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Heat and Chemical Shock Potentiation of Glucocorticoid Receptor Transactivation Requires Heat Shock Factor (HSF) Activity

MODULATION OF HSF BY VANADATE AND WORTMANNIN*

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Heat shock and other forms of stress increase glucocorticoid receptor (GR) activity in cells, suggesting cross-talk between the heat shock and GR signal pathways. An unresolved question concerning this cross-talk is whether heat shock factor (HSF1) activity is required for this response. We addressed this issue by modulating HSF1 activity with compounds acting by distinct mechanisms: sodium vanadate (SV), an inhibitor of protein phosphatases; and wortmannin, an inhibitor of DNA-dependent protein kinase. Using HSF1- and GR-responsive CAT reporters, we demonstrate that SV inhibits both HSF1 activity and the stress potentiation of GR, while having no effect on the hormone-free GR or HSF1. Paradoxically, SV increased hormone-induced GR activity in the absence of stress. In contrast, wortmannin increased HSF1 activity in stressed cells and had no effect on HSF1 in the absence of stress. Using the pMMTV-CAT reporter containing the negative regulatory element 1 site for DNA-dependent protein kinase, wortmannin was found to increase the GR response. However, in cells expressing a minimal promoter lacking negative regulatory element 1 sites, wortmannin had no effect on the GR in the absence of stress but increased the stress potentiation of GR. Our results show that the mechanism by which GR activity is increased in stressed cells requires intrinsic HSF1 activity.

The glucocorticoid receptor (GR)[1] is a member of the nuclear receptor family of proteins that act as hormone-activated transcription factors (1, 2). Since the GR is known to be involved in the organismal response to stress (3), and since the hormone-free GR is known to reside in the cytoplasm as a complex containing heat shock proteins (HSPs) (4), we and others have investigated the role of the heat shock response in controlling GR function. Early evidence to suggest a relationship between these responses includes the ability of heat shock or chemical stress (arsenite) to cause nuclear translocation of hormone-free GR in mouse L929 and Chinese hamster ovary cells (5, 6) and a partial increase in GR-mediated gene expression in Chinese hamster ovary cells subjected to heat or chemical shock in the absence of hormone (6). Heat shock-induced nuclear translocation of hormone-free GR has also been documented in the liver of rats subjected to whole-body hyperthermia (7, 8) as well as in COS cells expressing human GR (9). Evidence to suggest that heat shock-induced nuclear translocation of hormone-free GR can result in altered expression of endogenous genes has also been accumulating. For example, heat shock treatment of COS and Hela cells in the absence of hormone results in GR-mediated transrepression of the collagenase promoter (9), while heat shock treatment of murine macrophages results in a pattern of Fc receptor expression or repression that is identical to that observed in response to hormone (10). More recently, heat shock-induced translocation of hormone-free GR has been implicated in the process of stress-induced apoptosis in leukemic cells (11).

In the presence of hormone, heat shock has been shown to have a dramatic effect on reporter gene expression controlled by progesterone receptor (12) or GR (13, 14). In our laboratory, the potentiation of hormone-induced GR function by heat shock was observed at saturating concentrations of dexamethasone (Dex) agonist (1 μM) and was observed in cells stably transfected with complex (pMMTV-CAT) or minimal ($pGRE_2E1B$-CAT) promoters controlling expression of CAT (13). These observations suggest that the GR-mediated response typically seen in unstressed cells is not maximal, even in the presence of hormone concentrations that yield saturation results for reporter gene expression. Thus, it would appear that heat shock is a mechanism by which a latent response to hormone by the GR can be activated. However, the precise mechanism by which heat shock can control the GR has been little investigated. Since HSF1 is the principal factor regulating the heat shock response in cells, we have, in the present study, examined the role of HSF1 in the stress potentiation of GR. To test the role of HSF1 in this process, we sought the use of compounds that could either selectively inhibit HSF1 activity in stressed cells, or that could increase HSF activity without being an additional cause of stress. We have identified two such compounds, sodium vanadate (SV) and wortmannin.

Sodium vanadate has been widely used as an inhibitor of dual-specificity protein phosphatases (15), most notably the mitogen-activated protein kinase phosphatase MKP1 (also known as PAC1), inhibition of which appears to explain the ability of SV to induce MAPK activity (16, 17). Since MAPK family members have been shown to phosphorylate HSF1 within its transcriptional regulatory domain (18) and to inhibit HSF1 activity in response to stress (19–21), the ability of SV to

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[1] The abbreviations used are: GR, glucocorticoid receptor; GRE, glucocorticoid response element; HSF, heat shock transcription factor; HSE, heat shock response element; HSP, heat shock protein; SV, sodium vanadate; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; DNA-PK, DNA-dependent protein kinase; NRE1, negative regulatory element 1; NF-κB, nuclear factor-κB; MMTV, mouse mammary tumor virus; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay; Dex, dexamethasone.

induce MAPK activity has become a useful means by which to inhibit the heat shock response in stressed cells. Although two reports exist demonstrating an inhibitory effect of vanadate on heat shock-induced HSP70 expression (21, 22), no other studies on the effects of SV on HSF1 activity using endogenous genes or reporter constructs have been described. In this work, we demonstrate that SV can indeed cause inactivation of HSF1-mediated CAT reporter expression in stressed cells and that SV inhibition of HSF1 results in a corresponding inhibition of the stress effect on the GR.

Wortmannin is a fungal metabolite that has recently been shown to irreversibly inactivate members of the phosphatidylinositol 3-kinase-related kinase family, including DNA-dependent protein kinase (DNA-PK) (23, 24). DNA-PK is composed of two major components, a 460-kDa subunit containing catalytic activity (DNA-PK_{cs}) and a heteromeric 70- and 86-kDa subunit (Ku autoantigen) that binds DNA (25, 26). Inactivation of DNA-PK by wortmannin occurs by covalent binding at the catalytic domain of DNA-PK_{cs} (24). Although interaction of Ku with DNA can occur nonspecifically at ends of DNA (25), specific interaction of Ku with the negative regulatory element 1 (NRE1) response element has recently been described (27–29). A variety of reports exist to support the notion that DNA-PK can modulate HSF1 activity. First, overexpression of the 70-kDa component of Ku results in reduced levels of HSP70 expression following heat shock (30). In contrast, cells containing reduced levels of DNA-PK_{cs} showed reduced accumulation of HSP70 during recovery from stress, and these cells were much more sensitive to heat shock-induced apoptosis (31). Direct interaction between HSF1 and Ku or DNA-PK_{cs} has been observed (31, 32), along with DNA-PK_{cs}-mediated *in vitro* phosphorylation of HSF1 (32) in a region of HSF1 known to be negatively regulated by MAPKs (32–35). Based on the above observations, it can be concluded that wortmannin is an effective inhibitor of DNA-PK. However, the functional effects of DNA-PK on HSF1 remain somewhat contradictory. In order to resolve this issue and to hopefully develop an agent with positive, nonstressing effects on HSF1, we have tested the effects of wortmannin on HSF1 under stress and nonstress conditions. We report here that wortmannin treatment of intact cells results in a marked enhancement of HSF1-mediated CAT expression, but only under stress conditions, suggesting that DNA-PK does indeed act as a negative regulator of HSF1. In cells stably transfected with a GR-responsive CAT reporter, wortmannin treatment caused a corresponding increase in the stress potentiation of GR, under conditions where wortmannin had no effect on GR signaling in the absence of stress.

