Overexpression of HSF2-β Inhibits Hemin-induced Heat Shock Gene Expression and Erythroid Differentiation in K562 Cells

Sirpa Leppä‡‡, Lila Pirkkala‡‡, Helena Saareno‡, Kevin D. Sarge‡, and Lea Sistonen‡‡**

From the ‡Turku Centre for Biotechnology, University of Turku, Åbo Akademi University, P. O. Box 123, FIN-20521 Turku, Finland, ‡Department of Biology, Åbo Akademi University, Biocity, FIN-20550 Turku, Finland, and ‡Department of Biochemistry, Chandler Medical Center, University of Kentucky, Lexington, Kentucky 40536-0084

Acquisition of heat shock factor 2 (HSF2) DNA binding activity is accompanied by induced transcription of heat shock genes in hemin-treated K562 cells undergoing erythroid differentiation. Previous studies revealed that HSF2 consists of two alternatively spliced isoforms, HSF2-α and HSF2-β, whose relative abundance is developmentally regulated and varies between different tissues. To investigate whether the molar ratio of HSF2-α and HSF2-β isoforms is crucial for the activation of HSF2 and whether the HSF2 isoforms play functionally distinct roles during the hemin-mediated erythroid differentiation, we generated cell clones expressing different levels of HSF2-α and HSF2-β. We show that in parental K562 cells, the HSF2-α isoform is predominantly expressed and HSF2 can be activated upon hemin treatment. In contrast, when HSF2-β is expressed at levels exceeding those of endogenous HSF2-α, the hemin-induced DNA binding activity and transcription of heat shock genes are repressed, whereas overexpression of HSF2-α results in an enhanced hemin response. Furthermore, the hemin-induced accumulation of globin, known as a marker of erythroid differentiation, is decreased in cells overexpressing HSF2-β. We suggest that HSF2-β acts as a negative regulator of HSF2 activity during hemin-mediated erythroid differentiation of K562 cells.

Heat shock factors (HSFs)† function as transcriptional activators of the cellular stress response (1). To date, three mammalian HSF proteins, HSF1, HSF2, and HSF4, have been identified (2–5). HSFs have a common core structure consisting of the well conserved DNA binding and oligomerization domains. The DNA binding domain located in the amino-terminal part of the protein mediates binding to a highly conserved heat shock response element (HSE) found in multiple copies in the promoter of heat shock genes. The oligomerization domain adjacent to the DNA binding domain consists of the hydrophobic heptad repeats A/B (HR-A/B) (6) and is also termed the leucine zipper repeat (7). This domain is responsible for trimerization, which is essential for the high affinity binding of HSFs to the HSE. The carboxyl-terminal hydrophobic heptad repeat HR-C (leucine zipper repeat 4) is also well conserved and has been implicated in maintaining HSFs in the inactive non-DNA binding form (6, 7). External to DNA binding and oligomerization domains, however, the homology between HSF1 and HSF2 is limited (<40%).

A fundamental difference between HSF1 and HSF2 is that they respond to distinct signals. HSF1 is the functional homologue of the general HSF that is activated by diverse forms of stress, such as elevated temperatures, heavy metals, and amino acid analogs (8–12). Upon activation, HSF1 is rapidly converted from a monomer to a trimer, hyperphosphorylated, and translocated into the nucleus (6, 11–13). Unlike HSF1, HSF2 is not activated by acute stress but is constitutively active in mouse embryonal carcinoma cells, at the blastocyst stage during mouse embryogenesis, and during spermatogenesis (14–16). These findings suggest that HSF2 functions as a regulator of heat shock gene expression during development and differentiation. The functional significance of HSF2 DNA binding activity in these biological processes is, however, unclear. The inducible binding of HSF2 to the hsp70 promoter, which closely correlates with the transcriptional induction of the hsp70 gene during the hemin-mediated erythroid differentiation of K562 cells, provides a well studied example of HSF2 acting as a signal-responsive transcription factor (17, 18). Unlike HSF1, the non-DNA binding form of HSF2 is a dimer, but similar to HSF1, HSF2 trimerizes upon activation. Other significant differences between HSF1 and HSF2 are that HSF2 is activated by slower kinetics, is not constitutively or inducibly phosphorylated, recognizes HSEs slightly differently, and is a less potent activator of heat shock gene expression than HSF1 (12, 17, 19).

