Activation of Mitogen-activated Protein Kinase Pathways Induces Antioxidant Response Element-mediated Gene Expression via a Nrf2-dependent Mechanism*

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Antioxidant response element (ARE) regulates the induction of a number of cellular antioxidant and detoxifying enzymes. However, the signaling pathways that lead to ARE activation remain unknown. Here, we report that the expression of mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK) kinase 1 (MEKK1), transforming growth factor-β-activated kinase (TAK1), and apoptosis signal-regulating kinase (ASK1) in HepG2 cells activated the ARE reporter gene, whereas the expression of their dominant-negative mutants impaired ARE activation by the chemicals sodium arsenite and mercury chloride. Coexpression of downstream kinases, MAP kinase kinase 4, MAP kinase kinase 6, and c-Jun NH2-terminal kinase-1, but not MAP kinase kinase 3 and p38, augmented ARE activation by MEKK1, TAK1, and ASK1. The coexpression of a basic leucine zipper transcription factor Nrf2 but not c-Jun also greatly enhanced the activation of reporter gene by MEKK1, TAK1, and ASK1; however, a dominant-negative mutant of Nrf2 (NP-E2-related factor 2) blocked this event. Furthermore, when overexpressed, MEKK1, TAK1, and ASK1 induced the expression of heme oxygenase-1, a gene regulated by ARE, and the cotransfection with the dominant-negative mutant of Nrf2 abolished the induction. Taken together, these results suggest that MAP kinase pathways that are activated by MEKK1, TAK1, and ASK1 may link chemical signals to Nrf2, leading to the activation of ARE-dependent genes.

Mitogen-activated protein kinases (MAPKs) belong to the family of serine/threonine kinases and play a central role in coupling various extracellular signals to a variety of biological processes, such as gene expression, cell proliferation, differentiation, and cell death (1, 2). To date, at least six MAPK members have been identified in mammalian cells. Three of the MAPK members have been extensively studied: extracellular signal-regulated kinases (ERK) (3), c-Jun NH2-terminal kinases (JNKs, also called stress-activated protein kinases) (4, 5), and p38 (6, 7). The activity of these MAPKs is induced through the phosphorylation of their threonyl and tyrosyl residues within a tripeptide motif TXY by a dual specificity kinase termed MAP kinase (MKK), which in turn is phosphorylated and activated by an upstream kinase generally called MAP kinase kinase (MAPKK) (8). The first MAPKK identified is Raf-1. This kinase activates ERK through MEK1 or MEK2 but has little effect on JNK and p38 pathways (1). However, unlike Raf-1 MEKK1, a MAPKK isolated after Raf-1 predominantly stimulates JNK activity that is mediated by MKK4 or MKK7 (9). Recently, several other members of the MAPKK family have been identified including TAK1 (10) and ASK1 (11). These kinases are able to activate JNK through MKK4 and p38 through MKK3 or MKK6, but they do not affect the ERK pathway. Although MEKK1, TAK1, and ASK1 may regulate different downstream kinases, they are preferentially activated by various stress stimuli and are involved in the regulation of stress responses.

Exposure of human and rodent cells to phenolic antioxidants or certain electrophiles leads to the protection against the toxic and neoplastic effects of many carcinogens (12, 13). Such a chemopreventive property of these compounds has been presumably ascribed to the ability of the compounds to induce several detoxifying enzymes, such as glutathione S-transferase (GST) and quinone reductase (14). Subsequent studies revealed that the induction of detoxifying enzyme activity occurs at the transcriptional level and is regulated by a cis-acting element that is present in the promoters of detoxifying enzyme genes defined as an antioxidant response element (ARE) (15) or electrophilic response element (16). Later, ARE was also found in the promoters of genes that encode cellular antioxidant enzymes, such as γ-glutamylcysteine synthetase (17), heme oxygenase-1 (HO-1) (18), and ferritin L and H chains (19), and was activated by various oxidative stresses. Thus, ARE is not only responsive to antioxidants but also to oxidative stress. Understanding the mechanism by which ARE senses different signals will be of considerable interest. Because the ARE core sequence (GTGACNNNGC) shows a similarity with the activating protein-1 glutinin; TBS, Tris-buffered saline; HO-1, heme oxygenase-1; DN, dominant-negative; AP-1, active protein 1.

