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Dual control of heat shock response: Involvement of a constitutive heat shock element-binding factor

(gene regulation/heat shock protein/transcription factor/thermotolerance)

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ABSTRACT **Heat shock factor (HSF) has been implicated as the key regulatory protein in the heat shock response. Our studies on the response of rodent cells to heat shock or sodium arsenite indicate that a high level of HSF–DNA-binding activity, by itself, is not sufficient for the induction of hsp70 mRNA synthesis; furthermore, a high level of HSF binding is also not necessary for this induction. Analysis of the binding of protein factors to the heat shock element (HSE) in extracts of stressed rodent cells indicates that the regulation of heat shock response involves the heat-inducible HSF and a constitutive HSE-binding factor. Our results also suggest that overexpression of human hsp70 may decrease the level of heat-induced HSF–HSE-binding activity in rat cells.**

Heat shock genes are expressed in response to a wide range of physiological or chemically induced stresses (1, 2). In eukaryotes, transcriptional regulation of heat shock genes involves a highly conserved cis-acting sequence, termed the heat shock element (HSE), which is present in multiple copies upstream of the transcriptional start site. It is well documented that HSE is the binding site of the heat shock factor (HSF), and the binding of HSF to HSE activates heat shock gene transcription (3–7).

HSF prepared from unshocked mammalian cells can be induced to bind HSE *in vitro* by exposing cell extracts to elevated temperatures or to reagents that dissociate or denature protein complexes (8). Recently, genes encoding HSFs of a number of organisms including human and mouse have been cloned (9–14). Recombinant human or *Drosophila* HSF protein produced in *Escherichia coli* binds to HSE with high affinity without heat treatment (9–11). These results suggest that HSF in unstressed cells must be activated post-translationally, either by covalent modification or by modulation of its self-association or its interaction with one or more regulatory proteins. Extensive studies in *Drosophila* and yeast have provided strong evidence that protein modification and oligomerization are important factors that convert HSF in unstressed cells to an HSE-bound transcription activator upon stress (6, 9, 15–17). It has been postulated that suppression of HSF–HSE binding in unshocked cells is due to a block in multimerization of HSF, probably as a result of altered protein folding or the binding to HSF of an inhibitory substance (6, 17, 18).

The 70-kDa heat shock protein (hsp70) appears to play a key role in the cellular response to heat shock (1, 2). In *Drosophila*, there is evidence that hsp70 is autoregulated transcriptionally and translationally (19, 20); similar data exist for *E. coli* (21), the yeast *Saccharomyces cerevisiae*, and mammalian cells (22–24). Recently, it has been hypothesized that heat shock proteins, including hsp70, may be involved in the regulation of HSF (6, 7, 9, 25).

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HSF has been implicated as the key regulatory protein in the heat shock response. We report here that in rodent cells a high level of HSE-bound HSF, by itself, is not sufficient for the induction of hsp70 mRNA synthesis; furthermore, although HSF is presumably required to induce hsp70 mRNA synthesis, a high level of HSF–HSE-binding activity is not necessary for this induction. In addition to HSF, a constitutive HSE-binding factor (CHBF) appears to be involved in the regulation of hsp70 transcription. Our results also suggest that overexpression of human hsp70 may decrease the level of heat-induced HSF in rat cells.

MATERIALS AND METHODS

Cell Cultures and Heat Shock Treatment. Rat fibroblasts (Rat-1) and thermotolerant Rat-1 (TT Rat-1) cells were grown in Dulbecco's modified medium (DME-H21, GIBCO) supplemented with 10% fetal bovine serum. The infected Rat-1 cells (M21), constitutively expressing the exogenous human hsp70, were routinely maintained in Dulbecco's modified medium (DME-H21) supplemented with 10% fetal bovine serum and the antibiotic G418 (200 μg/ml) (26). The construction of plasmids containing human hsp70 and the procedures for DNA-mediated gene transfer have been described (26). M21 cells used in this study were derived from an individual colony.

