Mitogen-activated Protein Kinase Acts as a Negative Regulator of the Heat Shock Response in NIH3T3 Cells

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Abstract

Exposure of NIH3T3 cells to elevated temperatures induces the phosphorylation and activation of mitogen-activated protein (MAP) kinases [or extracellular signal-regulated kinases (ERKs)]. To investigate the significance of MAP kinase activation by heat shock, we examined the effect of inhibiting the activity of MAP kinase on heat shock protein 70 (hsp 70) expression. Overexpression of a dominant inhibitory mutant of ERK1, but not ERK2, in heat-shocked cells increased hsp70 reporter gene activity, suggesting that ERK1 acts as a repressor of hsp70 gene expression. Increases in ERK1 activity through treatment of cells with sodium vanadate (SV), an inhibitor of the dual-specificity MAP kinase phosphatase 1 (PAC1), resulted in increased phosphorylation of the heat shock transcription factor-1 (HSF-1) in unheated cells, delayed the activation of HSF-1 by heat shock, and inhibited the induction of hsp 70 by heat shock. Furthermore, the induction of thermotolerance was reduced significantly in cells that increased ERK1 activity by SV pretreatment. Immune complex kinase assays of heat shocked or SV-pretreated cells indicated that HSF-1 is a potential in vivo substrate for ERK1 phosphorylation. Taken together, these results suggest that agents that modulate MAP kinase act as negative regulators of the heat shock response in mammalian cells by modulating HSF-1 activity and hsp 70 expression.

Introduction

Activated Ras triggers a kinase cascade involving the serine/threonine kinase Raf-1 (1, 2), which phosphorylates and activates MEK3 (MAP/ERK kinase or MAP kinase kinase; Ref. 3), which then phosphorylates and activates MAP kinase via tyrosine and threonine phosphorylation (4). Therefore, constitutive activation of Ras or Raf-1 permits the cell to escape the regulation of normal growth factor control. In addition to growth factor regulation, MAP kinases have been shown to be induced by cellular exposure to free radical-generating agents, such as ionizing radiation, hydrogen peroxide, UV light, low oxygen conditions, and heat shock, indicating that MAP kinases respond also to non-growth-factor-mediated regulation as well (5–8). As a genetic means to demonstrate the role of MAP kinases in cell signaling after stress, inhibitory mutants of ERK1 and ERK2 were used to show that Ets-1 and API, but not c-Jun, activation was a direct consequence of MAP kinase activation (9). These dominant negative MAP kinases seemed to be specific in their activity, because they did not interfere with the pathway involved in the activation of the nuclear factor kB transcription factor, a pathway shown to be independent of MAP kinase activity (8).

One of the ways MAP kinases and their downstream effectors may modulate the heat shock response is through the regulation of HSF-1. Genes responding to a variety of stresses contain HSEs in their promoter regions that are composed of several NGAA repeats (10–14). Heat-activated HSF-1 binds in a sequence-specific manner to a HSE that results in increased transcriptional activity of heat shock genes (12, 14–20). Structurally, HSF-1 under unstressed growth conditions is a monomer that exhibits a low level of phosphorylation (17, 21, 22). In heat-shocked cells, HSF-1 trimerizes and becomes highly phosphorylated on serine residues. The heat-induced phosphorylation of HSF-1 can increase its apparent molecular weight by as much as M, 11,000 (17, 21–24). At present, the mechanism and function of HSF-1 phosphorylation are still unknown but are presumed to be important in modulating the heat shock response. Although the increased transcriptional activity of hsp70 seems to be mediated in large part by HSF-1, the promoter regions of heat shock genes contain several regulatory sequences that respond to diverse stimuli, such as changes in serum concentrations. For example, the increase in the level of hsp 70 transcription induced by serum is modulated through the serum response factor. It is still unclear whether these other elements interact synergistically with HSF to increase the heat shock response or act as negative regulators of the heat shock response under unstressed conditions (19, 20).