In summary, our results show that down-regulation or up-regulation of HSF1 will result in a corresponding modulation of GR activity in stressed cells. Since the agents used in this study to modulate HSF1 act by distinct mechanisms, it is highly unlikely that the effects of these agents on the GR is due to the existence of a common target controlling both the heat shock and GR pathways. Instead, we propose that the mechanism by which heat shock controls GR transactivation requires HSF1 activity.

EXPERIMENTAL PROCEDURES

Materials—[^{3}H]Acetate (10.3 μCi/mmol) and [^{125}I]conjugates of goat anti-mouse IgG (11.8 μCi/μg) and goat anti-rabbit IgG (9.0 μCi/μg) were obtained from ICN Radiochemicals. Sodium vanadate, wortmannin, ATP, Me_2SO, sodium arsenite, dexamethasone, G418 (Geneticin) antibiotic, acetyl-CoA synthetase, acetyl coenzyme A, Tris, Hepes, EDTA, protein A-Sepharose, Dulbecco's modified Eagle's medium powdered medium, and horseradish peroxidase conjugates of goat anti-mouse and goat anti-rabbit IgG were from Sigma. Iron-supplemented newborn calf serum and dialyzed fetal bovine serum were from Hyclone. Immobilon P membranes were obtained from Millipore Corp. GenePorter transfection reagent was obtained from Gene Therapy Systems, Inc. The SC-7383 monoclonal antibody against phosphorylated (active) ERK was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

In the p2500-CAT reporter used in this study, expression of chloramphenicol acetyltransferase (CAT) is controlled by the human HSP70 promoter containing consensus heat shock elements (HSEs) known to be activated by the binding of heat shock factor (36). The pMMTV-CAT plasmid contains the complete mouse mammary tumor virus-long terminal repeat promoter (MMTV-LTR) upstream of CAT (37). Hormonally driven expression of CAT by this reporter is controlled by glucocorticoid response elements (GREs) residing within the LTR region (38). The pGRE_2E1B-CAT minimal reporter is composed of two synthetic GREs derived from the tyrosine aminotransferase promoter linked to the adenovirus E1B TATA sequence (39).

MATERIALS AND METHODS

Cell Culture and Stress/Drug Treatment—The various CAT reporter-expressing cells lines were established as described previously (13, 40). Briefly, mouse L929 cells were co-transfected with pSV2neo and a 2-fold excess of p2500-CAT (LHSECAT cells), pMMTV-CAT (LMCAT2 cells), or pGRE_2E1B-CAT (LGEC cells) using Lipofectin as carrier. This was followed by selection for stably transfected, cloned cell lines using G418 (Geneticin) antibiotic at 0.4 mg/ml. Once established, all cell lines were grown in an atmosphere of 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium containing 0.2 mg/ml G418 and 10% iron-supplemented newborn calf serum. For all experiments, the newborn calf serum was stripped of endogenous steroids by extraction with dextran-coated charcoal. Most stress experiments were performed on cells that were at or near confluence, although similar results were obtained with subconfluent cultures. Heat shock treatment was achieved by shifting replicate flasks to a second 5% CO_2 incubator set at 43 °C. Typical duration of heat shock treatment was 2 h. Cells were also subjected to chemical shock by the addition of 200 μM sodium arsenite to the medium. In the chemical shock experiments, the arsenite-treated and nontreated cells were incubated at 37 °C for 2 h and were then washed with Dulbecco's modified Eagle's medium and allowed to recover.

In the experiments involving sodium vanadate or wortmannin, the drug was typically added to the culture medium for the indicated interval prior to either hormone or stress treatment. At the end of the stress or hormone treatments, the drug-containing medium was washed away, followed by continued culture as indicated for each experiment. Vanadate was prepared according to the method of Gordon (15) by dissolving sodium orthovanadate ($Na_3VO_4 \cdot nH_2O$) to 500 mM in sterile water and adjusting to approximately pH 10. Prior to use, SV stock solution was heated to boiling until solution was clear and to ensure the presence of the active monomers. Wortmannin stock was 1 mM in Me_2SO.

CAT Assay—Measurement of CAT enzyme activity was performed according to the method of Nordeen *et al.* (41) with minor modifications. In this assay, a reaction mixture containing acetyl-CoA synthetase, [^{3}H]sodium acetate, CoA, and ATP is briefly preincubated to enzymatically generate labeled acetyl coenzyme A from CoA and labeled acetate. Acetylation of chloramphenicol was then initiated by adding cell lysate containing CAT enzyme. The reaction was stopped by extraction with cold benzene, and 75% of the organic phase was counted. Cell lysates were prepared by sequential freezing and thawing in 0.25 M Tris, 5 mM EDTA (pH 7.5) and centrifugation at 14,000 × *g*. Aliquots of lysate containing equal protein content were added to the enzymatic reaction mixtures. Since the GRE- and HSE-containing promoters employed in this study have distinct basal and inducible activities, all data are represented as percentage of control, maximum, or the equivalent. In this way, the relative inhibitory or stimulatory effects of vanadate or wortmannin on HSF1- and GR-mediated CAT expression can be readily seen.

Gel Electrophoresis and Quantitative Western Blotting—Samples were resolved by electrophoresis in 10% polyacrylamide SDS gels as described by Laemmli (42), followed by transfer to Imobilon® polyvinylidene difluoride membranes. The relative amounts of MAPK were determined via a quantitative Western blotting technique previously described (43), which involves incubation of the blots with primary antibody, followed by peroxidase- and ^{125}I-conjugated counter antibodies. After color development, the ERK blots were exposed to Kodak XAR-5 film with an intensifying screen at −80 °C.

HSF1 EMSA—Electrophoretic mobility shift assays for HSF1 were performed according to the protocol of Mosser *et al.* (44), with minor modifications. Briefly, single 75-cm^2 flasks of LHSECAT cells were subjected to a variety of stress and drug conditions. Cells were har-

vested, centrifuged, and rapidly frozen at −80 °C. The frozen pellets were resuspended in WCE buffer (20 mM HEPES, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol, pH 7.9) and centrifuged at 100,000 × *g* for 10 min. The supernatants were either stored at −80 °C or used immediately. EMSA assays were performed by mixing 10 μg of whole cell extract with 0.1 ng (50,000 cpm) of ^{32}P-labeled HSE oligonucleotide (5′-GAT CTC GGC TGG AAT ATT CCC GAC CTG GCA GCC GA-3′) and 1.0 μg of poly(dI-dC) in 10 mM Tris (pH 7.8), 50 mM NaCl, 1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol in a final volume of 25 μl. For competition experiments, the binding reactions contained 0.1 ng of the [^{32}P]HSE and a 100-fold molar excess of unlabeled HSE. Reactions were incubated at 25 °C for 30 min and loaded onto 4% polyacrylamide gels in 0.5× TBE. The gels were run at room temperature for 1.5 h at 150 V and were exposed to Kodak XAR-5 film with an intensifying screen at −80 °C. The relative amounts of probe-bound HSF1 were then measured by densitometric scanning of the film using the Bio-Rad Molecular Analyst system.