For heat shock treatment, monolayers of cells were heated at 45°C for different times in hot water baths in specially designed incubators (27). TT Rat-1 cells were obtained by heating the cells at 45°C for 15 min and subsequently incubating them at 37°C for 16 hr (26, 28).

Isolation of RNA and Northern Hybridization. Total cellular RNA was isolated with a commercial kit (Biotecx Laboratories, Houston) according to the protocols provided by the manufacturer. RNA (10 μg) was denatured with glyoxal/dimethyl sulfoxide and incubated at 55°C for 1 hr, size-fractionated on 1% agarose gels, and transferred to Hybond-N membrane (Amersham) in 10× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7). The blotted membranes were probed with the 2.3-kb *Bam*HI–*Hin*dIII fragment of the human hsp70 gene, which was ^{32}P-labeled by the random primer method (27). After hybridization, the membranes were washed, dried, and autoradiographed with Kodak X-Omat film. For the quantification of hsp70 or hsc70 mRNA, autoradiograms were scanned using an AMBIS optical imaging system. The relative levels of hsp70 mRNA were expressed as ratios by dividing the optical intensity of hsp70 mRNA at various times after heat shock by

Abbreviations: HSE, heat shock element; HSF, heat shock factor; CHBF, constitutive HSE-binding factor; TT Rat-1, thermotolerant Rat-1.

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that of the constitutive hsc70 mRNA in the unheated control cells.

Preparation of Cell Extracts and Gel Mobility-Shift Assay. Preparation of the cell extracts and the gel mobility-shift assay were performed as described (29, 30). An equal amount of cellular proteins (40 μg) from each sample was incubated with a ^{32}P-labeled double-stranded oligonucleotide containing the HSE from rat heat shock promoter (5′-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3′; R. Mestril, personal communication). The protein-bound and free oligonucleotides were electrophorically separated on 4% native polyacrylamide gels in 0.5× TBE buffer (44.5 mM Tris, pH 8.0/1 mM EDTA/44.5 mM boric acid) for 4 hr at 140 V. The free ^{32}P-labeled oligonucleotides migrated to the bottom of the gel. Competition assays were performed by coincubating the cell extracts from control or heat-shocked cells with 50-fold molar excess of nonlabeled HSE oligonucleotides in addition to excess DNA of nonspecific sequences. The gels were dried and autoradiographed with Kodak X-Omat film and a DuPont Cranex Lightning Plus intensifying screen at −70°C. Autoradiograms were quantified using AMBIS.

RESULTS

Rat-1 Cells Contain Two HSE-Binding Factors: One Constitutive and One Heat Shock-Induced. Protein factors in Rat-1 cells that interact with the HSE were examined by the gel mobility-shift assay (29, 30). Fig. 1*A* depicts the electrophoretic migration patterns of HSE-binding proteins in a nondenaturing gel. Two distinct HSE–protein binding complexes were detected: a faster migrating complex in extracts of unshocked control Rat-1 cells (Fig. 1*A*, arrow) and a slower migrating complex in extracts of heat-shocked Rat-1 cells (Fig. 1*A*, arrowhead). The induction of the slower migrating HSE–protein complex by 45°C heat shock was rapid, reaching a maximum around 5 min after the heat shock (Fig. 1*A*); this complex corresponds to the well-documented HSE–HSF complex (31), and the DNA-binding protein component in this complex will be referred to as HSF. In contrast to that of HSF, the amount of the faster migrating HSE–protein complex present in unshocked Rat-1 cells decreases upon heat shock (Fig. 1*A*). This complex is probably the same as that of the so-called "constitutive HSE-binding activity (CHBA)" previously observed in HeLa cells (31), and the DNA-binding protein component in this complex will be referred to as CHBF. Cycloheximide had no effect on the appearance of HSF upon heat shock, indicating that *de novo* protein synthesis was not involved in its formation (data not shown).