Although the signal transduction pathway leading to HSF-1 activation is not known at the present time, there is some evidence that MAP kinases may regulate HSF-1 activity. For example, studies on Saccharomyces cerevisiae suggest that mutations in the Ras-cAMP pathway confer heat sensitivity. For example, S. cerevisiae that possess mutations in the protein kinase SLK1, a MAP kinase family member, fail to become thermotolerant and lose viability rapidly after heat shock compared with their wild-type counterparts (25, 26). Because the activation of MAP kinases by heat shock correlates closely with HSF-1 activity, we sought to determine the possible regulation of HSF-1 by MAP kinases.

In this report, we show through the coexpression of kinase dead alleles of ERK1 and hsp 70 reporter constructs that ERK1 exerts a significant negative regulatory effect on hsp70 gene expression. Because stable overexpression of ERK1 dominant inhibitory mutants results in growth inhibition, we used sodium vanadate (SV) as a rapid means to increase the activity of MAP kinases. Treatment of cells with SV, which induces a high sustained level of MAP kinase activity due to inhibition of the dual-specificity MAP kinase phosphatase MKP1 (PAC1), inhibited the sequence-specific DNA binding activity and increased the phosphorylation of HSF-1. Cells pretreated with SV were 100-fold more sensitive to repeated heating compared with cells that were heated in the absence of SV pretreatment. Taken together, these data suggest a role for MAP kinases in the regulation of the heat shock response.

Materials and Methods

Cell Lines. NIH3T3 cells were maintained routinely in DMEM supplemented with 10% FCS. Ha-ras-inducible NIH3T3 cells were cultured in the presence of 400 µg/ml G418 in DMEM supplemented with 10% FCS. This cell...
line has been characterized extensively previously and has been shown to assume a transformed phenotype in the presence of IPTG (6, 8, 27, 28).

**Heat Survival Assays.** Cell survival studies were performed using colony formation analysis as an end point (24, 29). Briefly, cells were plated in 60-mm tissue culture dishes. Where appropriate, 20 mM IPTG was added 24 h prior to the onset of the experiments. On the day of the experiment, cells were heated in a specially designed circulating water bath that is temperature and CO₂ regulated. In the case of thermotolerance experiments, cells were given a heat shock of 43°C for 20 min to induce thermotolerance then returned to 37°C for varying time intervals before they were given a second heat exposure of 45°C for 50 min. After the heat treatment, cells were trypsinized and counted, and defined numbers of cells were plated for colony formation. After 8–10 days incubation at 37°C, plates were rinsed with PBS, colonies were stained with Coomasie blue, and colonies with 50 or more cells were counted as viable survivors. Survival data are presented as surviving fraction (number of colonies obtained for the treated groups/number of colonies obtained for the untreated groups; Refs. 24 and 29).

**Plasmids.** Plasmids used in these studies were as follows: plasmid human hsp70-luciferase, which contains ~188 bp of human hsp 70 promoter ligated to the luciferase reporter gene, was provided Dr. Richard Morimoto (Northwestern University, Evanston, IL; Ref. 30). Plasmid pCMVβGal, which expresses the β-galactosidase gene constitutively under the control of the cytomegalovirus promoter, was obtained from Dr. Chaim Jacobs (University of Southern California, Los Angeles, CA). Plasmids containing the human cDNAs for wild-type ERK1, wild-type ERK2, dominant negative allele of ERK1 (ERK1KR) and dominant negative allele of ERK2 (ERK2—52R) under the control of the constitutive cytomegalovirus promoter were provided by Drs. Melanie Cobb (University of Texas Southwestern Medical Center, Dallas, TX) and David Brenner (University of North Carolina, Chapel Hill, NC; Refs. 9 and 31).