RESULTS

The Phosphatase Inhibitor, Sodium Vanadate, Decreases Stress-induced HSF1 Transcription Enhancement Activity by a Mechanism Involving MAPK-mediated Phosphorylation—In order to test the role of HSF1 in the heat shock-induced potentiation of GR, we have taken advantage of the recently described ability of SV to inhibit HSF1 in stressed cells (20–22). In two of these reports, the inhibitory effect of SV on HSF1 activity was based on measurements of heat shock-induced HSP70 expression (21, 22). However, no studies on the effects of SV on HSF1 transactivation using reporter constructs have been described. To accurately measure the effects of SV on this HSF1 function, we have stably transfected L929 cells with the p2500-CAT construct (LHSECAT cells), in which high level CAT gene expression is controlled by the human HSP70 promoter (36). Dose-dependent inhibition by SV of heat shock-induced CAT expression in the LHSECAT cells can be seen in Fig. 1*A*, with maximal inhibition (88.5%) occurring at 500 μM SV. We chose 500 μM SV for the next set of experiments (Fig. 1*B*), in which the effects of SV on both heat and chemical shock activation of HSF1 transactivation were measured in the LHSECAT cells. The results show that SV can effectively inhibit both heat and arsenite activation of HSF1. The results also show that SV can inhibit HSF1-mediated transactivation when added to the cells immediately after the stress event, a point in time at which HSF1 is presumably already bound to DNA. This result would suggest that the ultimate effect of SV is to primarily inhibit the transcription enhancement activity of HSF1.

In order to test the above conclusion, we measured the effects of SV on HSF1 DNA binding ability by use of the EMSA seen in Fig. 2. As expected, the results show that heat shock can activate HSF1 binding to HSE-containing oligomers. However, SV did not inhibit the ability of heat shock to activate the DNA binding function of HSF1. Quantitation of our results by densitometric scanning and normalization to the heat shock-activated control (Fig. 2*B*) shows that SV treatment of the cells before (SV-HS) or after heat shock (HS-SV) results in almost no change in this function, 82.4 and 89.5%, respectively. This result contrasts with a report by Mivechi and Giaccia (21) in which the authors show that pretreatment of NIH3T3 cells with 500 μM SV results in a near complete inhibition of DNA binding by heat shock-activated HSF1. A similar report by He and Fox (22) also showed a 2-fold reduction in HSF1 DNA-binding in Chinese hamster ovary cells exposed to 10 μM SV for 24 h. However, our results are consistent with those of Chu *et al.* (19), in which inactivation of HSF1 by overexpression of MAPK kinase and subsequent activation of ERK does not result in loss of HSF1 DNA binding ability. Taken as a whole then, our reporter gene (Fig. 1) and gel shift data (Fig. 2) point to a mechanism in which the primary effect of SV on HSF1 function is at the level of HSF-induced transactivation.

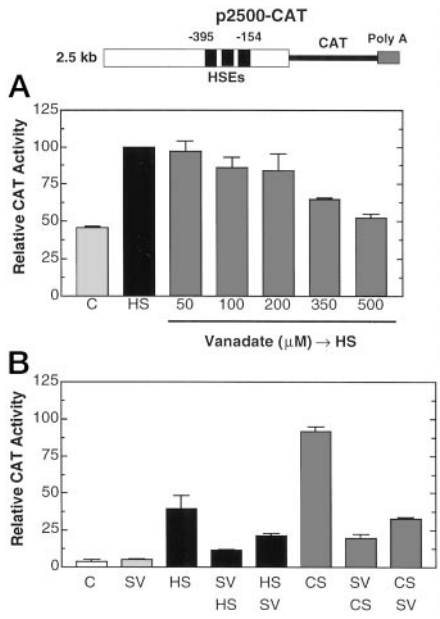

FIG. 1. **Vanadate inhibits stress-induced activation of HSF1.** *A*, concentration dependence curve for inhibition of HSF1 activity by vanadate. L929 cells stably transfected with the p2500-CAT reporter (LHSECAT cells) were left untreated (*C*), were subjected to heat shock at 43 °C for 2 h (*HS*), or were preincubated for 2 h with vanadate at the indicated concentrations prior to heat shock (43 °C, 2 h). After removal of the vanadate, the cells were allowed to grow under nonstress conditions for 20 h. Cell lysates were prepared and analyzed for CAT activity, expressed as percentage of the HS control. The results shown represent the means ± S.E. of two independent experiments. *B*, vanadate can inhibit both heat and chemical shock activation of HSF1. LHSECAT cells were subjected to the following conditions: no treatment (*C*); 500 μM vanadate for 2 h (*SV*); 43 °C for 2 h (*HS*); 500 μM vanadate for 2 h followed by 43 °C for 2 h (*SV-HS*); heat shock (43 °C for 2 h) followed by 500 μM vanadate for 2 h (*HS-SV*); 200 μM sodium arsenite for 2 h (*CS*); 200 μM sodium arsenite for 2 h followed by 200 μM sodium arsenite for 2 h (*SV-CS*); and chemical shock (200 μM sodium arsenite for 2 h) followed by 500 μM vanadate for 2 h (*CS-SV*). All cells were washed free of vanadate prior to culture under nonstress conditions for 20 h. Cell lysates were prepared and analyzed for CAT activity, expressed as a percentage of the maximum response (*CS*). The results shown represent the means ± S.E. of 5–11 independent experiments.

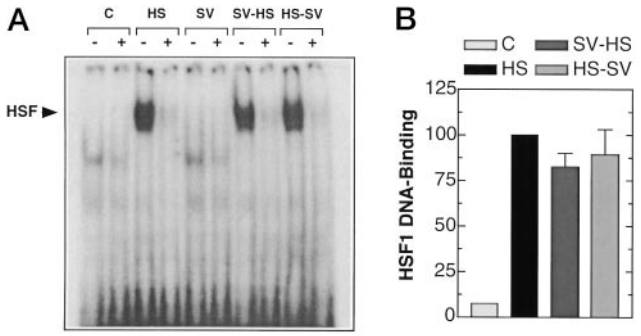

FIG. 2. **Vanadate does not affect the DNA-binding function of heat shock-activated HSF1.** *A*, LHSECAT cells were subjected to the indicated conditions followed by analysis of HSF1 DNA-binding function by EMSA assay, as described under "Experimental Procedures." *C*, no treatment. *HS*, 43 °C for 2 h; *SV*, 500 μM vanadate for 2 h; *SV-HS*, 500 μM vanadate for 2 h followed by heat shock (43 °C for 2 h); *HS-SV*, heat shock (43 °C for 2 h) followed by vanadate (500 μM) at 37 °C for 2 h. Where indicated (+), a 100-fold excess of unlabeled HSE oligomer was added as competitor. *B*, bands corresponding to the HSF1-bound probes were analyzed by densitometric scanning of the films. Values are expressed as percentages of the heat shock control (*HS*). Results represent the means ± S.E. of two or three independent experiments.