The recovery kinetics of HSF- and CHBF–HSE-binding activity at 37°C after a 15-min heat shock of 45°C are illustrated in Fig. 1 *B* and *C*. The level of HSF–HSE binding decreased rapidly and disappeared by 30 min (Fig. 1 *B* and *C*, open bar). The recovery of CHBF–HSE binding took longer; it returned to the pre-heat shock level in ≈8 hr (Fig. 1 *B* and *C*, hatched bars).

For simplicity, throughout the text the level of HSF or CHBF always refers to the level of HSF–HSE- or CHBF–HSE-binding activity as determined by the gel mobility-shift assay.

Relation Between hsp70 mRNA Synthesis and Levels of CHBF and HSF. To determine whether the level of HSF correlated with the increase in hsp70 transcription, the dose dependence of the induced hsp70 mRNA transcription was examined by heating Rat-1 cells at 45°C for 5, 15, and 30 min. The hsp70 mRNA level (Fig. 2*A*, arrow indicating 70_i), determined 4 hr after treatment, demonstrated that its accumulation depended upon the severity of heat shock treatment. This dose–response of hsp70 mRNA correlates well with that of HSF (compare Fig. 1*A* and Fig. 2*A*).

Fig. 2*B* shows the kinetics of hsp70 mRNA in Rat-1 cells after a heat treatment of 15 min at 45°C (unless otherwise stated, a 15-min heat treatment at 45°C was always used). In unstressed Rat-1 cells, the level of the constitutive hsc71 mRNA is low, and hsp70 mRNA is not detectable (Fig. 2*B*, arrow indicating 70_c). Heat-induced transcription is evidenced by the gradual accumulation of the newly synthesized hsp70 mRNA, with a maximum at about 6–8 hr, and a subsequent decline to 20% of its maximum value by 16 hr (Fig. 2*B*, arrow indicating 70_i). The time required for com-

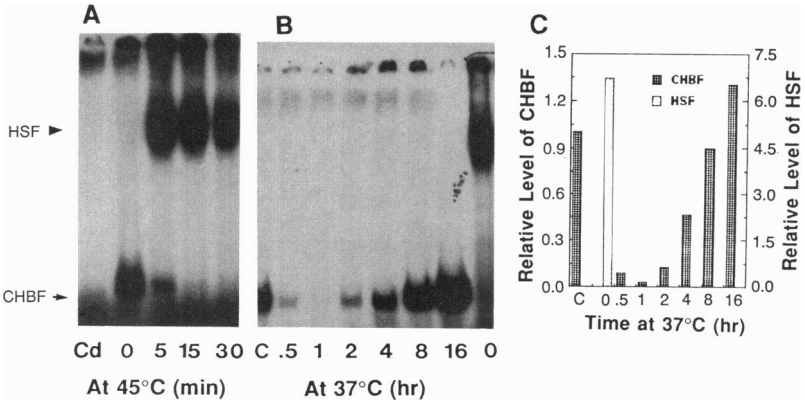

FIG. 1. Analysis of HSE-binding activities in control and heat-shocked Rat-1 cells. (*A*) Gel mobility-shift analysis of whole cell extracts from control and 45°C heat-shocked Rat-1 cells in 4% nondenaturing polyacrylamide gels at 25°C. Analysis was performed as described (29, 30) with a ^{32}P-labeled oligonucleotide containing the HSE from rat heat shock promoter (5′-GGGCCAAGAATCTTCCAGCAGTTTCGGG-3′; R. Mestril, personal communication). The free ^{32}P-labeled oligonucleotide migrated to the bottom of the gel. Competition assays were also performed by coincubating the cell extracts from control or heat-shocked cells with 50-fold molar excess of nonlabeled HSE oligonucleotide in addition to excess DNA of nonspecific sequences (lane Cd). Bands of HSE–protein complexes were visualized by autoradiography. The constitutive HSF–HSE-binding complex (CHBF) is indicated by an arrow, and the heat-shock induced HSF–HSE complex (HSF) is indicated by an arrowhead. Rat-1 cells (26, 27) were heat shocked at 45°C for 0–30 min and whole cell extracts were prepared immediately afterward for gel mobility-shift assays. (*B*) Autoradiogram showing levels of CHBF and HSF during recovery at 37°C. Rat-1 cells were heat shocked at 45°C for 15 min and returned to 37°C incubation for 0.5, 1, 2, 4, 8, and 16 hr and whole cell extracts were used for gel mobility-shift analysis. Equal amounts of total cellular proteins were loaded for each lane. C, control Rat-1 cells; 0, Rat-1 cells heat shocked at 45°C for 15 min and cell extracts were prepared immediately. (*C*) Autoradiogram from *B* was quantified using an AMBIS optical imaging system. Relative levels of HSF and CHBF are expressed as ratios by dividing the optical intensity of CHBF or HSF at various times after heat shock by that of CHBF in the control Rat-1 cells receiving no heat shock treatment.