**Transient Transfection.** Transient transfection studies were performed by the calcium phosphate method and followed by glycerol shock (32). DNA precipitate was added dropwise onto 2 × 10⁶ cells in a 60-mm tissue culture dish containing 1 ml serum free-growth medium. After 5 h incubation with the DNA precipitate, cells were reseeded with 4 ml growth medium that was supplemented with 10% FCS and were incubated overnight at 37°C. On the following day, cells were glycerol shocked [15% (v/v)] for 1 min at room temperature, washed once with PBS, and incubated at 37°C for 24 h. In experiments that examined the combined effects of oncogenic Ras expression and heat shock, IPTG-induced cells were heat shocked at 43°C for 20 min and incubated at 37°C for 6 h to permit luciferase accumulation before cell lysis. Luciferase activity following transient transfections was assayed as follows. Cells were lysed in 100 μl 50 mM Tris-HCl (pH 7.8) and subjected to three cycles of freezing and thawing. The lysates were then microfuged, and an aliquot was added to the luciferase assay reagent [20 mM Tricine, 1 mM (MgCO₃)Mg(OH)₂ H₂O, 2.67 mM MgSO₄, 0.1 mM EDTA, and 530 μM ATP (final pH 7.8)]. The reactions were measured in a luminometer for 20 s (33). In transfection studies, 1 μg hsp 70-luciferase was mixed either alone or in combination with 4 μg ERK1, ERK2, ERK1KR, and ERK2—52R plasmids. The amount of total DNA added to the reaction was adjusted to 25 μg by the addition of the plasmid pBR322. Four micrograms of pCMVβGal were co-transfected as an internal control for transfection efficiency. All transfection experiments were performed five times.
MAP KINASE AS A REGULATOR OF HEAT SHOCK RESPONSE IN NIH3T3 CELLS

Fig. 2. SV treatment of NIH3T3 cells increases ERK1 and ERK2 activity. A, NIH3T3 cells either were left untreated (Lane 1) or were treated with 500 µM SV for 30 min (Lane 2), 1 h (Lane 3), or 2 h (Lane 4). After exposure to SV, lysates were analyzed by immunoblotting with anti-ERK1/ERK2 antibody. MW, molecular weight. B, NIH3T3 cells were treated with 500 µM SV for 30 min or 2 h at 37°C. Cells were then lysed, and equal amounts of protein were immunoprecipitated using anti ERK1 antibody. The immunoprecipitated materials were used in an immune complex kinase assay with myelin basic protein as a substrate. The 32P incorporation into myelin basic protein was quantitated by scintillation counting.

Gel Retardation Assays. Electrophoretic mobility shift assays using whole-cell extracts have been described in detail previously (15, 23, 34). Briefly, after various treatments, cells were rinsed with cold PBS and were lysed by homogenization in 100 ml extraction buffer [10 mM HEPES (pH 7.9), 0.4 mM NaCl, 0.1 mM EDTA, 0.5 mM DTT, 5% glycerol, and 0.5 mM PMSF]. The protein concentration of each sample was estimated by the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL). Equal amounts of protein (15 µg) in extraction buffer (volume not exceeding 15–25 µl) were added to 4 µl binding buffer [37.5 mM NaCl, 15 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 0.5 mM DTT, and 5% glycerol] that also contained 1 µl yeast tRNA (10 µg), 1 µg sheared Escherichia coli DNA, 10 µg poly(dl-dC), and 1 ng 32P-labeled HSE. This mixture was incubated at 25°C for 15 min and electrophoresed on a nondenaturing 4.5% polyacrylamide gel. After electrophoresis, gels were fixed in 7% (v/v) acetic acid for 5 min, rinsed once in distilled water, dried under vacuum, and exposed to X-ray film. The nucleotide sequence for HSE was the same as that described originally by Zimarino and Wu (34) and was 5'-GTCCAGCGATGCAGGCCTCGAATGTTCAGAAAAGG (28). The double-stranded oligonucleotide was labeled by the fill-in reaction using the Klenow fragment of the DNA polymerase I and [γ-32P]ATP.