Since SV inhibition of HSF1 is reported to involve activation

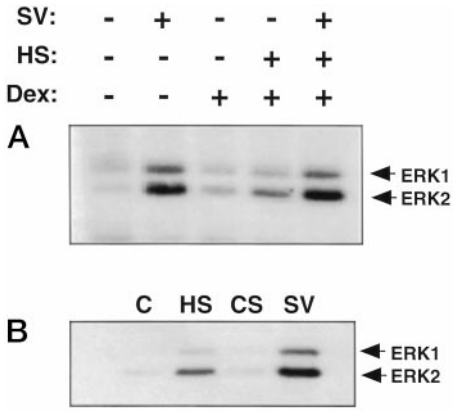

FIG. 3. **Effects of vanadate, hormone, and stress on ERK1 and ERK2 activity in LHSECAT cells.** In response to the indicated treatments, MAPK activity in LHSECAT cells was analyzed by SDS-PAGE (240 μg of protein) and immunoblotting with the sc-7383 antibody against phosphorylated (active) ERK1 and ERK2. In *A* is shown no treatment (*C*) and treatment with 500 μM sodium vanadate for 2 h followed by wash and additional culture for 4 h (*SV*), 1 μM dexamethasone for 2 h (*Dex*) 43 °C for 2 h followed by 1 μM Dex for 2 h at 37 °C (*HS-Dex*), and 500 μM vanadate for 2 h followed by heat shock (43 °C for 2 h) and 1 μM Dex for 2 h at 37 °C (*SV-HS-Dex*). *B*, in this set of samples, MAPK activity was assayed immediately after the indicated treatments (no recovery or additional culture as in *A*). *C*, no treatment; *HS*, 43 °C for 2 h; *CS*, 200 μM sodium arsenite for 2 h; *SV*, 500 μM sodium vanadate for 2 h. Results are representative of three independent experiments.

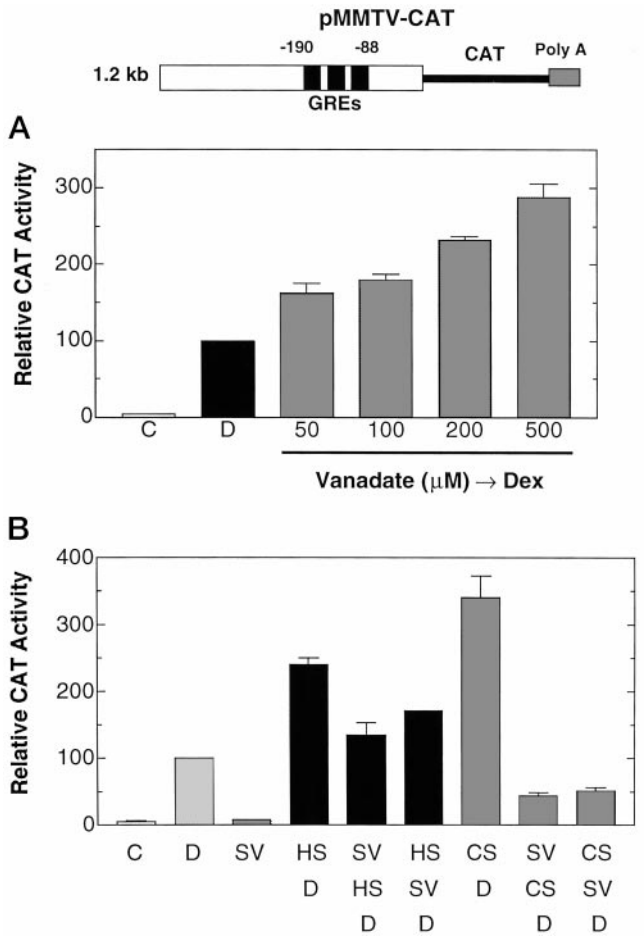

FIG. 4. **Vanadate exerts diametrically opposed effects on GR-mediated gene expression in nonstressed and heat-shocked cells.** *A*, concentration-dependent potentiation of GR-mediated CAT gene expression by vanadate in nonstressed cells. L929 cells stably transfected with the pMMTV-CAT reporter (LMCAT2 cells) were left untreated (*C*), were incubated with 1 μM Dex for 20 h (*D*), or were preincubated for 2 h with vanadate at the indicated concentrations, followed by removal of the vanadate and incubation with Dex (1 μM) for 20 h. Cell lysates were prepared and analyzed for CAT activity, expressed as a percentage of the Dex control. The results shown represent the means ± S.E. of six independent experiments. *B*, vanadate inhibits heat shock potentiation of GR-mediated CAT gene expression. LMCAT2 cells were subjected to the following conditions: no treatment (*C*), 1 μM Dex for 20 h (*D*); 500 μM vanadate for 2 h (*SV*); heat shock (43 °C) for 2 h followed by 1 μM Dex for 20 h (*HS-D*); 500 μM vanadate for 2 h followed by heat shock (43 °C) for 2 h and 1 μM Dex for 20 h (*SV-HS-D*); heat shock (43 °C, 2 h) followed by 500 μM vanadate for 2 h and 1 μM Dex for 20 h (*HS-SV-D*); chemical shock (200 μM arsenite for 2 h) followed by 1 μM Dex for 20 h (*CS-D*); 500 μM vanadate for 2 h followed by chemical shock and 1 μM Dex for 20 h (*SV-CS-D*); or chemical shock followed by 500 μM vanadate for 2 h and 1 μM Dex for 20 h (*CS-SV-D*). All cells were washed free of vanadate prior to stress or Dex treatment. Cell lysates were prepared and analyzed for CAT activity, expressed as a percentage of the Dex control (*D*). The results shown represent the means ± S.E. of 7–9 independent experiments.

of mitogen-activated protein kinases, notably ERK1 and ERK2, and subsequent phosphorylation of HSF1 (20, 21), we confirmed these responses to SV in our cells. In preliminary experiments, we have observed a time-dependent activation of ERK1 and -2 in response to SV treatment (500 μM) of LHSECAT cells, as measured by Western blotting of whole cell extracts with the SC-7383 antibody against phosphorylated (active) ERK (data not shown). To test the ability of SV-activated ERK to phosphorylate HSF1, we have used an immune complex kinase assay in which total (active and inactive) ERK1, adsorbed to protein A-Sepharose beads with the SC93 antibody against ERK1, was allowed to phosphorylate human recombinant HSF1. The results demonstrate that HSF1 can serve as a substrate for SV-activated ERK1 phosphorylation (data not shown), in keeping with published results (19–21, 33). More important to the goals of our study, however, was the need to measure the extent of ERK1 and -2 activation in response to combined SV, stress, and hormone treatments, since these would be the conditions under which we would determine the role of HSF in the GR response to stress (see below). As can be seen in Fig. 3, SV treatment alone resulted in a level of ERK1 and ERK2 activation that is comparable with that seen under combined SV, heat shock, and Dex treatment. Thus, if HSF1 activity is indeed modulated by MAPK, then we would expect about the same level of HSF inactivation under both of these conditions.

It was interesting to note from the results of Fig. 3*A* that heat shock treatment alone also caused activation of ERK1 and -2, albeit to a lesser degree than vanadate alone. Although this apparent contradiction calls into question the role of MAPK as a negative regulator of HSF1, it is also possible that heat shock activation of MAPK is actually a mechanism by which to achieve autoregulation of the heat shock response. As a test of this idea, we reasoned that other forms of stress that yield higher levels of HSF1 activity should show reduced induction of MAPK. In our LHSECAT cells, we have consistently observed that chemical shock using arsenite produces a stronger CAT response than does heat shock (see Fig. 1*B*). We therefore compared MAPK activation in response to both forms of stress (Fig. 3*B*). The results show that once again HS activation of ERK1 and -2 is intermediate between control levels and those seen in response to vanadate, while chemical shock had almost no effect on this activity. Thus, the differences we and others have observed in the heat shock response to various forms of stress could be due, at least in part, to variable effects of these stressors on MAPK activity.