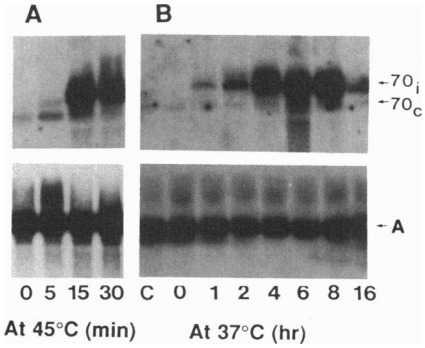

FIG. 2. (*A*) Levels of hsp70 mRNA after different doses of heat shock. Rat-1 cells were heated at 45°C for 5, 15, or 30 min and then returned to 37°C for 4 hr. Total cellular RNA was isolated, size fractionated on 1% agarose gel, transferred to Hybond-N membrane, and probed with a ^{32}P-labeled 2.3-kb *Bam*HI–*Hin*dIII fragment of the human hsp70 gene (upper blot) or human β-actin (lower blot). The time interval was chosen because hsp70 mRNA reaches its maximal level in about 4–8 hr. (*B*) Rat-1 cells were exposed to 45°C for 15 min and then returned to 37°C for 0, 1, 2, 4, 6, 8, and 16 hr. Total cellular RNA was isolated and analyzed as described in *A*. The inducible rat hsp70 mRNA (70_i) and constitutive hsc71 mRNA (70_c) are indicated by arrows; actin is indicated as A.

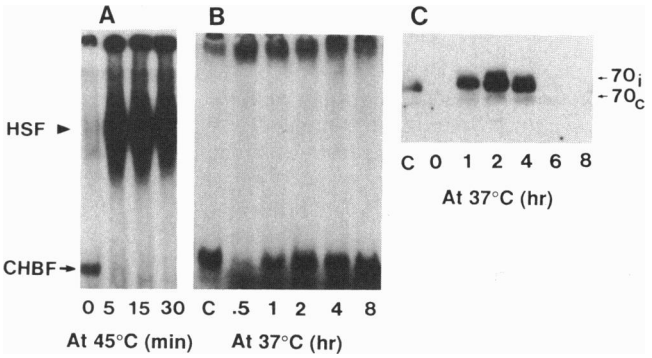

FIG. 3. Analysis of HSF and CHBF levels and hsp70 transcription in heat-shocked TT Rat-1 cells. TT Rat-1 cells were obtained by exposing Rat-1 cells to 45°C for 15 min followed by 16 hr of incubation at 37°C. (*A*) Gel mobility-shift analysis of whole cell extracts from TT Rat-1 cells heat shocked at 45°C for 0, 5, 15, and 30 min. CHBF and HSF are indicated by the arrow and arrowhead, respectively. (*B*) Levels of CHBF and HSF in TT Rat-1 cells during recovery at 37°C after the 45°C, 15-min heat treatment. Equal amounts of total cellular proteins were loaded per lane. C, control TT Rat-1 cells before the second heat shock treatment; 0.5, 1, 2, 4, and 8, recovery times in hr at 37°C. (*C*) hsp70 mRNA levels in heat-shocked TT Rat-1 cells during recovery at 37°C. TT Rat-1 cells were heated at 45°C for 15 min and returned to 37°C for 0, 1, 2, 4, 6, and 8 hr. Total cellular RNA was isolated and analyzed as described in the legend to Fig. 2. hsp70 mRNA and hsc71 mRNA are indicated by arrows. There is no difference in the level of actin mRNA in TT Rat-1 cells before and immediately after the second heat treatment and during the subsequent recovery period at 37°C (data not shown).