In most cell types, HSF2 is present in a latent non-DNA binding form. However, in cells in which HSF2 is constitutively active such as embryonal carcinoma cells, mouse blastocysts, and mouse testis, the expression levels of HSF2 are markedly elevated when compared with other cell types (14–16). Similarly, the acquisition of HSF2 DNA binding activity correlates with the increased levels of HSF2 in hemin-treated K562 cells (18). The existence of two distinct isoforms, HSF2-α and HSF2-β, provides another level of regulation that adds further complexity to the function of HSF2 (20, 21). The smaller isoform, HSF2-β, is generated from the nascent transcript by an additional splice, resulting in the deletion of an 18-amino acid sequence present in the larger HSF2-α isoform (21). The deletion is located adjacent to the HR-C and may cause modulation of the transcriptional activity of HSF2 since the HSF2-α isoform has been shown to be a more potent transcriptional activator than HSF2-β in NIH 3T3 cells (21). Interestingly, the ratio of HSF2-α and HSF2-β isoforms varies significantly between different mouse tissues such as brain, heart, and testis (21). In this study, we show that the hemin-mediated induction
of hsp70 transcription is negatively regulated in K562 cells overexpressing HSF2-β. Our results also suggest that the molar ratio of HSF2-α and HSF2-β is likely to be of central relevance in the regulation of HSF2 activity. Finally, we propose that HSF2 activation is essential for the hemin-mediated erythroid differentiation of K562 cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—Human K562 erythroleukemia cells were cultured in a humidified 5% CO2 atmosphere at 37°C in RPMI 1640 supplemented with 10% fetal calf serum. Cells were heat-shocked at 42°C or treated with 30 μM hemin as described earlier (22). To generate permanent cell clones overexpressing HSF2-α and HSF2-β, 10 μg of the expression plasmids β-actin-HSF2-α and β-actin-HSF2-β (12, 21) were transfected to K562 cells using electroporation. Host cells were washed twice with phosphate-buffered saline and electroporated (Gene Pulser™, Bio-Rad) in phosphate-buffered saline with 25 μM ascorbic acid and 1.35 kV. Transfected cells were allowed to recover for 2 days, and neomycin-resistant cells were selected in medium containing G418 (500 μg/ml, Life Technologies, Inc.) for 2 weeks. Drug-resistant cells were diluted and selected for single cell clones for another 2 weeks. Cell clones that stably express HSF2-α and HSF2-β were routinely maintained in medium containing G418 (500 μg/ml). Mock-transfected cells were used as a control. Before exposure to heat shock or hemin treatment, cells were plated in medium without G418.

SDS-PAGE and Western Blot Analysis—Whole cell extracts were prepared as described (10). Protein samples (10–20 μg) were separated on SDS-polyacrylamide gels (23) and transferred to a nitrocellulose filter using a semi-dry transfer apparatus (Bio-Rad). Western blotting was performed using immuno-serum against mouse HSF1 and HSF2 as described (12). The inducible form of Hsf70 was detected by monoclonal antibody 4g4 (Affinity Bioreagents, Inc.). Hsp90 was detected by monoclonal antibody SPA-835 (StressGen Biotechnologies Corp.) and Hsc70 by monoclonal antibody SPA-815 (StressGen). Horseradish peroxidase-conjugated secondary antibodies were purchased from Promega and Amersham Life Science, Inc. The blots were developed with an enhanced chemiluminescence method (Amersham). Globin content was detected by Coomassie staining. Quantitation was performed using a computerized image analysis (Microcomputer Imaging Device version M4, Imaging Research Inc.).

Gel Mobility Shift Assay—A gel mobility shift analysis of protein-DNA complexes was performed as described previously (10). Briefly, whole cell extracts were incubated with a 32P-labeled oligonucleotide representing the proximal HSE of the human hsp70 promoter. Protein-DNA complexes were resolved on a 4% nondenaturing polyacrylamide gel.

Nuclear Run-on Analysis—Transcription run-on analysis was performed with equal numbers of isolated nuclei in the presence of 100 μCi of [α-32P]dUTP as described previously (24). Radiolabeled RNA was isolated and hybridized to nitrocellulose-immobilized plasmids specific for hsp70 (25), hsp90 (26), and β-actin (27). Bluescript (Stratagene) was used as a vector control. The hybridization and washing conditions were as described (17). Quantitation was performed using phosphoimaging (Bio-Rad).