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(AP-1)-binding site (TGACTCA), a number of studies have examined the roles of AP-1 proteins such as c-Jun and c-Fos in the activation of ARE and have yielded controversial results (20–23). However, several lines of present evidence indicate that another member of the basic leucine zipper transcription factor family, Nrf2 (NF-E2-related factor 2), may be involved in the activation of ARE. First, Nrf2 is able to bind to the ARE in association with other members of the basic leucine zipper transcription factor family, such as Maf G or Maf K (24). Second, the transient expression of Nrf2 transactivates the ARE-linked reporter gene (17, 18, 25). Furthermore, the Nrf2 knockout mice (Nrf2−/−) show a defect in the induction of GST by butylated hydroxyanisole (24) and are extremely susceptible to the pulmonary injury induced by butylated hydroxytoluene (26).

Although progress has been made in the identification of an ARE-binding protein, the signal transduction pathways that are able to activate the Nrf2/ARE complex remain unknown. Previously, we have demonstrated that blocking the ERK pathway attenuates the induction of ARE-mediated gene expression by tert-butylhydroquinone and sulforaphane (27), whereas the inhibition of the p38 pathway shows an opposite effect (28) implying the involvement of MAPKs in the modulation of ARE-mediated gene expression. In this study, we explored the role of MAPK pathways that are preferentially activated by the stress stimuli in the regulation of ARE activity. As described below, the activation of MAPK pathways by the overexpression of MEKK1, TAK1, or ASK1 induced ARE reporter gene activity, which was mediated by a Nrf2-dependent mechanism. Activation of ARE by MEKK1, TAK1, and ASK1 also involved MKK4, MKK6, and JNK1 but not MKK3 and p38.

MATERIALS AND METHODS

Cell Culture, Antibodies, and Chemicals—Human hepatoma HepG2 cells were obtained from ATCC (Manassas, VA) and maintained as monolayer cultures in F12 medium supplemented with 10% fetal bovine serum, 1.7 mg/ml sodium bicarbonate, 0.1 unit/ml insulin, 0.5 mM minimal essential medium amino acid, 100 units/ml penicillin, and 100 μg/ml streptomycin. For experiments, HepG2 cells were cultured in minimal essential medium supplemented with 10% fetal bovine serum, 2.2 mg/ml sodium bicarbonate, 100 units/ml penicillin, and 100 μg/ml streptomycin. The mouse anti-HA monoclonal antibody (12CA5) was purchased from Roche Molecular Biochemicals. Polyclonal rabbit antibodies against human heme oxygenase-1 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). γ32P ATP (6000 Ci/mmol) was purchased from PerkinElmer Life Sciences. Myelin basic protein, tert-butylhydroquinone, sodium arsenite, and mercury chloride were purchased from Sigma.