Immunoprecipitation. Immunoprecipitation of 32P-labeled HSF-1 was performed by lysing the cells in RIPA buffer [10 mM Tris (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.5 mM PMSF, and 100 µM SV]. The lysate was microfuged for 10 min at 4°C. Approximately 500 µg protein were immunoprecipitated using the antimouse HSF-1 antibody for 1 h at 4°C. Afterward, 30 µl 50% solution of protein A-Sepharose were added to the mixture, and the mixture was incubated at 4°C for an additional 1 h. The protein A-antibody complex was washed four times with the lysis buffer and once with kinase buffer [20 mM β-glycerophosphate (pH 7.3), 5 mM MgCl2, 1 mM EGTA, 10% glycerol, 1 mM DTT, 1 mM SV, and 0.2 mM PMSF; Ref. 35]. The protein A-antibody-antigen complex was then incubated for 20 min at 37°C in 10 µl kinase buffer with 50 µM unlaoded ATP, 20 µCi [γ-32P]ATP, and 100 µM segment of myelin basic protein (Santa Cruz Biotechnology) or 100 µM purified recombinant human HSF-1 as substrates. When human HSF-1 was used as a substrate, the reaction was terminated by the addition of 2X SDS sample buffer before electrophoresis on SDS-PAGE gels. The amount of radioactivity in bands corresponding to phosphorylated HSF-1 was quantitated by densitometry. When the MBP peptide was used as a substrate, the reaction was stopped by spotting 20 µl reaction mixture on chromatography paper (P81; Whatmann), followed by seven washes with 1% (v/v) phosphoric acid. The filters were dried and quantitated by scintillation counting.

Immunoblotting. Immunoblotts were performed as described previously (6, 8, 23). After treatments, cells were washed with ice-cold PBS, lysed in SDS sample buffer, and denatured by heating to 97°C for 5 min. The amount of protein in each lysate was determined by precipitating an aliquot of each sample in trichloroacetic acid and measuring the protein concentration by bicinchoninic acid (Pierce, Bradford IL). Equal amounts of protein from each sample were then resolved in 10% PAGE and transferred onto a nylon membrane (Amersham, Arlington Heights, IL). Membranes were stained with Ponceau S solution (Sigma Chemical Co., St. Louis, MO) to ensure equal transfer of the proteins, followed by blocking with 3% nonfat dry milk in PBS and incubation with the primary antibody. The ERK1-ERK2 polyclonal antibody was obtained from Santa Cruz. The anti-HSF-1 antibody was provided by Dr. Richard Morimoto (21). The membranes were visualized using enhanced chemoluminescent according to the manufacturer’s instruction (Amersham). The dilution of the anti-ERK1-ERK2 polyclonal antibody (sc-94) was 1:2000.

Results

Negative Regulatory Effect of ERK1 on hsp70 Gene Expression. To examine the role of MAP kinases (ERK1 and ERK2) in the regulation of the heat shock response, we cotransfected a hsp70 reporter gene construct with different combinations of wild-type
ERK1, ERK2, or dominant negative alleles of ERK1 and ERK2 (ERK1KR and ERK2–52R) into Ha-ras-inducible NIH3T3 cells (8). The hsp70 reporter gene contained 188 bp of the hsp 70 promoter from the start site of transcription (30). In Ha-ras-uninduced cells, wild-type ERK1 and ERK2 had little effect on heat-inducible hsp70 gene expression (Fig. 1A). Similar results were obtained when kinase-defective alleles of ERK1 (ERK1KR) and ERK2 (ERK2–52R) were cotransfected transiently with the hsp70-luciferase reporter construct in Ha-ras-uninduced NIH3T3 cells (Fig. 1B). However, in Ha-ras induced cells, wild type ERK1 inhibited hsp 70-luciferase expression by 50% after heat shock, and kinase dead ERK1KR increased hsp 70-luciferase activity by 30-fold after heat shock (Fig. 1B). This inhibitory effect seemed to be specific for kinase-defective and wild-type ERK1, because wild-type ERK2 and the kinase dead allele of ERK2 had little effect on hsp 70-luciferase induction by heat shock. These results indicate that ERK1 acts as a negative regulator of hsp70 gene expression in cells that possess oncogenic Ras mutations.