Northern Analysis—Total RNA was extracted from hemin-treated cells using a single-step method (28). 10 μg of RNA was separated on a 1% formaldehyde-agarose gel and transferred to a nylon membrane (Hybond-N, Amersham). The membranes were hybridized with [α-32P]dCTP-labeled probes specific for hsp70 (25) and β-actin (27) according to the manufacturer’s instructions.

RESULTS

Accumulation of HSF2 Is Increased in K562 Cells upon Hemin Treatment—To examine the molar ratio of the HSF2-α and HSF2-β isoforms and the effect of hemin treatment on the expression of HSF2, K562 cells were exposed to hemin for various time periods and analyzed by Western blotting (Fig. 1A). In untreated cells, the levels of HSF2-α were approximately 2-fold higher as compared with those of HSF2-β (Fig. 1C). Hemin treatment increased the abundance of both HSF2-α and HSF2-β isoforms, and the increased amounts of HSF2 isoforms were observed within 6 h, in close correlation with the appearance of HSF2 DNA binding activity (Fig. 1B).

The amounts of HSF1 as well as Hsp70 and Hsp90 were analyzed in comparison with HSF2 in the same samples (Fig. 1A). Hemin treatment of K562 cells did not affect the accumulation of HSF1. The heat shock response was shown to be typical (Fig. 1A), as characterized by the retarded mobility on SDS-PAGE due to the inducibly phosphorylated HSF1 (12, 29) and the kinetics of DNA binding activity (Fig. 1B). Accumulation of Hsp70 protein in cells exposed to either heat shock or hemin treatment correlated well with previous results showing transcriptional induction of hsp70 upon heat shock and hemin treatments (Refs. 17 and 18; see also Fig. 3), whereas the levels of Hsp90 did not markedly change upon exposure to heat shock or hemin.

Altering the Molar Ratios of HSF2 Isoforms by Overexpression of HSF2-α and HSF2-β in K562 Cells—The result showing that HSF2-α is predominantly expressed in K562 cells (Fig. 1C) raised the possibility that the relative abundance of HSF2 isoforms, and the increased amounts of HSF2 isoforms, might play functionally distinct roles in the hemin-mediated regulation of heat shock gene expression. For this purpose, we generated cell clones that stably express mouse HSF2-α and HSF2-β under the control of the human β-actin promoter (30). K562 cells were used as recipients for the expression plasmids (12, 21). More than 15 single cell clones for each transfection were isolated, and the expression of the distinct HSF2 isoforms was determined by Western blotting. Table I summarizes the characteristics of the cell clones.
that were chosen for further analysis. As the mouse HSF2 protein shows extensive homology with the human HSF2 (>95%), it is likely to function also in human cells. However, different mobilities of mouse and human HSF2 on SDS-PAGE (12) allowed us to estimate the relative expression levels of the exogenous HSF2 isoforms. Among several single cell clones transfected with β-actin-HSF2-α, the 2α-F4 and 2α-C7 cells expressed the highest levels of HSF2-α and were chosen for further studies. Quantitation revealed that the ratio of total HSF2-α to HSF2-β was increased 2–2.5-fold in 2α-F4 cells and 2α-C7 cells, respectively, as compared with the parental K562 cells and the mock-transfected cells (vector). The representative HSF2-β clones (2β-C8, 2β-D5, 2β-E7) expressed 3–5-fold higher levels of HSF2-β than the control cells.

To examine the effect of the exogenously expressed HSF2-α and HSF2-β on the hemin-mediated increase in HSF2 levels, the transfectants were treated with hemin and analyzed by Western blotting (Fig. 2). Similar to the parental K562 cells (Fig. 1), hemin treatment resulted in increased accumulation of the HSF2 isoforms in mock-transfected cells (Fig. 2, vector). In 2α-C7 cells expressing exogenous HSF2-α, both endogenous and exogenous HSF2 levels were elevated. Surprisingly, the hemin-mediated increase in the endogenous HSF2 was repressed in HSF2-β-overexpressing cells (2β-D5). This repression was detected in all HSF2-β cell clones. Analysis of HSF1 revealed that none of the mock-transfected cells or cells expressing exogenous HSF2 isoforms showed changes in accumulation or phosphorylation state of the endogenous HSF1 (data not shown).