Expression Constructs and Fusion Proteins—pcDNA3-HA-JNK1 was kindly provided by Dr. Michael Karin (University of California, San Diego, CA). pcDNA3-HA-ASK1 and pcDNA3-HA-ASK1(K709R) were gifts from Dr. Tatyana A. Voyno-Yasenetskaya (University of Illinois). pcDNA3-HA-MKK6 and pcDNA3-HA-MKK6(DN) were kindly provided by Dr. Zhengbin Yao (Amgen, Inc., Boulder, CO), pcDNA3-FLAG-MKK4, pcDNA3-FLAG-MKK4(Ala), pRSV-FLAG-MKK3, and pRSV-FLAG-MKK3(Ala) were kindly provided by Dr. Roger J. Davis (University of Massachusetts, Worcester, MA). pcDNA1.1-Nrf2 construct was kindly provided by Dres. Yuet W. Kan and Jeffery Y. Chan (University of California, San Francisco, CA). pcDNA3.1-c-Jun and pcDNA3.1-c-Jun (TAM 67) were gifts from Dr. Michael J. Birrer (National Institutes of Health). pARE-TI-luciferase reporter construct (containing a single copy of the 41-base pair murine GST-Ya ARE and a minimal TATA-Inr promoter) was described previously (27). p3T-TP-luciferase reporter construct containing three 12-O-tetradecanoylphorbol-13-acetate-responsive elements was kindly provided by Dr. John Massague (Memorial Sloan-Kettering Cancer Center). pcDNA3-HA-MKK1(Ala), pcDNA3-FLAG-MKK1(Ala), pcDNA3-FLAG-MKK1(Ala), pRSV-FLAG-MKK1, pRSV-FLAG-MKK3, pRSV-FLAG-MKK3(Ala), and the fusion proteins GST-c-Jun(1–76) and GST-activating transcription factor 2(1–96) were described previously (27–30).

To generate a dominant-negative Nrf2 mutant, a DNA fragment that contains a Cap’n/homolog collar region and a basic leucine zipper domain (amino acids 399–589) was amplified by polymerase chain reaction using the following primers: 5′-AACGTTACTACTAAGCTGATGAGTGGCCTAAG-3′ (sense) and 5′-CCTCGGCTAGATTTGATTTAATCTGC- G-3′ (antisense). The amplified fragment was cloned into pCR2.1 (Invitrogen) and then inserted in-frame into pEGFP-C3 at HindIII/SstI sites (CLONTECH).

Transient Transfection and Reporter Gene Activity Assays—HepG2 cells were plated in six-well plates at a density of 1.5 × 105 cells/well. 24 h after plating, cells were transfected with expression vectors using the calcium phosphate precipitation method as indicated in the figure legends. In each transfection, 0.5 μg of pCH110-β-galactosidase plasmid and 1 μg of ARE-TI-luciferase reporter construct were used, and the total amount of DNA in each well was adjusted to 5.5 μg with empty vector. Cells were incubated with transfection mixtures for 7 h and then cultured in fresh medium for an additional 36 h before harvesting. β-Galactosidase activity was determined as described previously (31). Luciferase activity was determined by the method provided by the manufacturer (Promega, Madison, WI). After treatment, cells were washed twice with ice-cold phosphate-buffered saline and harvested in 1× reporter lysis buffer. After brief centrifugation, a 20-μl supernatant was aliquoted and assayed for luciferase activity with a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). The luciferase activity was normalized against β-galactosidase activity.

Immunocomplex Kinase Assays of ERK, JNK, and p38—HepG2 cells were plated in 10-cm-diameter plates at a density of 105 cells/plate. Cells were transfected as described above except that the total DNA in 25 μg. After the transfection, cells were washed twice with ice-cold phosphate-buffered saline and harvested in a cell lysis buffer containing 10 mM Tris-HCl, pH 7.1, 50 mM NaCl, 50 mM NaF, 30 mM Na3P04, 100 μM Na3VO4, 5 μM ZnCl2, 2 mM iodoacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 0.5% Triton X-100. Cell lysates were homogenized by passing through a 23-gauge needle three times and cleared by centrifugation at 12,500 × g for 15 min at 4 °C. HA-tagged ERK, JNK, or p38 was immunoprecipitated with anti-HA antibody in the presence of protein A-Sepharose 4B conjugate (Zymed Laboratories Inc., San Francisco, CA) and assayed for kinase activity as described previously (27). The immunoprecipitated kinase was resuspended in a 30-μl kinase assay buffer (20 mM HEPES, pH 7.5, 10 mM MgCl2, 2 mM MnCl2, 0.1 mM Na3VO4, 50 mM β-glycerophosphate, and 1 mM dithiothreitol) and incubated with [γ32P]ATP, 20 μM ATP, and 5 μg of the indicated substrates. After incubation at 30 °C for 30 min in JNK1 and p38 assay or 15 min in ERK2 assay, kinase reaction was terminated by adding 10 μl of 4× Laemml buffer and heating at 95 °C for 5 min. Phosphorylated products were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