**SV has a Negative Regulatory Effect on Heat Shock Response through Activation of ERK1 Protein Kinase.** To investigate the negative regulatory effect of MAP kinase on the heat shock response further, we took advantage of the stimulatory effect of SV on ERK activity. This approach allowed us to modulate MAP kinase activity for the desired time interval and circumvented the problem of obtaining a cell line that produced high constitutive levels of MAP kinase activity. SV, a specific phosphotyrosine phosphatase inhibitor, is known to be a positive effector of the mitogenic effects of growth factors on cells (36). No significant effect of SV has been detected on phosphatidic acid, phosphatidylinositol, phosphatidylinositol-4-phosphate, phosphatidylinositol-4,5-biphosphate, or phosphatidylcholine (37). Tsao and Greene (38) have reported that SV treatment at concentrations of 0.5–1 mα results in the activation of MAP kinases. Additionally, nerve growth factor down-regulation of MAP kinase activity can be reactivated by SV, suggesting that SV regulates MAP kinase by effecting nonreceptor tyrosine kinase activity. Further, inhibition of the dual-specificity tyrosine/threonine phosphatase PAC1/MKP1, a physiological regulator of MAP kinases (39, 40), by SV offers a potential mechanism for the direct action of SV in increasing MAP kinase activity. SV has a negative regulatory effect on heat shock response through activation of ERK1 protein kinase.

![Graph showing the effect of IPTG and Sodium vanadate on HSF-1](image)

**Fig. 3. SV increases 3P incorporation of HSF-1.** NIH3T3 cells were labeled with 32P for 6 h at 37°C. Some cells were treated for the final 2 h of labeling with 500 μm SV. Before termination of labeling, HSF-1 was immunoprecipitated from cell lysates with anti-HSF-1 antibody. Immunoprecipitated samples were then analyzed by gel electrophoresis and were quantitated. Lane 1, HSF-1 from NIH3T3 cells that were treated with SV only; Lane 2, HSF-1 from NIH3T3 cells that were treated with SV and were heat shocked at 43°C for 20 min; Lane 3, HSF-1 from uninduced control NIH3T3 cells; Lane 5, HSF-1 from heat shocked (43°C, 20 min) NIH3T3 cells; Lane 4, HSF-1 from control NIH3T3 cells; Lane 6, HSF-1 from heat shocked (43°C, 20 min), induced NIH3T3 cells.
lysed, ERK1 was immunoprecipitated using anti-ERK1 antibody, and the immunoprecipitated ERK1 was used to phosphorylate purified recombinant human HSF-1. ERK1 from heat-shocked cells phosphorylated HSF-1 efficiently, resulting in a 3-fold increase in phosphorylation of HSF-1 (Fig. 4, A and B). ERK1 immunoprecipitated from SV-treated cells displayed a 6-fold increase in the phosphorylation of HSF-1. Because HSF-1 is a substrate for ERK1 phosphorylation in vitro, ERK1 protein kinase also may regulate the heat shock response negatively through its direct phosphorylation of the HSF-1 protein in vivo.

To examine whether SV-pretreated NIH3T3 cells also display a reduction in the sequence-specific DNA-binding activity of HSF-1, NIH3T3 cells were pretreated with 500 μM SV for 2 h, rinsed twice with PBS, and heat shocked at 43°C for 20 min. Cell lysates were prepared immediately after heat shock and after 1 or 2 h recovery at 37°C. Extracts from SV-pretreated cells showed an immediate reduction in HSF-1 DNA-binding activity that lasted for 1 h (Fig. 5A). However, by 2 h after heat shock, HSF-1-binding activity in SV extracts was similar to extracts from untreated cells. This reduction in the DNA-binding activity of HSF-1 in extracts from SV-pretreated cells correlated with an inhibition of hsp 70 induction after heat shock (Fig. 5B). The inhibition of the endogenous mouse hsp70 gene supports our previous results of ERK inhibiting human hsp 70 promoter expression (Fig. 1).

