated transactivation (Fig. 6A).

Effects of p38 Inhibition and MKK6 on NF-κB-controlled

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**Fig. 2. Substrate specificity of p38 MAP kinase.** Bacterially expressed Gal4, Gal4-p50, Gal4-p65, GST-1xB, GST, and GST-ATF-2 (each 5 μg of protein) were incubated with [γ-32P]ATP and p38 MAP kinase expressed in PMA-stimulated 293 cells. The phosphorylation reaction was terminated after 30 min by the addition of Laemmli buffer, resolved by 12.5% SDS-polyacrylamide gel electrophoresis, and visualized by autoradiography.

**Fig. 3. Activation of AP-1 in response to different stress-inducing agents.** The same extracts of cells used in Fig. 1, panels A and B were incubated with a radiolabeled AP-1-specific oligonucleotide and analyzed for AP-1 activation by EMSA. AP-1 DNA binding activity was detected by autoradiography (A) and quantitated by PhosphorImager analysis (B).
Gene Expression—The activity of p38 is specifically inhibited by the pyridinyl imidazole SB203580. In 293 cells we found that SB203580 also strongly prevented expression of a NF-κB-controlled reporter gene in response to a combination of PMA and TNF (Fig. 7B). As measured in EMSA electrophoretic mobility shift assay, SB203580, however, was unable to prevent activation of NF-κB DNA binding activity (Fig. 7B).

We further studied the effects of the dual specificity kinase MKK6, which has been identified as a selective activator of p38 (25, 46). Expression of a kinase inactive MKK6 mutant abolished p38 activation triggered by PMA and TNF (Fig. 8A). In parallel experiments, a potent inhibition of NF-κB transactivating activity was also observed (Fig. 8B). Collectively, these results therefore indicate a complex mode of regulation of p38 and NF-κB. As suggested by the differential activation pattern of both molecules, triggering of NF-κB by the release of IκB in the cytoplasm involves effectors other than p38 activation. Convergence may occur further downstream in the cell nucleus, either by effects of p38 on NF-κB-mediated transactivation or post-transcriptional mechanisms.

DISCUSSION

NF-κB represents a prototypic stress-responsive transcription factor that is activated by a plethora of noxious conditions that also cause activation of MAP kinases. In the course of activation, its inhibitor IκB is phosphorylated and proteolytically degraded thereby allowing the DNA binding NF-κB dimer to enter the cell nucleus. Although the critical protein kinase mediating this key event is unknown, various kinases including Src, Raf, protein kinase C isoforms e and f, and a proteasome-associated kinase have been proposed to either directly or indirectly participate in this process (reviewed in Refs. 29 and 47). Molecules of the MAP kinase modules have further been implicated in NF-κB activation. Activation of JNK by upstream MEK kinase (MEKK-1) resulted in NF-κB activation, and kinase inactive MEKK-1 blocked TNF-induced NF-κB activity in some cellular models (48). However, since this manuscript was submitted, it has been shown that at least in some cell types JNK and NF-κB activation are two separate TNF responses (49, 50). In addition, p38 may be linked to NF-κB activation since a specific pharmacological inhibitor of p38 blocked NF-κB-dependent transactivation (Ref. 24 and present data).

In this study we demonstrate that the primary signaling pathways leading to p38 and NF-κB activation can be dissociated, since many stimuli activating either of these molecules are not overlapping. Although several stress-inducing agents such as cytokines, Taxol, mitogens, and UV irradiation activate both p38 and NF-κB (6, 13, 17, 19, 30), in particular, efficient agonists of p38 including arsenite and hyperosmotic shock virtually do not activate NF-κB. Also other classical stimuli of stress-responsive MAP kinases such as DNA-damaging agents or heat shock were not found to trigger NF-κB activation. This observation indicates that no common cellular stress sensor or denominator of a stress response exists where different forms
of cellular stress converge. A distinct responsiveness to cellular stresses is underlined by the fact that PMA, which is a potent NF-κB activator in several cell types, selectively induced p38 but not NF-κB activation in 293 cells. In contrast to NF-κB many stress stimuli were found to lead to the activation of AP-1. This convergence of multiple stress stimuli at the level of AP-1 is reasonable, because different forms of stimuli preferentially affect distinct MAP kinase cascades (9, 41, 42). These in turn all modulate AP-1 activation at different control points including regulation at the transcriptional and post-translational level and AP-1 subunit composition. The involvement of p38 in induction of AP-1 has been implicated recently by the finding that inhibition of p38 kinase prevented the synthesis of c-Fos and c-Jun in response to stress stimuli (26).