Western Blotting—Cell lysates were prepared as described in immunocomplex kinase assays. 25 μg of protein, as determined by the Bradford method, was resolved by 10% SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

RESULTS

Overexpression of MEKK1, TAK1, and ASK1 Induces ARE-mediated Gene Expression, Whereas Their Dominant-negative Mutants Diminished ARE Activation by Chemicals—MAPK pathways are activated through serial phosphorylation. To address the roles of MAPKs in the activation of ARE-dependent genes, we focused on the upstream kinases, MEKK1, TAK1, and ASK1. Because MEKK1 and ASK1 but not TAK1 are highly active when overexpressed (11, 32), we examined the effects of cotransfection with the wild-type MEKK1 or ASK1 or a constitutively active TAK1 mutant, TAK1ΔN, on the activity of the ARE reporter gene. As shown in Fig. 1A, overexpression of MEKK1, TAK1ΔN, and ASK1 in HepG2 cells stimulated ARE reporter gene activity in a dose-dependent manner. To demonstrate a specific role of ARE in this event, we also con-
ARE is activated by a variety of chemicals. Activation of ARE by MEKK1, TAK1, and ASK1 implicates a potential role of these kinases in the ARE activation by exogenous stimuli. To address this issue, we examined the effect of their dominant-negative mutants on the activation of ARE-dependent reporter gene by several electrophilic compounds. Although enforced expression of dominant-negative mutants of MEKK1, TAK1, or ASK1 did not affect ARE activation by tert-butylhydroquinone, these mutants significantly diminished the induction of reporter gene activity by sodium arsenite and to a lesser extent, decreased the reporter gene activity induced by mercury chloride (Fig. 1C). This result suggests that the activation of MEKK1-, TAK1-, or ASK1-dependent pathways may contribute to the induction of ARE-mediated gene expression by some chemicals.

**JNK and p38 Show Opposite Effects on the Activation of ARE Reporter Gene by MEKK1, TAK1, and ASK1—Activation of MEKK1, TAK1, and ASK1 leads to the activation of downstream MAPKs. To identify the MAPKs that were activated in the cells overexpressing the MEKK1, TAK1AN, and ASK1, we performed transient transfection with the vectors encoding HA-tagged ERK2, JNK1, or p38 and different forms of MEKK1, TAK1, or ASK1. The kinase activity of ERK2, JNK1, and p38 was then analyzed by immunocomplex kinase assays with anti-HA antibody. Cotransfection of HepG2 cells with MEKK1, TAK1AN, or ASK1 resulted in substantial activation of JNK and p38 but not ERK2, although a modest induction of ERK2 activity was observed in MEKK1-transfected cells as compared with vector-transfected cells (Fig. 2A). As a negative control, cotransfection with the kinase-defective mutants, MEKK1-KR, TAK1-KW, or ASK1-KR, did not activate JNK and p38. Furthermore, Western blotting analysis with anti-HA antibody revealed an equal expression level of epitope HA-ERK, HA-JNK, and HA-p38 in different transfections. Thus, the overexpression of MEKK1, TAK1AN, and ASK1 preferentially activated JNK and p38 pathways but not ERK.

Because both JNK and p38 were activated by three MAPKKks, we next examined the roles of JNK and p38 in ARE activation by MEKK1, TAK1, and ASK1. As shown in Fig. 2B, the coexpression of JNK1 enhanced the activity of the ARE reporter gene that was induced by all three MAPKKks. Consistent with such a positive role of JNK, the cotransfection with JNK1(APF), a dominant-negative mutant of JNK1, suppressed the activation of ARE reporter gene by the three MAPKKks. However, unlike JNK, the expression of wild-type p38 inhibited the induction of the ARE reporter gene by MEKK1, TAK1AN, and ASK1, whereas a dominant-negative mutant of p38, p38so(AGF), potentiated such induction. This result substantiates a negative role of p38 in the ARE-mediated induction of phase II detoxifying enzymes reported previously by Yu et al. (28).