**Effect of Increase in ERK1 Activity on Thermotolerance Response in NIH3T3 Cells.** To determine the effect of increasing ERK1 activity on the development of thermotolerance, cells were exposed to 43°C for 20 min with or without SV pretreatment. This brief heat exposure is used to induce hsp70 that presumably will protect the cell against future heat shock exposure. SV-treated cells displayed a 100-fold decrease in the development of thermotolerance for 5 h following the first priming heat exposure, suggesting that reduction of HSF-1 activation and hsp 70 induction inhibits development of thermotolerance to a great extent (Fig. 6). However, cells pretreated with SV do develop thermotolerance independent of any detectable increase in hsp 70 induction (Fig. 6). This increase in thermotolerance in SV-treated cells could be attributed to the induction of a small amount of hsp 70 (or other hsps) that is undetectable (41) or development of thermotolerance by a mechanism independent of hsp 70 induction after heat shock (42).

**Discussion**

In this article, we have presented evidence that, when taken together, indicate strongly that MAP kinase acts as a negative regulator of the heat shock response. Expression of dominant negative alleles of ERK1 but not ERK2 increases hsp 70 promoter activity specifically following heat shock. Although forced overexpression of dominant negative alleles of MAP kinase could inhibit other mitogen- or stress-activated protein kinases potentially, we have not found that to be true in our hands (6). As a means of circumventing the problems associated with obtaining cell clones that possessed increased MAP kinase expression, we used the tyrosine phosphatase inhibitor SV. It should be emphasized that not only does SV inhibit the dual-specificity MAP kinase phosphatase MKP1 (PAC1; Refs. 39 and 40), but it also inhibits other tyrosine phosphatases as well. Although results obtained with use of this agent should be interpreted cautiously, they suggest that the effect of SV on the heat shock response is related most likely to inhibition of PAC1 rather than on the inhibition of other receptor and nonreceptor tyrosine phosphatases. This conclusion is supported by the following results: (a) the rapid kinetics of MAP kinase activation correlate with the rapid increase in HSF-1 phosphorylation in unheated cells; (b) HSF-1 is a good substrate for MAP kinase in immune complex kinase assays; (c) SV pretreatment of cells before heat shock inhibits HSF-1 activity and hsp70 induction; and (d) SV delays the induction of thermotolerance. These results suggest
strongly that PAC1 or a closely related tyrosine phosphatase that modulates MAP kinase activity also regulates the transcriptional activity of heat shock genes in mammalian cells. Future experiments will be directed at developing genetic approaches to inhibit the activity of the dual-specificity phosphatase PAC-1 to demonstrate a role for MAP kinase as a negative regulator of the heat shock response more directly.

If MAP kinase acts as a negative regulator of hsp70 gene expression, is its target HSF-1? MAP kinases phosphorylate a large range of substrates, such as other protein kinases (e.g., ribosomal protein S6 kinase), transcription factors (e.g., ternary complex factor ELK-1), growth factor receptors, and cytoskeletal components (1, 43, 44). The human, inducible hsp 70 used in our studies does contain a serum response element, which binds a protein complex composed of a homodimer of serum response factor and ternary complex factor. However, it is unlikely that the serum response factor is acting as a negative regulator of hsp 70 transcription. One recently documented case of MAP kinase-induced transrepression involves phosphorylation of the C-terminal region of the c-Fos protein (45). When this region of c-Fos is deleted, it becomes transforming. If one would apply the c-Fos paradigm to HSF-1 activation, then ERK1 may act as a trans-repressor of the heat shock response by phosphorylating HSF-1 to inhibit it from transcriptionally regulating hsp70 gene expression. Our data indicate that ERK1 is directly able to phosphorylate HSF-1 in vitro and is supported by the identification of several potential MAP kinase phosphorylation sites between amino acid residues 270 and 380 in the HSF-1 monomer.