plete recovery of CHBF—i.e., about 8 hr (Fig. 1 *B* and *C*)—coincides with the onset of decline of hsp70 mRNA after the identical heat treatment (compare Fig. 1*B* and Fig. 2*B*). This temporal correlation between the recovery of CHBF and the accumulation and disappearance of hsp70 mRNA after heat shock is also clearly seen in TT Rat-1 cells and in Rat-1 cells overexpressing a cloned human hsp70 protein (see below).

Rat-1 cells, exposed to 45°C for 15 min and then incubated at 37°C for 16 hr, develop a transient state of resistance to subsequent heat challenge. This phenomenon, termed thermotolerance, has been observed in many mammalian cells and animal model systems and appears to correlate well with the elevated level of hsp70 (32–35). Thus we examined TT Rat-1 cells, before and after a second heat shock of 15 min at 45°C, to ascertain the influence of elevated hsp70 level on HSF and CHBF as well as the associated hsp70 mRNA synthesis (Fig. 3). Prior to the second heat treatment, TT Rat-1 cells exhibited levels of CHBF and HSF similar to that of the control nontolerant cells. After the second heat shock, CHBF drastically decreased and HSF increased rapidly with levels comparable to those of similarly heated nontolerant Rat-1 cells (Fig. 3*A*).

The kinetics of induced hsp70 mRNA synthesis by the second 45°C, 15-min heat shock are considerably different for the TT Rat-1 cells when compared to the control nontolerant Rat-1 cells after a similar heat treatment (compare Fig. 3*C* and Fig. 2*B*). Specifically, the induction of hsp70 mRNA synthesis is more rapid in TT Rat-1 cells, with a maximum at about 2 hr after the second heat shock, as compared to 6–8 hr for the nontolerant Rat-1 cells (Fig. 4*A*). After the maximal accumulation, the hsp70 mRNA level declined rapidly to about 10% of its maximum by 6 hr for the TT Rat-1 cells (Fig. 4*A*).

The recovery kinetics of HSF in TT Rat-1 cells, after a second heat shock, are similar to that in nontolerant cells, with HSF decreasing rapidly to an undetectable level by 30 min (Fig. 3*B*). The recovery of CHBF at 37°C, however, is more rapid than that of the nontolerant Rat-1 cells after an identical heat treatment; the level of CHBF in TT Rat-1 cells returns to the pre-heat-shock level within 2 hr following the down-shift of temperature (Figs. 3*B* and 4*B*). Again, the time required for the full recovery of CHBF appears to coincide with the time for the onset of decline of hsp70 mRNA, and the faster recovery of CHBF in TT Rat-1 cells correlates well with the faster accumulation and disappearance of hsp70 mRNA in the same cells (Fig. 4 *A* and *B*).

Constitutive Expression of Human hsp70 Gene in Rat-1 Cells Suppresses HSF but not CHBF. One plausible feedback loop in the regulation of cellular response to heat shock is that the heat shock proteins may negatively regulate their production (19–25). Recently, using a DNA-mediated gene transfer technique, we have established rat cell lines stably and constitutively expressing a cloned human hsp70 gene (26–28). The expression of human hsp70 confers heat resistance to the rat cells, as evidenced by increased survival and enhanced recovery of transcriptional and translational activity after heat shock. These cell lines provide a way of testing whether