**Activation of ARE-dependent Genes by MEKK1, TAK1, and ASK1 Involves the Downstream Kinases, MKK4 and MKK6—**MEKK1 can directly phosphorylate and activate MKK4 that, in turn, activates JNK and p38 pathways but not ERK. Hence, we asked whether MKK3, MKK4, and MKK6 are involved in the ARE activation by MEKK1, TAK1, and ASK1. As shown in Fig. 3A, the expression of MKK4 and MKK6 caused the induction of ARE-mediated gene expression in a dose-dependent fashion. In contrast, the expression of MKK3 decreased the basal activity of the ARE reporter gene.

To provide further evidence for the roles of MKK3, MKK4, and MKK6 in the MEKK1-, TAK1-, and ASK1-induced ARE activation, we used the dominant-negative mutants. Coexpression of a dominant-negative MKK4 mutant (MKK4(Ala)) di-
The activation of ARE reporter gene by all three MAPKKKs (Fig. 3B). Coexpression of a dominant-negative mutant of MKK6 (DN-MKK6) reduced the ARE activation by TAK1ΔN and ASK1 but not by MEKK1 (Fig. 3B). This result suggests that the activation of ARE by MEKK1 involves MKK4, whereas the activation of ARE by TAK1 and ASK1 may involve both MKK4 and MKK6. Unlike MKK4(Ala) and DN-MKK6, MKK3(Ala), a dominant-negative mutant of MKK3, augmented the induction of ARE reporter gene by TAK1ΔN, ASK1, and, to a lesser extent, MEKK1 (Fig. 3B), supporting a negative role of MKK3 in the regulation of ARE-mediated gene expression as described previously (28).

**Activation of ARE-dependent Genes by MEKK1, TAK1, and ASK1 Is Not Mediated by the Transcription Factor c-Jun but Is by Nrf2—** After demonstrating the downstream signaling kinases involved in the activation of ARE by MEKK1, TAK1, and ASK1, we sought to identify the transcription factors that convey the kinase signaling to the ARE. A well known target of these MAPK pathways is c-Jun (33) that has also been shown to bind to the ARE sequence (20). Accordingly, we examined the role of c-Jun in ARE-mediated gene expression induced by MEKK1, TAK1, and ASK1. HepG2 cells were cotransfected with c-Jun or its dominant-negative mutant (TAM 67) and with different MAPKKKs. Subsequently, the induction of ARE reporter gene was analyzed by luciferase activity assays. As shown in Fig. 4A, the expression of c-Jun alone induced approximately a 2-fold increase in the activity of ARE reporter gene as compared with vector-transfected cells. However, the coexpression of c-Jun had no effect on the activation of ARE reporter gene by MEKK1, TAK1, and ASK1, and, to a lesser extent, the coexpression with TAM 67 inhibited such activation (Fig. 4B). However, the induction of ARE reporter gene by c-Jun was modest, and more interestingly, a relatively higher amount of c-Jun (more than 2 μg) suppressed the ARE reporter gene activity (Fig. 4B). Thus, we conclude that c-Jun is unlikely to be a mediator of MEKK1-, TAK1-, or ASK1-induced expression of ARE-dependent genes.