We also analyzed the accumulation of Hsp70 and Hsc70 during hemin treatment of the transfected cell clones (Fig. 2). Interestingly, the accumulation of Hsp70 was markedly reduced in the hemin-treated cells overexpressing HSF2-β as compared with the mock-transfected and HSF2-α-transfected cells. As expected, the levels of the constitutively expressed Hsc70 were not affected. Based on these results, we conclude that overexpression of HSF2-β inhibits the increase in HSF2 amounts and consequently prevents the hemin-mediated accumulation of Hsp70.

**Overexpression of HSF2-β Represses the Hemin-induced Heat Shock Gene Transcription Due to Inhibition of HSF2 DNA Binding Activity**—Since our results presented in Fig. 2 suggest that HSF2-β functions as a repressor of the hemin-induced increase in Hsp70 accumulation, we wanted to determine at which molecular level the repression occurs. First, we analyzed whether the reduced accumulation of Hsp70 was caused by a corresponding decrease in hsp70 transcription. The production of hsp70 and hsp90 transcripts were measured by nuclear run-on assay using nuclei isolated from the parental K562 cells, the mock-transfected cells (vector), and the cells transfected with HSF2-α (2α-F4) and HSF2-β (2β-D5, 2β-C8). The results presented in Fig. 3A and quantitated in Fig. 3B show that the repression of Hsp70 indeed occurs at the transcriptional level in the hemin-treated cells overexpressing HSF2-β, as only a weak, if any, hemin-mediated induction of hsp70 transcription could be detected. Similarly, the hemin-mediated induction of hsp90 transcription was repressed due to overexpression of HSF2-β. In contrast, all other cell clones showed the characteristic transcriptional induction of heat shock genes upon hemin treatment. The transcriptional repression of hsp70 and hsp90 genes in HSF2-β-overexpressing cells was specific for hemin treatment since exposure to heat shock resulted in an equally prominent induction of heat shock gene transcription in all cells. The repression of hsp70 gene expression in HSF2-β-overexpressing cells was observed also at the steady-state mRNA level as analyzed by Northern blotting (Fig. 3C).

HSF2-α and HSF2-β have identical DNA binding and oligomerization domains. The only alteration in HSF2-β is the deletion of an 18-amino acid sequence located immediately adjacent to the carboxyl-terminal HR-C (21). Thus, HSF2-β would be expected to compete with HSF2-α for binding to HSE. To test this assumption, we next analyzed HSE binding activity in untreated and hemin-treated cells (Fig. 4). As expected, hemin treatment induced the HSE binding activity in parental K562 cells (Fig. 1B), mock-transfected cells (vector), and cells overexpressing HSF2-α (2α-C7, 2α-F4). Surprisingly, despite the presence of a DNA binding domain in the HSF2-β isoform, the formation of a protein-HSE complex was prevented in the hemin-treated cells overexpressing HSF2-β. This inhibition of DNA binding activity was detected in all HSF2-β cell clones (2β-D5, 2β-C8, 2β-E7). Quantitation revealed that overexpression of HSF2-α in 2α-C7 cells potentiated the binding to HSE.

In accordance with the transcriptional induction of heat shock

---

**TABLE I**

Characteristics of the transfected cell lines

| Cell type             | Cell clone | HSF2-α/HSF2-β<sup>a</sup> | Hemin response<sup>b</sup> | Heat shock response<sup>b</sup> |
|-----------------------|------------|---------------------------|-----------------------------|-------------------------------|
|                       |            | HSF2 binding              | hsp70 transcription         | HSF2 binding                  | hsp70 transcription         |
| Parental cells        | K562       | 2.1                       | +                           | +                             | ++                           |
| Mock-transfected cells| vector     | 2.0                       | +                           | +                             | ++                           |
| HSF2-α-transfected cells| 2α-C7    | 4.9                       | +                           | ++                            | ++                           |
| HSF2-β-transfected cells| 2β-C8     | 0.6                       | –                           | –                             | +                            |
|                       | 2β-D5      | 0.4                       | –                           | –                             | –                            |
|                       | 2β-E7      | 0.5                       | –                           | –                             | –                            |

<sup>a</sup> The ratio of HSF2-α to HSF2-β was quantitated from the Western blot using computerized image analysis.