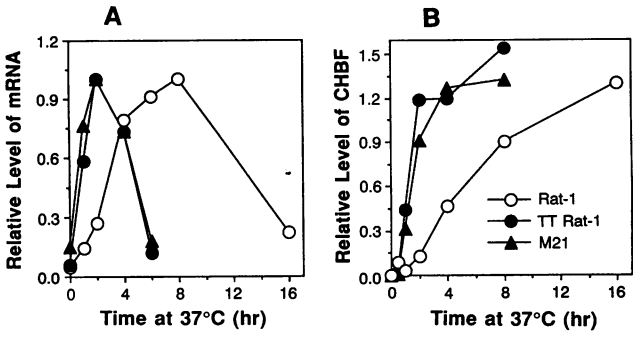

FIG. 4. Relative levels of CHBF and hsp70 mRNA during recovery at 37°C after a 45°C, 15-min heat shock treatment: Relative levels of hsp70 mRNA (*A*) and relative levels of CHBF (*B*) in heat-shocked cells during recovery at 37°C (see Figs. 1–3 and 5 for details). Autoradiograms from Figs. 1*B*, 3*B*, and 5*B* (for CHBF) and Figs. 2*B*, 3*C*, and 5*C* (for hsp70 mRNA) were quantified using AMBIS. Relative levels of CHBF are expressed as a ratio by dividing the optical intensity of CHBF at various times after heat shock to that of CHBF in the control cells before heat shock treatment. The relative levels of hsp70 mRNA are calculated similarly with the maximal level of hsp70 mRNA normalized to 1.

the cellular level of human hsp70 regulates the HSF–HSE binding and heat-induced hsp70 mRNA transcription.

When rat M21 cells overexpressing human hsp70 were heat shocked at 45°C, HSF was induced rapidly but at a level much reduced (Fig. 5*A*) from that of similarly treated control Rat-1 cells (Fig. 1*A*). In Rat-1 and M21 cells, the heat induction of HSF is associated with a concomitant decline of CHBF (Figs. 1*A*, 3*A*, and 5*A*). Similar data were obtained when M21 cells were heat shocked at 46°C or 47°C (results not shown).

The recovery kinetics of CHBF at 37°C in M21 cells showed a steeper time dependence than that of Rat-1 cells. Similar to that of TT Rat-1 cells, the level of CHBF in M21 cells returned to the pre-heat-shock level within 2 hr following the down-shift in temperature (Figs. 5*B* and 4*B*).

In spite of the much lower level of HSF thermally induced in M21 cells overproducing human hsp70, the thermal induction of the endogenous rat hsp70 mRNA appears to be unaffected. As shown in Fig. 5*C*, the level of rat hsp70 mRNA was much more elevated in response to heat shock, reaching a maximum in about 2 hr and decreasing to about 10% of its maximum by 6 hr. Such a time course is akin to that for hsp70 mRNA in TT Rat-1 cells: both show a faster rise and fall of the cellular level of hsp70 mRNA following heat shock relative to that in control nontolerant Rat-1 cells following a similar heat shock treatment (Fig. 4*A*). Again, there appears to be an inverse correlation between CHBF and hsp70 mRNA; a more rapid recovery of CHBF level is associated with a faster accumulation followed by a faster decrease of endogenous rat hsp70 mRNA in M21 cells after a 45°C, 15-min heat shock (compare Fig. 4 *A* and *B*).

Does Induction of HSF Necessarily Activate the Expression of Heat Shock Genes? The above results suggest that a high level of HSF is not necessary for the induction of the endogenous rat hsp70 gene transcription. We show below that a high level of HSF is insufficient for the expression of hsp70.