As discussed earlier, accumulating evidence indicates that a basic leucine zipper transcription factor Nrf2 plays an essential role in ARE-mediated induction of phase II detoxifying enzymes (34, 35). We next explored the role of Nrf2 in mediating the activation of ARE by MEKK1, TAK1, and ASK1. Upon overexpression, Nrf2 induced dose-dependent activation of the ARE reporter gene (Fig. 5A). This transcriptional activity of Nrf2 was dramatically enhanced by cotransfection with...
MEKK1, TAK1ΔN, or ASK1 (Fig. 5B). More interestingly, transfection with a dominant-negative Nrf2 mutant (DN-Nrf2) that lacks the N-terminal transactivation domain not only inhibited ARE basal activity but also abolished ARE activation by MEKK1, TAK1ΔN, and ASK1 (Fig. 5B). Similar results were obtained when Nrf2or its dominant-negative mutant (DN-Nrf2) was cotransfected with MKK4 or MKK6 (Fig. 5C). However, MKK3 inhibited Nrf2-induced expression of ARE reporter gene (Fig. 5C), consistent with its negative role in ARE gene expression. These data suggest that the activation of ARE by MEKK1, TAK1, ASK1, and the downstream kinases is mediated by a Nrf2-dependent mechanism.

Overexpression of MEKK1, TAK1, and ASK1 Induces Nrf2-dependent Expression of HO-1—ARE regulates the induction of a number of antioxidant and detoxifying enzymes, such as GST, quinone reductase, and HO-1. To demonstrate the physiological relevance of ARE activation by MEKK1, TAK1, and ASK1, we examined the induction of HO-1 in HepG2 cells transfected with these MAPKKks. As shown in Fig. 6A, when overexpressed, MEKK1, TAK1, and ASK1 induced the expression of HO-1 as revealed by Western blotting analysis. Treatment with sodium arsenite also induced HO-1 expression, which has been shown by others (18, 35). Coexpression of a dominant-negative mutant of Nrf2 (DN-Nrf2) abolished the MEKK1 induction of HO-1 (Fig. 6B). Consistent with the previous result (18), DN-Nrf2 also blocked the induction of HO-1 by sodium arsenite (Fig. 6B). A similar result was obtained when TAK1 or ASK1 was cotransfected with DN-Nrf2 (data not shown). Therefore, MEKK1, TAK1, and ASK1 are able to activate endogenous genes that are regulated by ARE and Nrf2.

**DISCUSSION**

In this study, we provide evidence for the roles of MAP kinase pathways in the activation of ARE-dependent genes. The expression of MEKK1, TAK1, and ASK1 induces ARE reporter gene expression, which is potentiated by the coexpression of downstream kinases, MKK4, MKK6, and JNK1, but not by MKK3 or p38. Coexpression of a transcription factor Nrf2 also greatly enhanced ARE activation by MEKK1, TAK1, and ASK1, which was abolished by a dominant-negative mutant of Nrf2. In contrast, cotransfection with c-Jun or its dominant-negative mutant (c-Jun TAM 67) had no effect. Thus, this study demonstrates for the first time that the kinase pathways acti-
sodium arsenite. HepG2 cells were plated as in negative mutant of Nrf2 on the induction of HO-1 by MEKK1 and lane 6

for 36 h before challenging with 30 sodium arsenite treatment, cells were either transfected with vector). For

lanes 5 and 6) for 12 h. Cells were then analyzed for HO-1 expression by Western blotting as in lane 5

and ASK1. Thus, this study presents the signaling pathways that show the opposite effect, indicative of a positive role for JNK. Unlike JNK, however, when p38 was overexpressed, it not only inhibited ARE basal activity but also suppressed ARE activation by the MEKK1, TAK1, and ASK1. This result supports our previous observation that p38 acts as a negative regulator of the ARE-mediated induction of phase II detoxifying enzymes by different chemicals (28). In our previous studies, we also found that the ERK pathway but not JNK is potently activated by tert-butyldihydroquinone and sulforaphane. Blockade of ERK activation impairs the induction of quinone reductase and ARE reporter gene expression by the inducers (27), suggesting that the ERK and JNK pathways may link different signals to the ARE reporter gene. Therefore, differential activation of MAPKs, such as JNK and ERK versus p38, will have different consequences on the ARE-mediated gene expression and may provide a mechanism for controlling intracellular levels of protective enzymes after challenges with different stress stimuli.