Sodium Vanadate Prevents the Stress Potentiation of GR Transactivity—Given the results of Figs. 1–3, it appeared that SV could be used as an effective inhibitor of HSF1 activity in

stressed cells and as a potential reagent by which to test the role of HSF1 in the stress potentiation of GR. In order for SV to be useful in this approach, we reasoned that SV could not have a similar inhibitory effect on the GR response in nonstressed cells. To test this, we subjected L929 cells stably transfected with the pMMTV-CAT reporter (LMCAT2 cells) to increasing concentrations of SV prior to the addition of 1 μM Dex (Fig. 4*A*). To our surprise, the results showed that SV pretreatment caused an increase in Dex-induced CAT expression. Thus, SV appears able to independently target both the GR and heat shock pathways. Because SV exhibits diametrically opposed actions on each of these pathways, it was difficult to postulate how SV would affect the potentiation of GR under stress conditions, although one possible outcome was that inhibition by SV of HSF1's role in this process would be "masked" by a compensatory potentiation of GR by SV. To determine if this was indeed the case, we performed the experiments of Fig. 4*B*, in which LMCAT2 cells were subjected to a variety of Dex, SV, and stress conditions. As expected, the results show a strong potentiation of Dex-induced CAT activity by both heat shock and arsenite. Somewhat surprisingly, however, was the fact that SV could effectively block potentiation of the GR by both forms of stress. Moreover, this inhibition of the stress potentiation of the GR occurred whether SV was added before or after the stress event, a response pattern similar to that obtained for the effect of SV on HSF1 activity (Fig. 1). Thus, the data as a whole are consistent with a model requiring HSF1 activity for the stress potentiation of GR. Although at present it is not clear why the inhibitory effect of SV on HSF1 should show "dominance" over the stimulatory effect of SV on the GR, one possibility is that the latter response cannot occur in stressed cells, perhaps because SV targets multiple phosphatases, one or more of which act on the GR but only under nonstress conditions.

The DNA-dependent Protein Kinase Inhibitor, Wortmannin, Increases both Stress-induced HSF1 Activity and the Stress Potentiation of GR—To corroborate the results obtained with vanadate, we sought the use of a compound that could selectively increase HSF1 activity without itself causing stress. For this purpose, we chose wortmannin, an agent commonly used as an inhibitor of DNA-PK, a member of the phosphatidylinositol 3-kinase-related kinase family (23, 24). DNA-PK has been shown to directly interact with HSF1 *in vitro* (31, 32) and to modulate its activity *in vivo* (30, 31). However, it has yet to be shown whether wortmannin treatment of cells can affect HSF1 activity. Because increased DNA-PK activity is, in at least one report (30), purported to be inhibitory of HSF1, we reasoned that inhibition of DNA-PK by wortmannin should enhance the HSF1 response in cells. To test this idea, we performed the experiments of Fig. 5, in which LHSECAT cells were preincubated with wortmannin prior to either heat or chemical shock. As expected, both heat and arsenite induced HSF1-mediated CAT expression. Interestingly, wortmannin increased both of these responses but did not affect HSF1 in the absence of stress. Thus, it appears that inactivation of DNA-PK by wortmannin can result in derepression of HSF1 activity in intact cells. It also appears that wortmannin meets the criteria needed of a non-stress-inducing agent that can enhance the HSF response.

In order to take advantage of these properties of wortmannin and to test the involvement of HSF1 in the stress potentiation of GR, we first had to document the effects of this agent on GR signaling in the absence of stress. In Fig. 6*A*, LMCAT2 cells were pretreated with wortmannin prior to the addition of hormone. The results show that wortmannin alone had no effect but that it could enhance Dex-induced CAT expression. This

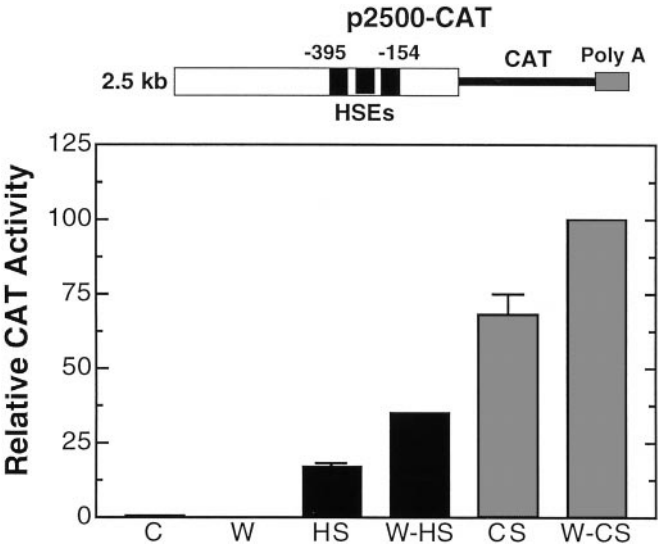

FIG. 5. **Wortmannin enhances heat and chemical shock activation of HSF1-mediated gene expression.** L929 cells stably transfected with the p2500-CAT reporter (LHSECAT cells) were subjected to the following conditions: no treatment (*C*); 1 μM wortmannin for 30 min (*W*); 43 °C for 2 h (*HS*); 1 μM for 30 min followed by washing and heat shock (43 °C for 2 h) (*W-HS*); 200 μM sodium arsenite for 2 h (*CS*); or 1 μM wortmannin for 30 min followed by washing and chemical shock (200 μM arsenite for 2 h) (*W-CS*). All cells were cultured for an additional 20 h under nonstress conditions. Cell lysates were prepared and assayed for CAT activity, expressed as a percentage of maximum (*W-CS*). The results shown represent the means ± S.E. of two independent experiments.

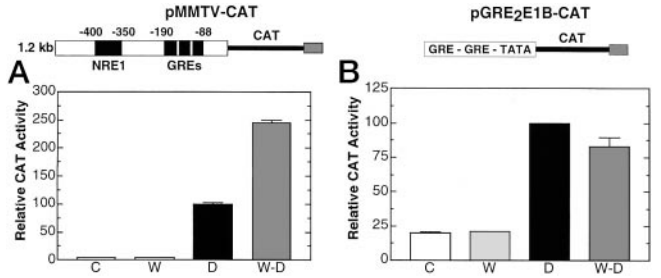

FIG. 6. **Enhancement of GR-mediated CAT gene expression by wortmannin does not occur with a minimal promoter containing only GREs and a TATA box.** L929 cells stably transfected with CAT reporter constructs controlled by the complex MMTV-LTR promoter (*A*) or by the synthetic, GRE_2E1B minimal promoter (*B*) were subjected to the indicated conditions and analyzed for CAT activity. Values are expressed as percentages of the Dex control (*D*). The results shown represent the means ± S.E. of three (*A*) or two (*B*) independent experiments. *C*, no treatment. *W*, 1 μM wortmannin for 30 min followed by washing and additional culture for 20 h. *D*, 1 μM Dex for 20 h. *W-D*, 1 μM wortmannin for 30 min followed by washing and additional culture in the presence of 1 μM Dex for 20 h.