<sup>b</sup> DNA binding activities and transcription of hsp70 were quantitated by phosphoimaging and scored as follows: −, no induction or only a weak induction; +, moderate induction; ++, strong induction.

---

**FIG. 2**. Overexpression of HSF2-β represses the hemin-mediated increase in HSF2 and Hsp70 protein levels. Mock-transfected cells (vector) and K562 cells transfected with mouse HSF2-α (2α-C7) or HSF2-β (2β-D5) isoforms were exposed to hemin (HE) for 16 and 24 h. Equal amounts of protein (15 µg) were analyzed by Western blotting as described in Fig. 1. In the panel showing HSF2 protein levels, the upper double band corresponds to the endogenous HSF2 isoforms as indicated in Fig. 1. The exogenously expressed mouse HSF2-α and HSF2-β are marked by arrows.
Overexpression of HSF2-β suppresses the hemin-mediated induction of heat shock gene expression. A, transcription rates of hsp70, hsp90, and β-actin genes were analyzed by nuclear run-on assay. Equal numbers of nuclei from the control (C), heat shocked (HS) and hemin-treated (HE) K562 cells, mock-transfectants, and cells overexpressing HSF2 isoforms (see Table I for details of cell clones) were used to isolate labeled transcripts that were hybridized to the immobilized probes. Bluescript was used as a vector control. B, quantitative analysis of hsp70 and hsp90 transcription upon 16- and 24-h exposure to hemin as compared with basal transcription in untreated cells (value 1). β-Actin was used as an internal control. C, steady-state levels of hsp70 and β-actin mRNAs in untreated (C) and hemin-treated (HE) K562 cells. Transfectants were analyzed by Northern blotting. The mRNA sizes are indicated on the right, kb, kilobases.

Fig. 3. Overexpression of HSF2-β suppresses the hemin-mediated induction of heat shock gene expression. A, transcription rates of hsp70, hsp90, and β-actin genes were analyzed by nuclear run-on assay. Equal numbers of nuclei from the control (C), heat shocked (HS) and hemin-treated (HE) K562 cells, mock-transfectants, and cells overexpressing HSF2 isoforms (see Table I for details of cell clones) were used to isolate labeled transcripts that were hybridized to the immobilized probes. Bluescript was used as a vector control. B, quantitative analysis of hsp70 and hsp90 transcription upon 16- and 24-h exposure to hemin as compared with basal transcription in untreated cells (value 1). β-Actin was used as an internal control. C, steady-state levels of hsp70 and β-actin mRNAs in untreated (C) and hemin-treated (HE) K562 cells. Transfectants were analyzed by Northern blotting. The mRNA sizes are indicated on the right, kb, kilobases.
Figure 4. Inhibition of hemin-mediated DNA binding activity in cells overexpressing HSF2-β. Whole cell extracts from untreated (C) and hemin-treated (HE) transfectants (see Table 1 for details of cell clones) were analyzed by gel mobility shift assay using an HSE-oligonucleotide probe. HSF, specific inducible HSP-HSF complex; CHBA, constitutive HSE binding activity; NS, nonspecific protein-DNA interaction.

DISCUSSION

Signal-dependent regulation of transcription factors plays a critical role in the processes underlying cellular differentiation and development. HSF2 has been shown to be active during mouse embryogenesis at blastocyst stage, during mouse spermatogenesis, during hemin-induced erythroid differentiation of K562 cells, and in mouse embryonal carcinoma cells (14–17). Characteristic of the cells involved in these processes is abundant expression of HSF2 as compared with other cell types. Furthermore, HSF2-α is the predominantly expressed isoform in spermatogenic cell types in testis (21). Based on these results, it has been suggested that increased expression of HSF2 as well as the relative abundance of the HSF2 isoforms might contribute to HSF2 activation. Our results provide evidence that besides increased expression of HSF2, a molar excess of HSF2-α is required for HSF2 activation in K562 cells. Our results further suggest that HSF2-α and HSF2-β have distinct functions during hemin-mediated erythroid differentiation, as HSF2-α acts as a potential activator and HSF2-β as a suppressor of the hemin-induced transcription of heat shock genes in K562 cells.