FIG. 6. Nrf2-dependent induction of heme oxygenase-1 by MEKK1, TAK1, and ASK1. A, induction of HO-1 by overexpression of

western blotting. As a positive control, nontransfected cells were treated with 30 μM NaAsO2 (lane 5) for 12 h before harvesting. Western blotting of β-actin was also included as loading control. B, effect of dominant-negative mutant of Nrf2 on the induction of HO-1 by MEKK1 and

nodate arsenite. HepG2 cells were plated as in A and transfected with MEKK1 alone (lane 2) or cotransfected with DN-Nrf2 (lane 3). For

sodium arsenite treatment, cells were either transfected with vector (lane 4) or DN-Nrf2 (lane 6) for 36 h before challenging with 30 μM NaAsO2 (lanes 5 and 6) for 12 h. Cells were then analyzed for HO-1 expression by Western blotting as in A. The blots shown are examples of two separate experiments.

vated by MEKK1, TAK1, and ASK1 are able to induce ARE-mediated gene expression and suggests that the transcriptional activity of Nrf2 can be regulated as a phosphorylation mechanism. MEKK1, TAK1, and ASK1 are activated by a variety of stress stimuli, such as UV irradiation (29), osmotic stress (36), oxidative stress (37), anti-cancer agent (38), and pro-inflammatory cytokines (11). Although the precise biological roles of their activation are not clear, MEKK1, TAK1, and ASK1 have been implicated in the induction of apoptosis. For example, overexpression of MEKK1 has been shown to induce apoptosis in Swiss 3T3 and REF52 fibroblasts (39), whereas overexpression of a kinase-defective mutant of MEKK1 has been shown to block the apoptosis induced by nerve growth factor withdrawal (40) and anti-cancer agent chelerythrine (38). Activation of ASK1 has also been shown to be required for the apoptosis induced by tumor necrosis factor-α (11). In this study, we showed that the activation of MEKK1, TAK1, and ASK1 leads to the induction of ARE-mediated reporter gene expression, suggesting that these kinases can be involved in the regulation of genes that encode detoxifying or antioxidant enzymes, such as glutathione S-transferase, quinone reductase, heme oxygenase-1, and γ-glutamylcysteine synthetase. As discussed earlier, induction of these detoxifying enzymes facilitates the excretion of toxic compounds and the removal of reactive oxygen species, thereby protecting cells against chemical insults and oxidative stresses. In this respect, MEKK1, TAK1, and ASK1 may also regulate cell protective and/or survival signaling, contrasting their roles in the induction of apoptosis. The biological consequence of the activation of these signaling pathways may depend on the nature of stimuli and/or cellular context.

Although both JNK1 and p38 are activated by the overexpression of MEKK1, TAK1, and ASK1, our results indicate that they may play different roles in the regulation of ARE-mediated gene expression. Coexpression of JNK1 potentiates the induction of ARE reporter gene expression by MEKK1, TAK1, and ASK1, whereas its dominant-negative mutant JNK1(APF) shows the opposite effect, indicative of a positive role for JNK. In contrast to JNK, however, when p38 was overexpressed, it not only inhibited ARE basal activity but also suppressed ARE activation by the MEKK1, TAK1, and ASK1. This result supports our previous observation that p38 acts as a negative regulator of the ARE-mediated induction of phase II detoxifying enzymes by different chemicals (28). In our previous studies, we also found that the ERK pathway but not JNK is potently activated by tert-butyldihydroquinone and sulforaphane. Blockade of ERK activation impairs the induction of quinone reductase and ARE reporter gene expression by the inducers (27), suggesting that the ERK and JNK pathways may link different signals to the ARE reporter gene. Therefore, differential activation of MAPKs, such as JNK and ERK versus p38, will have different consequences on the ARE-mediated gene expression and may provide a mechanism for controlling intracellular levels of protective enzymes after challenges with different stress stimuli.

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