result was not entirely unexpected, since the MMTV promoter has been shown to contain a sequence termed NRE1, residing between 350 and 400 base pairs upstream of the transcription start site, that serves as the binding site for the Ku subunit of DNA-PK (27). As DNA-PK localized to NRE1 has been shown to phosphorylate the GR and to inhibit its hormone-induced transactivity (28, 29), the results obtained with wortmannin in Fig. 6*A* are as predicted based on these prior findings. To further test this model of action and to circumvent any direct effect of wortmannin on GR signaling, we reasoned that the stimulatory effect of wortmannin should not be seen in promoters lacking the NRE1 site. To this end, L929 cells stably transfected with a minimal promoter construct ($pGRE_2E1B$-CAT) in which CAT expression is controlled by two synthetic GREs and a TATA box (39) were employed. Using these cells, it was found

that wortmannin no longer had a stimulatory effect on GR-mediated CAT expression (Fig. 6*B*), providing further evidence that the NRE1 site within the MMTV-LTR is indeed responsible for directing DNA-PK-mediated inhibition of GR activity. More importantly, these results with the pGRE$_2$E1B-CAT reporter now made it possible to use wortmannin to test the role of HSF1 in the stress potentiation of GR. The results of such experiments are seen in Fig. 7. Although this reporter typically yields a much smaller response to Dex than the MMTV promoter, the relative increase due to heat shock is much more dramatic with pGRE$_2$E1B-CAT (13, 40), making it look as if there is no response to Dex alone in Fig. 7. In reality, the Dex-induced responses in both Figs. 6*B* and 7 are each approximately 4-fold. Regardless, it can also be clearly seen that wortmannin can further potentiate the heat shock effect on the GR. Thus, like the experiments with vanadate, modulation of HSF1 activity with wortmannin results in a corresponding modulation of the stress potentiation effect on the GR.

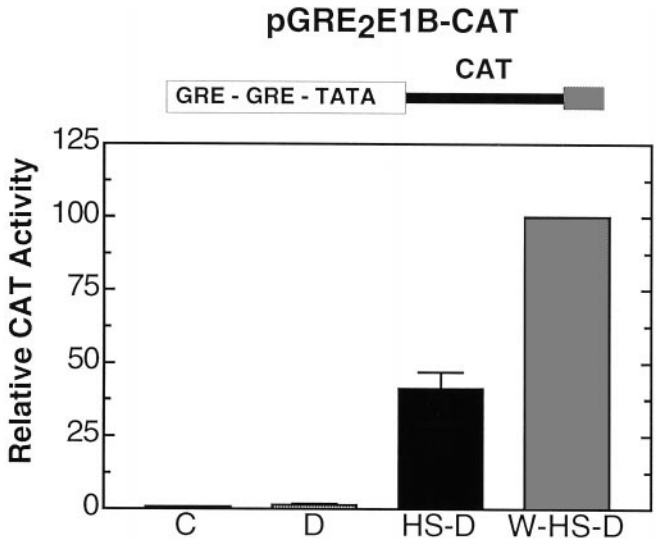

FIG. 7. **Wortmannin enhances heat shock potentiation of GR transactivation in cells transfected with a minimal CAT reporter.** L929 cells stably transfected with the pGRE$_2$E1B-CAT reporter were subjected to the indicated conditions and analyzed for CAT activity. Values are expressed as percentages of the maximum (*W-HS-D*). The results shown represent the means ± S.E. of two independent experiments. *C*, no treatment. *D*, 1 μM Dex for 20 h. *HS-D*, heat shock at 43 °C for 2 h followed by 1 μM Dex for 20 h. *W-HS-D*, 1 μM wortmannin for 30 min followed by washing, heat shock (43 °C for 2 h), and 1 μM Dex for 20 h. Because the magnitude of the heat shock potentiation effect on the GR using this promoter is so large, the Dex control appears to be unchanged compared with basal. Actual -fold induction value for the Dex control is 3.5-fold, compared with 4.2-fold induction for this condition in Fig. 6*B*.

DISCUSSION

In recent work from our laboratory (40), we obtained our first evidence of a correlation between HSF1 activity and the heat shock potentiation of GR by demonstrating a similar pattern of HSF1 and GR activity in stressed cells and by showing that inhibition of HSF1 activity by the flavonoid compound quercetin results in a similar inhibition of the stress effect on the GR. In those experiments, inhibition of HSF1 and of the stress potentiation effect on the GR were observed only when cells were exposed to quercetin before the stress event, suggesting that quercetin was acting to inhibit an early event in the stress activation of HSF1. However, since quercetin appears to have multiple targets within cells (45–48), based primarily on its ability to block ATP-binding sites, it was possible that quercetin was acting by inhibiting one or more enzymes (*e.g.* that independently controlled activation of HSF and potentiation of the GR in stressed cells). If the latter were true, it should be possible to separate the HSF1 response from the stress potentiation effect on the GR. In the experiments reported here, we have attempted to do this by modulating HSF1 activity by two compounds known to have distinct targets and mechanisms of action. Our results demonstrate that either inhibition of HSF1 activity by sodium vanadate or enhanced stimulation of HSF1 activity by wortmannin results in a corresponding modulation of GR activity in stressed cells. Thus, the mechanism by which heat shock increases the GR response must now be thought to require intrinsic activity on the part of HSF1.

If HSF1 is fundamentally involved in the stress potentiation of the GR, what then is the nature of this involvement? Our current working hypothesis is illustrated in Fig. 8. Under this model, there are two overall mechanisms by which a heat shock-induced gene product (*X*) can potentiate GR-mediated gene expression, a process referred to in this model as the heat shock potentiation effect (*HSPE*). In one of these mechanisms, we postulate that *X* may directly promote or enhance the transactivation function of the GR. In this respect, product *X* could be viewed as a heat shock-inducible co-activator. Consistent with this mechanism are our previous observations that poten-

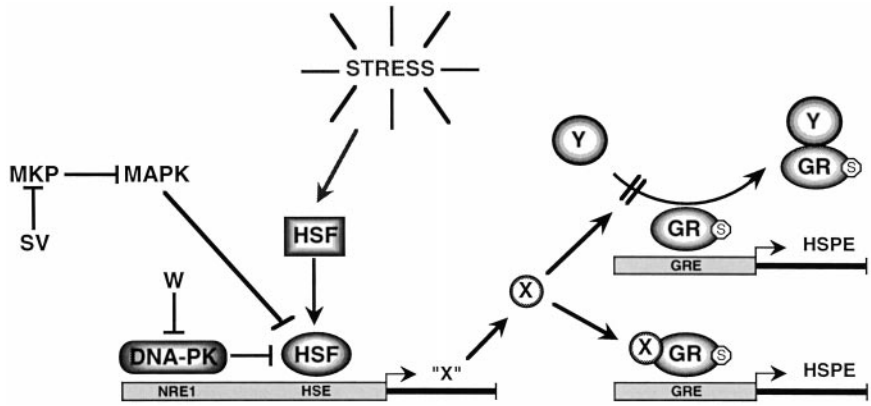

FIG. 8. **Model for the involvement of HSF1 in the stress potentiation of GR.** In the present study, we have used sodium vanadate (*SV*) or wortmannin (*W*) to modulate HSF1 activity. Our results show that SV, presumably by its direct effect on MAPK phosphatases (*MKP*), can inhibit HSF1 activity by a mechanism involving increased MAPK (ERK1/2) activity and subsequent phosphorylation of HSF1. Wortmannin was shown to increase HSF1 without being an additional form of stress, presumably by inhibiting the negative regulatory effects of DNA-PK. With each drug, a corresponding decrease (*SV*) or increase (*W*) of the heat shock potentiation effect (*HSPE*) on the GR was observed, suggesting that the heat shock potentiation effect requires HSF1 activity. Based on these results, we propose that the heat shock potentiation effect response is mediated by a heat shock-induced gene product (*X*) that can act on the GR by one of two overall mechanisms: 1) as a stress-induced co-activator or 2) as an inhibitor of a factor(s) that antagonizes the activity of GR. (See "Discussion" for further details).