Using cell lines that express altered molar ratios of HSF2-α and HSF2-β, we examined the regulation and the functional significance of the alternatively spliced HSF2 isoforms during the hemin-mediated erythroid differentiation. We observed that excess of HSF2-β prevents the hemin-mediated HSE binding activity and subsequent induction of heat shock gene transcription. Furthermore, overexpression of HSF2-β specifically inhibits the hemin-mediated increase in the levels of both endogenous and exogenous HSF2. These results raise the interesting possibility that HSF2-β negatively controls the amount of HSF2 protein accumulated in a cell. This type of regulation could be a relevant mechanism to attenuate the hemin-mediated induction of heat shock gene expression. Our results further suggest that HSF2-α could counteract the inhibitory effect of HSF2-β on HSF2 DNA binding and transcriptional activity only when the molar ratio of HSF2-α to HSF2-β is favorable, i.e., HSF2-α is predominantly expressed. However, overexpression of HSF2-α alone is not sufficient to activate HSF2 in K562 cells, but another factor, such as hemin, is required for activation.

Yet, it is not clear whether hemin mediates the action on HSF2 directly by binding to the molecule or by triggering some post-translational modifications thereby resulting in conformational change in HSF2. Interestingly, as hemin-mediated activation of HSF2 is accompanied by an increase in the levels of both HSF2 isoforms, the conformations of HSF2-α and HSF2-β might be differentially affected by hemin. An example of a hemin-regulated transcription factor is HAP1, which in yeast activates transcription of genes encoding cytochromes in response to oxygen and hemin (33, 34). HSF2, however, does not contain a sequence similar to the HAP1 repeat, termed hemin regulatory motif, that binds hemin directly (35). Furthermore, the kinetics of HSF2 activation upon hemin treatment is relatively slow, varying between 6 and 16 h of hemin exposure (Ref. 17 and this study). Based on these results and the fact that hemin is known to be an important regulator of protein synthesis (36), it is tempting to speculate that the hemin-induced increase in HSF2 levels and acquisition of HSF2 DNA binding activity could be consequences of changes in protein synthesis leading to a requirement for elevated levels of molecular chaperones.

There are multiple examples where changes in RNA splicing result in transcription factor isoforms with altered activities. For example, certain AP-2 isoforms function as inhibitors of transactivation due to a deletion in the oligomerization domain, which is necessary for DNA binding (37). Splicing can also result in deletion of sequences required for full transactivation potential as exemplified by the Fos-related proteins (38–40). Analyses of different family members of nuclear steroid hormone receptors, c-ErbA and RORα, have revealed that their distinct DNA binding properties are dictated by the specific amino-terminal domains (41, 42). In the case of HSF2-β, alternative splicing eliminates 18 amino acids adjacent to the

Suppression of HSF2 Activity by the HSF2-β Isoform

Figure 5. Accumulation of globin in differentiating K562 cells is decreased by overexpression of HSF2-β. Soluble proteins (15 µg) from untreated (C) and hemin-treated (HE, 72 h) K562 cells and cells overexpressing HSF2-α and HSF2-β were separated on a 15% SDS-PAGE and stained with Coomassie Blue. Quantitation of globin (filled arrow) was performed by computerized image analysis. Globin signals were corrected for an internal control (open arrow), and the induced accumulation of globin in transfectants was compared with that in K562 cells (100%). The relative values of globin accumulation are listed below the gel image.
HR-C, and HR-C has been implicated in maintaining HSFs in the inactive, non-DNA binding form (6, 7). According to Goodson et al. (21), the deletion in HSF2-β resulted in poor trans-activation capacity in transiently transfected NIH 3T3 cells without affecting the DNA binding activity. However, our results showing that overexpression of HSF2-β in K562 cells interferes with HSF2 DNA binding lead us to conclude that the hemin-induced HSF2 DNA binding activity is controlled by this specific sequence adjacent to the HR-C located in the carboxy-terminal region of the protein. We propose that the different results obtained earlier and in this study are likely to be due to experimental differences reflecting specific features of the different cell types and distinct expression levels of HSF2-β. Interestingly, HSF4, a novel human HSF lacking the properties of a transcriptional activator, has recently been identified (5). In HeLa cells, which do not express endogenous HSF4, overexpression of HSF4 was shown to lead to Constitutive HSE binding activity and repression of target genes, which could be related to the fact that HSF4 lacks the HR-C. Thus, despite the similar effects of overexpression of HSF4 and HSF2-β on gene expression, these factors seem to be functionally distinct, and the mechanism(s) by which overexpression of HSF2-β prevents HSF2 DNA binding activity remains to be established.