It is known that arsenite can induce thermotolerance (36). However, arsenite-treated rat cells only express an elevated level of the constitutive form of rat hsc71 but not of the heat-inducible hsp70 proteins. Arsenite-treated Rat-1 cells were therefore examined for the presence of HSF. As shown in Fig. 6*A*, there were distinct differences in the HSE-binding

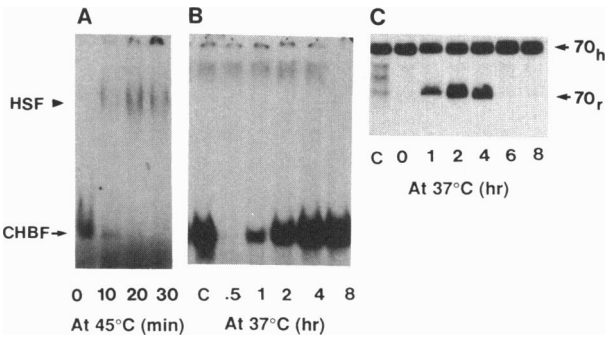

FIG. 5. Analysis of HSF and CHBF levels and hsp70 transcription in heat-shocked M21 cells. (*A*) Monolayers of exponentially growing M21 cells were exposed to 45°C for 0, 10, 20, and 30 min. Whole cell extracts were prepared, and the constitutive CHBF and the heat-induced HSF were analyzed by gel mobility-shift assay as described in the legend to Fig. 1. (*B*) Levels of CHBF and HSF during recovery at 37°C after a 45°C, 15-min heat treatment. Equal amounts of total cellular proteins were loaded per lane. (*C*) hsp70 mRNA levels in heat-shocked M21 cells during recovery at 37°C. M21 cells were heated at 45°C for 15 min and returned to 37°C for 0, 1, 2, 4, 6, and 8 hr. hsp70 mRNA levels were determined as in Fig. 2. The human hsp70 mRNA (70_h) and rat hsp70 mRNA (70_r) are indicated. M21 cells stably and constitutively expressed intact human hsp70 and are derived from a clone isolated from Rat-1 cells infected with MVH retroviruses containing a cloned human hsp70 gene (26).

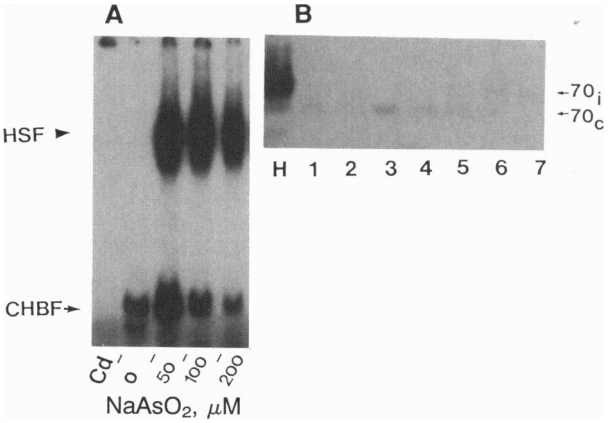

FIG. 6. Effect of sodium arsenite on HSE and CHBF levels and hsp70 transcription. (*A*) Rat-1 cells were exposed to sodium arsenite (50, 100, and 200 μM) for 1 hr at 37°C. CHBF and HSF were assayed as described in the legend to Fig. 1; lane Cd, competition assay was performed by co-incubating cell extracts from sodium arsenite-treated cells with a 50-molar excess of nonlabeled HSE oligonucleotide as described. (*B*) Northern hybridization analysis of hsp70 mRNA in Rat-1 cells during and after exposure to graded doses of sodium arsenite. Lane H, Rat-1 cells heat shocked at 45°C for 30 min and incubated at 37°C for 4 hr; lane 1, control Rat-1 cells with no treatment; lanes 2–5, Rat-1 cells exposed to 10, 50, 100, and 200 μM sodium arsenite at 37°C for 1 hr; lanes 6 and 7, cells exposed to 100 μM sodium arsenite at 37°C for 1 hr and then incubated at 37°C in drug-free medium for 1 hr and 6 hr, respectively. Cell extract preparation and hsp70 mRNA levels were analyzed as described in the legend to Fig. 2.

activity between the arsenite-treated and heat-shocked Rat-1 cells. Exposure of Rat-1 cells to arsenite greatly increased the level of binding of HSF to HSE. In contrast to heat-shocked Rat-1 cells, arsenite exposure also increased the CHBF level. Even though a high level of HSF is present in arsenite-treated cells, at the transcriptional level there is little induction of rat hsp70 mRNA (Fig. 6*B*). That high levels of HSF and CHBF were associated with low levels of hsp70 mRNA suggested that CHBF may act as a negative factor or repressor of hsp70 mRNA synthesis.