tiation of the GR can occur for GR that is already localized to the nucleus in response to hormone and that the potentiation requires only a minimal promoter containing just GREs (13). Although it is interesting to speculate that one of the major known heat shock proteins may serve the function of product *X* in our system, to date no direct evidence for this possibility has been found. However, there is evidence that HSPs can affect the DNA-binding properties of steroid receptors, since the glucocorticoid (49), progesterone (50, 51), and estrogen receptors (52) have been shown to remain tightly complexed to HSP70 even after hormone-induced transformation of these receptors to the DNA-binding state. Moreover, purified HSP70 has been shown to enhance the *in vitro* DNA binding properties of the estrogen receptor (52). In contrast, HSP90 may have a negative regulatory effect on GR transactivity, since overexpression of a form of HSP90 modified for nuclear targeting results in an inhibition of GR activity at the MMTV promoter (53).

In the second mechanism, we speculate that a HSF1-induced product can regulate the GR by binding to and sequestering a factor that serves to inhibit the GR. A plausible and interesting example of this mechanism has recently come to light, namely the mutual antagonism between GR and nuclear factor-κB (NF-κB). Direct interaction between GR and NF-κB has recently been demonstrated (54–56), resulting in inhibition of the transactivation functions of each of these factors (56, 57). More importantly, heat shock has recently been shown to increase the expression of IκB, the negative regulator of NF-κB, by a mechanism that probably involves direct transcriptional enhancement of the IκB gene by HSF1 (58–60). Heat shock-induced IκB expression has also been shown to inhibit NF-κB activity following stress (58–60). These observations, thus, present the interesting possibility that the heat shock potentiation of GR could result from a release of the negative regulatory effects of NF-κB on the GR by a mechanism involving increased expression of IκB. Although the role of NF-κB/IκB in the stress regulation of GR is an intriguing line of investigation, other transcription factors could also be involved. For example, mutual antagonism between the GR and AP-1 has also been demonstrated. In this case, GR has been shown to form dimers with both the Fos and Jun subunits of AP-1, resulting in reciprocal inhibition of GR and AP-1 transactivation functions (61–63). Since cell stress has also been shown to activate AP-1 function by promoting formation of Fos/Jun heterodimers (64), it is possible that heat shock could potentiate the actions of GR by essentially shifting the interaction of the AP-1 subunits away from the GR.

Other possible mechanisms exist by which a heat shock-induced gene product could modulate the GR response. For example, an effect of this product to increase nuclear translocation or retention of the GR in stressed cells would result in potentiation of the GR. However, using a quantitative Western blotting technique, we have shown that the amount of GR in the nucleus is actually slightly decreased in cells subjected to both stress and hormone, as compared with hormone-alone controls (13). Thus, this mechanism is not likely to be operating in our cells. A stress-induced protein may also serve to directly or indirectly modify the GR, perhaps through phosphorylation, leading to increased transactivation activity. As a test of this possibility, experiments are under way to determine the phosphorylation states of GR protein obtained from cells subjected to various combinations of hormone and stress treatment.

An alternative explanation for our data (one that does not require an HSF-induced gene product) is that HSF1 can control GR transactivation function by directly binding to the GR. However, to the best of our knowledge, no interaction between HSF1 and any other transacting factor has yet to be described, perhaps due to the rather unique requirement of HSF1 to undergo trimerization, whereas most other transcription factors are active as dimers. An aspect of our current study that also makes a direct HSF/GR interaction less likely is that both SV (Fig. 2) and wortmannin (data not shown) did not affect the amount of HSF1 bound to DNA, suggesting that oligomerization and all prior steps are not involved in stress potentiation mechanism.

Taken as a whole, the results of this study provide evidence that HSF1 activity within stressed cells is necessary for the observed ability of stress to cause a superactivation of the GR response. Moreover, the essential property of HSF1 activity that is required for the stress effect on the GR appears to be its transactivation function. As a test of this hypothesis, future experiments will include expression studies using wild-type HSF1 as well as mutant forms of HSF1 with altered levels of transcription enhancement activity.