ROI formation has been considered as a common denominator involved in signal transduction of a variety of different forms of stress. Indeed, activation of NF-κB by all stimuli analyzed so far is inhibited by antioxidants implying that ROIs are the key messengers for NF-κB activation (30, 31). Activation of ERK1 and ERK2, as well as JNKs in response to growth factors, and cytokines has also been shown to be inhibited by antioxidants (51, 52). Thus, we were surprised to find that p38 activity was not inhibited but even augmented by antioxidants. An increase in p38 activity was paralleled by an antioxidant-induced activation of AP-1 confirming earlier findings that several unrelated antioxidants cause AP-1 activation (43, 44). Similar to AP-1 (43), p38 was also modestly activated by hydrogen peroxide. This is in line with reports showing that, in contrast to JNKs and ERKs, the p38 pathway is only weakly

![Image](http://www.jbc.org/)

**Fig. 6.** Effect of ATF-2 overexpression on NF-κB-mediated transactivation. 293 cells (A) and HeLa cells (B) were transiently transfected with different concentrations of an expression construct encoding ATF-2 together with the NF-κB-dependent luciferase vector (0.5 μg/plate). In the titration experiments 125, 250, 500, and 1000 μg of ATF-2 construct were used, and the plasmid concentrations were adjusted to 2 μg/plate with the parental vector pJ6 (9). In some experiments, expression constructs (1 μg) of NF-κB p65 and p38 were included in the transfection mixture. Cells were stimulated with TNF (10 ng/ml) and PMA (50 ng/ml) and processed for measurement of NF-κB reporter gene activity as described in Fig. 5. The experiments were repeated twice with similar results.

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**Fig. 7.** Inhibition of NF-κB transactivating but not DNA binding activity by the p38-specific inhibitor SB203580. A, prevention of NF-κB-dependent reporter gene expression. 293 cells were transfected with the NF-κB-controlled luciferase construct as described in Fig. 5. 18 h later, cells were pretreated for 90 min with the indicated drug concentrations and then either left untreated or stimulated with PMA and TNF for additional 6 h. Mean values ± S.D. of NF-κB activity indicated as relative light units (RLU) from three experiments are shown. B, effect on p38 activation (top panel) and NF-κB DNA binding (bottom panel). p38 was expressed in 293 cells as described in Fig. 1. After 18 h cells were pretreated for 90 min with the indicated concentrations of SB203580 and then either left untreated or stimulated for 45 min with PMA and TNF. Only sections of the fluorograms are shown.

![Image](http://www.jbc.org/)

**Fig. 8.** The effect of expression of a dominant-negative MKK6 mutant on NF-κB and p38 activity. A, the effect of dominant-negative MKK6 on the expression of a NF-κB-controlled luciferase gene is shown. The indicated amounts of the MKK6 (K82A) mutant construct were cotransfected with 0.5 μg of the NF-κB reporter gene. Cells were then processed as described in Fig. 5. NF-κB-transactivating activity is shown as mean ± S.D. of relative light units (RLU) from four experiments. B, inhibition of p38 activation is shown. The indicated amounts of the MKK6 mutant construct were cotransfected with the p38 expression plasmid. The total amount of transfected DNA was adjusted to 2 μg/plate with parental plasmid. After 18 h, 293 cells were either left untreated or stimulated for 60 min with PMA and TNF.
activated by several prooxidants such as hydrogen peroxide, nitric oxide, or organic peroxides (53, 54). Although many stimulatory conditions of JNKs overlap that of p38, there are other examples of distinct modes of regulation (55, 56). A physiological correlate of antioxidant treatment may be hypoxia. Interestingly, it has been observed that in hypoxia-reperfusion treatment of cells JNKs are specifically activated during reperfusion where ROI formation occurs, whereas p38 is only activated in initial hypoxia (57, 58). Thus, a shift in the cellular redox balance may allow cells to rather selectively trigger p38 or the JNK and ERK pathways.

An independent regulation of the pathways leading to activation of p38 and NF-κB was found by studying the involvement of small GTPase proteins. Members of the Rho subfamily such as Rac and Cdc42 that mediate p38 and JNK activation (4, 5) are specifically inhibited by the Clostridium enterotoxin toxin A (45). Whereas toxin A did not interfere with NF-κB DNA binding activity, p38 activation was strongly reduced.

Although we show that p38 and NF-κB activation are not correlated indicating divergent primary pathways, our results also suggest that convergence may occur further downstream. Thus, a dominant-negative mutant of MKK6 as well as a p38-specific pharmacological inhibitor strongly abrogated NF-κB reporter gene assays. In these experiments we did not find an increase of NF-κB DNA binding activity, p38 activation was strongly reduced. Therefore, a dominant-negative mutant of MKK6 as well as a p38-specific pharmacological inhibitor strongly abrogated NF-κB DNA binding activity, p38 activation was strongly reduced.

Motivated indicators of JNKs overlap that of p38, there are other examples of distinct modes of regulation (55, 56). A physiological correlate of antioxidant treatment may be hypoxia. Interestingly, it has been observed that in hypoxia-reperfusion treatment of cells JNKs are specifically activated during reperfusion where ROI formation occurs, whereas p38 is only activated in initial hypoxia (57, 58). Thus, a shift in the cellular redox balance may allow cells to rather selectively trigger p38 or the JNK and ERK pathways.

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Separate Pathways of NF-κB and p38 MAP Kinase Activation

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Activation of Transcription Factor NF-κB and p38 Mitogen-activated Protein Kinase Is Mediated by Distinct and Separate Stress Effector Pathways

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