DISCUSSION

The major findings of the present study are as follows. First, the induction of a high level of HSF capable of binding to HSE is insufficient to induce hsp70 mRNA synthesis; arsenite-treated cells exhibited minimal hsp70 mRNA synthesis in spite of the high level of induced HSF. Recently, sodium salicylate, an antiinflammatory agent, was also shown to induce a high level of HSE-bound HSF in cultured human cells, yet it did not elicit the transcription of heat shock genes (37); binding of HSF to HSE was also found to be insufficient for transcriptional activation in murine erythroleukemic cells (38).

It also appears that a high level of HSF is not necessary for the activation of hsp70 mRNA transcription; whereas the HSF level in M21 cells overexpressing human hsp70 is much lower than that in Rat-1 and TT Rat-1 cells, the induction of rat hsp70 mRNA upon heat shock is similar to the latter.

Second, it appears that CHBF may repress the heat shock response. (*i*) This study showed that kinetically the heat-induced decrease of CHBF correlated with the increase of HSF in a dose-dependent manner. (*ii*) During post-heat-shock recovery at 37°C, HSF disappeared within 30 min. On the other hand, CHBF recovered with much slower kinetics. The time required for complete recovery of CHBF, and not that of HSF disappearance, coincided with the onset of the decline of hsp70 mRNA in Rat-1, TT Rat-1, and M21 cells.

(*iii*) For the TT Rat-1 cells and the M21 cells, during 37°C recovery after heat shock, a faster reappearance of CHBF correlated well with a faster rise followed by a faster disappearance of hsp70 mRNA.

The above results suggest that CHBF may also be actively involved in the regulation of heat shock response; CHBF appears to have the characteristics of a negative factor in the regulation of hsp70 gene transcription. This hypothesis is supported by two additional findings: (*i*) arsenite induces high levels of CHBF and HSF but only a minimal amount of hsp70 mRNA and (*ii*) in heat-shocked M21 cells overexpressing human hsp70 the amount of HSF is negligible, but CHBF declines normally, and the induction of hsp70 mRNA is comparable to Rat-1 cells. Recently, we have also examined the effect of salicylate on CHBF and HSF activity in Rat-1 and Chinese hamster HA-1 cells. Similar to the results obtained from arsenite-treated cells, salicylate activated HSF–HSE binding in both cell lines but has little effect on CHBF binding activity, and does not induce hsp70 protein (data not shown).

It is not clear how CHBF may affect hsp70 transcription. We have performed additional experiments to examine the level of transcription using the nuclear run-off assay and the stability of hsp70 mRNA in the presence of actinomycin D. Our results show that (*i*) the rate of transcription after a 45°C, 15-min heat shock reaches its maximum much sooner in TT Rat-1 and M21 cells than in control Rat-1 cells (at ≈3–5 hr for Rat-1 and ≈0–1 hr for TT Rat-1 and M21 cells, respectively) and (*ii*) the degradation of hsp70 mRNA is also more rapid in TT Rat-1 and M21 cells than in control Rat-1 cells (data not shown). It is likely that the binding of CHBF to HSE affects the heat shock response at transcriptional and post-transcriptional levels.

Finally, the constitutive overexpression of the human hsp70 protein in M21 cells decreases the heat-induced HSF. It is unknown, however, whether this is due to a decrease in the steady-state HSF level or to the suppression of HSF–HSE-binding activity. It is possible that the abundant human hsp70 in M21 cells may bind to HSF, affecting directly or indirectly the latter's activity. Indeed, complexes containing HSF and hsp70 have been detected by a gel mobility-shift assay and hsp70 has been found to interact with HSF *in vitro* (25, 39).

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