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REFERENCES

1. Evans, R. M. (1988) *Science* **240,** 889–895
2. Tsai, M.-J., and O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63,** 451–486
3. Munck, A., Guyre, P. M., and Holbrook, N. J. (1984) *Endocr. Rev.* **5,** 25–44
4. Pratt, W. B., and Toft, D. O. (1997) *Endocr. Rev.* **18,** 306–360
5. Sanchez, E. R. (1992) *J. Biol. Chem.* **267,** 17–20
6. Shen, P., Xie, Z.-J., Li, H., and Sanchez, E. R. (1993) *J. Steroid Biochem. Mol. Biol.* **47,** 55–64
7. Matic, G. (1995) *Jugoslov. Med. Biochem.* **14,** 89–102
8. Matic, G., Kipic, J., Ristic, B., Dundjerski, J., and Trajkovic, D. (1995) *Cell Biol. Int.* **19,** 203–213
9. Liu, W., Hillmann, A. G., and Harmon, J. M. (1995) *Mol. Cell. Biol.* **15,** 1005–1013
10. Sivo, J., Harmon, J. M., and Vogel, S. N. (1996) *J. Immunol.* **156,** 3450–3454
11. Smets, L. A., Salomons, G., Van Rooij, H., and van den Berg, J. (1998) *Leukemia* **12,** 406–413
12. Edwards, D. P., Estes, P. A., Fadok, V. A., Bona, B. J., Onate, S., Nordeen, S. K., and Welch, W. J. (1992) *Biochemistry* **31,** 2482–2491
13. Sanchez, E. R., Hu, J. L., Zhong, S. J., Shen, P., Green, M. J., and Housley, P. R. (1994) *Mol. Endocrinol.* **8,** 408–421
14. Hu, J. L., Guan, X. J., and Sanchez, E. R. (1996) *Cell Stress Chaperones* **1,** 197–205
15. Gordon, J. A. (1991) *Methods Enzymol.* **201,** 477–482
16. Sun, H., Charles, C. H., Lau, L. F., and Tonks, N. K. (1993) *Cell* **75,** 487–493
17. Ward, Y., Gupta, S., Jensen, P., Wartmann, M., Davis, R. J., and Kelly, K. (1994) *Nature* **367,** 651–654
18. Zou, J., Salminen, W. F., Roberts, S. M., and Voellmy, R. (1998) *Cell Stress Chaperones* **3,** 130–141
19. Chu, B., Soncin, F., Price, B. D., Stevenson, M. A., and Calderwood, S. K. (1996) *J. Biol. Chem.* **271,** 30847–30857
20. Kim, J., Nueda, A., Meng, Y., Dynan, W. S., and Mivechi, N. F. (1997) *J. Cell. Biochem.* **67,** 43–54
21. Mivechi, N. F., and Giaccia, A. J. (1995) *Cancer Res.* **55,** 5512–5519
22. He, L., and Fox, M. H. (1999) *Radiation Res.* **151,** 283–292
23. Sarkaria, J. N., Tibbetts, R. S., Busby, E. C., Kennedy, A. P., Hill, D. E., and Abraham, R. T. (1998) *Cancer Res.* **58,** 4375–4382
24. Izzard, R. A., Jackson, S. P., and Smith, G. C. (1999) *Cancer Res.* **59,** 2581–2586
25. Gottlieb, T. M., and Jackson, S. P. (1993) *Cell* **72,** 131–142
26. Dvir, A., Peterson, S. R., Knuth, M. W., Lu, H., and Dynan, W. S. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11920–11924
27. Giffen, W., Torrance, H., Rodda, D. J., Prefontaine, G. G., Pope, L., and Hache, R. J. G. (1994) *J. Biol. Chem.* **269,** 1449–1459
28. Giffen, W., Kwast-Welfeld, J., Rodda, D. J., Prefontaine, G. G., Traykova-Andonova, M., Zhang, Y., Weigel, N. L., Lefebvre, Y. A., and Hache, R. J. G. (1997) *J. Biol. Chem.* **272,** 5647–5658
29. Giffen, W., Gong, W., Schild-Poulter, C., and Hache, R. J. G. (1999) *Mol. Cell. Biol.* **19,** 4065–4078
30. Yang, S. H., Nussenzweig, A., Li, L., Kim, D., Ouyang, H., Burgman, P., and Li, G. C. (1996) *Mol. Cell. Biol.* **16,** 3799–3806
31. Nueda, A., Hudson, F., Mivechi, N. F., and Dynan, W. S. (1999) *J. Biol. Chem.* **274,** 14988–14996
32. Huang, J., Nueda, A., Yoo, S., and Dynan, W. S. (1997) *J. Biol. Chem.* **272,** 26009–26016
33. Knauf, U., Newton, E. M., Kyriakis, J., and Kingston, R. E. (1996) *Genes Dev.* **10,** 2782–2793
34. Zou, J., Rungger, D., and Voellmy, R. (1995) *Mol. Cell. Biol.* **15,** 4319–4330
35. Shi, Y., Kroeger, P. E., and Morimoto, R. I. (1995) *Mol. Cell. Biol.* **15,** 4309–4318
36. Dreano, M., Brochot, J., Myers, A., Cheng-Meyer, C., Rungger, C., Voellmy, R., and Bromley, P. (1986) *Gene* (*Amst.*) **49,** 1–8
37. Danielsen, M., Northrop, J. P., and Ringold, G. M. (1986) *EMBO J.* **5,** 2513–2522

38. Cato, A. C. B., Miksicek, R., Schutz, G., Arnemann, J., and Beato, M. (1986) *EMBO J.* **5,** 2237–2240
39. Allgood, V. E., Oakley, R. H., and Cidlowski, J. A. (1993) *J. Biol. Chem.* **268,** 20870–20876
40. Li, D.-P., Li Calzi, S., and Sanchez, E. R. (1999) *Cell Stress Chaperones* **4,** 223–234
41. Nordeen, S. K., Green, P. P. I., and Fowlkes, D. M. (1987) *DNA* **6,** 173–178
42. Laemmli, U. K. (1970) *Nature* **227,** 680–685
43. Tienrungroj, W., Sanchez, E. R., Housley, P. R., Harrison, R. W., and Pratt, W. B. (1987) *J. Biol. Chem.* **262,** 17342–17349
44. Mosser, D. D., Theodorakis, N. G., and Morimoto, R. I. (1988) *Mol. Cell. Biol.* **8,** 4736–4744
45. Gschwendt, M., Horn, F., Kittstein, W., and Marks, F. (1983) *Biochem. Biophys. Res. Commun.* **117,** 444–447
46. Kuriki, Y., and Racker, E. (1976) *Biochemistry* **15,** 4951–4956
47. Graziani, Y., and Chayoth, R. (1979) *Biochem. Pharmacol.* **28,** 397–403
48. End, D., Look, R. A., Shaffer, N. L., Balles, E. A., and Persico, F. J. (1987) *Res. Commun. Chem. Pathol. Pharmacol.* **56,** 75–86
49. Srinivasan, G., Patel, N. T., and Thompson, E. B. (1994) *Mol. Endocrinol.* **8,** 189–196
50. Estes, P. A., Suba, E. J., Lawler-Heavner, J., Elashry-Stowers, D., Wei, L. L., Toft, D. O., Sullivan, W. P., Horwitz, K. B., and Edwards, D. P. (1987) *Biochemistry* **26,** 6250–6262
51. Kost, S. L., Smith, D. F., Sullivan, W. P., Welch, W. J., and Toft, D. O. (1989) *Mol. Cell. Biol.* **9,** 3829–3838
52. Landel, C. C., Kushner, P. J., and Greene, G. L. (1994) *Mol. Endocrinol.* **8,** 1407–1419
53. Kang, K. I., Meng, X., Devin-Leclerc, J., Bouhouche, I., Chadli, A., Cadepond, F., Baulieu, E.-E., and Catelli, M.-G. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 1439–1444
54. Scheinman, R. I., Gualberto, A., Jewell, C. M., Cidlowski, J. A., and Baldwin Jr., A. S. (1995) *Mol. Cell. Biol.* **15,** 943–953
55. Ray, A., and Prefontaine, K. E. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 752–756
56. Caldenhoven, E., Liden, J., Wissink, S., Van de Stolpe, A., Raaijimakers, J., Koenderman, L., Okret, S., Gustafsson, J.-A., and Van der Saag, P. T. (1995) *Mol. Endocrinol.* **9,** 401–412
57. McKay, L. I., and Cidlowski, J. A. (1998) *Mol. Endocrinol.* **12,** 45–56
58. Wong, H. R., Ryan, M., Menendez, I. Y., and Wispe, J. R. (1999) *Cell Stress Chaperones* **4,** 1–7
59. Wong, H. R., Ryan, M., and Wispe, J. R. (1997) *J. Clin. Invest.* **99,** 2423–2428
60. Rossi, A., Elia, G., and Santoro, M. G. (1998) *J. Biol. Chem.* **273,** 16446–16452
61. Konig, H., Ponta, H., Rahmsdorf, H.-J., and Herrlich, P. (1992) *EMBO J.* **11,** 2241–2246
62. Schule, R., Rangarajan, P. N., Kliewer, S., Ransone, L. J., Bolado, J., Yang, N., Verma, I. M., and Evans, R. M. (1990) *Cell* **62,** 1217–1226
63. Yang-Yen, H.-F., Chambard, J.-C., Sun, Y.-L., Smeal, T., Schmidt, T. J., Drouin, J., and Karin, M. (1990) *Cell* **62,** 1205–1215
64. Erdos, G., Lee, Y. J., Cho, J. M., and Corry, P. M. (1995) *J. Cell. Physiol.* **164,** 404–413