Under conditions in which MAP kinase activity is increased, such as SV pretreatment of cells, HSF-1 becomes hyperphosphorylated.

4 N. Mivechi, unpublished observation.
This increase in the phosphorylated form of HSF-1 by ERK1 correlates with its reduced DNA-binding activity and the abolishment of hsp 70 induction after heat shock. It should be noted that although HSF in SV-pretreated cells contains some DNA-binding ability as measured by gel mobility shift analysis of cell extracts, this assay measures the binding activity of both HSF-1 and HSF-2 transcription factors (46). Unlike HSF-1, HSF-2 is a weak inducer of heat shock gene transcription, and it does not seem to be phosphorylated (21). It is also possible that the delayed binding activity of HSF to the HSE in SV-pretreated cells may not be meaningful in terms of the ability of HSF to trans-activate hsp genes. Regardless of these two possibilities, these results suggest that phosphorylation of HSF-1 by ERK1 inhibits its transcriptional regulatory activity at the step of DNA binding.

We propose that ERK1 may regulate the heat shock response negatively in the following model. ERK1 may be activated continuously by signals from growth factors resulting in phosphorylation of HSF-1 by MAP kinase. Phosphorylation of HSF-1 inactivates it, presumably by keeping it in the folded state. During heat shock, HSF-1 may be dephosphorylated at MAP kinase phosphorylation residues that potentiate HSF-1 trimerization. After trimerization, the level of HSF-1 phosphorylation increases with heat shock (47). Whether phosphorylation of HSF-1 by heat shock is modulated by a different protein kinase(s) than ERK1 or whether, following trimerization and activation, HSF-1 is phosphorylated by ERK1 to signal HSF-1 deactivation may still remain to be addressed. The latter possibility is favored, because recent evidence from yeast suggests that HSF phosphorylation may be required for its deactivation after heat shock (48). The fact that SV can increase phosphorylation of HSF-1 under control conditions suggests that MAP kinase phosphorylation sites on the HSF-1 protein are normally in an equilibrium between phosphorylated and unphosphorylated states. Because both endogenous and exogenous mouse and human hsp70 genes are modulated in a negative manner by the MAP kinase pathway, future experiments are directed at determining whether other hsp genes are also down-regulated by MAP kinase in mammalian cells.

It is interesting to note that there is a fundamental difference in HSF-1 activation between yeast and mammalian cells. In yeast, HSF-1 is bound constitutively to the DNA, and heat shock is required for its activation (49, 50). In mammalian cells, however, HSF-1 is associated with cytoplasmic hsp70s under unstressed conditions, and a stress such as heat shock is required to cause binding competence and activation of HSF-1 (14, 15, 49, 51, 52). This difference between yeast and mammalian cells may indicate that only in mammalian cells is HSF-1 phosphorylated by ERK1 under unstressed conditions. Therefore, due to differences between HSF-1 localization in mammalian cells and yeast, the negative regulatory effect of the Ras-cAMP pathway on the heat shock response in yeast may not be directly on HSF-1, as has been suggested previously by Engelberg et al. (26), whereas HSF-1 is regulated negatively by ERK1 in mammalian cells.

A great deal of evidence has been accrued to support a role for MAP kinase as a mediator of extracellular growth factor signals and sensors to a variety of stress signals, such as UV light, ionizing radiation, low oxygen conditions, and elevated temperatures (8, 26, 53–56). Although MAP kinase seems to have a positive regulatory role in the activation of early response genes, in response to heat shock, MAP kinase seems to act as a negative regulator of gene transcription. Although we do not know yet why this negative effect on the heat shock response would be conserved from yeast to mammals, understanding its mechanism will give us further insights into the complex regulation of the heat shock response.

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References


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