The biologically relevant finding that HSF2 activity is essential for the regulation of hemin-induced erythroid differentiation of K562 cells is based on our results showing that globin accumulation upon hemin treatment is differentially affected by overexpression of HSF2 isoforms. The enhancing and inhibiting effects of HSF2-α and HSF2-β, respectively, on the signaling cascades leading to activation of HSF2 and subsequently to accumulation of Hsp70 and globin suggest that HSF2 plays a critical role during progression of cells to erythroid differentiation. We also demonstrate that the HSF1-mediated heat shock response is not affected by overexpression of HSF2-β and thus provides support for earlier studies implying that hemin-induced accumulation of Hsp70 in the differentiated erythroblasts of both K562 and bone marrow reflects a differentiation event rather than a general response to stress conditions (43). Future studies will be directed toward elucidating whether activation of HSF2 functions as a trigger in the initiation phase of erythroid differentiation and determining by which mechanisms HSF2 activity is regulated in cells undergoing differentiation.

Acknowledgments—We thank Minna Löneström for expert technical assistance and Fiorenzo Peverali for valuable advice on photography. Dirk Bohmann, John E. Eriksson, and Päivi J. Koskinen are acknowledged for discussions and critical review of the manuscript.

REFERENCES

1. Wu, C. (1995) Annu. Rev. Cell Dev. Biol. 11, 441–469
2. Rabindran, S. K., Giorgi, G., Clos, J., and Wu, C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6906–6910
3. Sarge, K. D., Zimarino, V., Holm, K., Wu, C., and Morimoto, R. I. (1991) Genes Dev. 5, 1902–1911
4. Schuetz, T. J., Gallo, G. J., Sheldon, L., Tempst, P., and Kingston, R. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6911–6915
5. Nakai, A., Tanabe, M., Kawaoze, Y., Inazawa, J., Morimoto, R. I., and Nagata, K. (1997) Mol. Cell. Biol. 17, 469–481
6. Rabindran, S. K., Haroun, R. I., Clos, J., Wisniewski, J., and Wu, C. (1993) Science 259, 230–234
7. Zuo, J., Baler, R., Dahl, G., and Voellmy, R. (1994) Mol. Cell. Biol. 14, 5288–5293
8. Sorger, P. K., Lewis, M. J., and Pelham, H. R. B. (1987) Nature 329, 81–84
9. Amin, J., Ananthan, J., and Voellmy, R. (1988) Mol. Cell. Biol. 8, 3761–3769
10. Mosser, D. D., Theodorakis, N. G., and Morimoto, R. I. (1988) Mol. Cell. Biol. 8, 4736–4744
11. Baler, R., Dahl, G., and Voellmy, R. (1993) Mol. Cell. Biol. 13, 2486–2496
12. Sarge, K. D., Murphy, S. P., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 3370–3383
13. Westwood, J. T., Clos, J., and Wu, C. (1991) Nature 353, 822–827
14. Meeger, V., Rainui, M., Morimoto, R. I., Morange, M., and Renard, J.-P. (1994) Dev. Biol. 166, 819–822
15. Murphy, S. P., Gorzowski, J. J., Sarge, K. D., and Phillips, B. (1994) Mol. Cell. Biol. 14, 5309–5317
16. Sarge, K. D., Park-Sarge, O.-K., Kirby, J. D., Mayo, K. E., and Morimoto, R. I. (1994) Biochem. Biophys. Res. Commun. 200, 528–531
17. Sistonen, L., Sarge, K. D., Phillips, B., Abravaya, K., and Morimoto, R. I. (1992) Mol. Cell. Biol. 12, 4104–4111
18. Sistonen, L., Sarge, K. D., and Morimoto, R. I. (1994) Mol. Cell. Biol. 14, 2087–2099
19. Kroeger, P. E., Sarge, K. D., and Morimoto, R. I. (1993) Mol. Cell. Biol. 13, 3570–3583
20. Fiorenza, M. T., Parkas, T., Molding, K., and Zamarino, V. (1995) Nucleic Acids Res. 23, 467–474
21. Goodson, M. L., Park-Sarge, O.-K., and Sarge, K. D. (1995) Mol. Cell. Biol. 15, 5298–5303
22. Theodorakis, N. G., Zand, D. J., Kotzbauer, P. T., Williams, G. T., and Morimoto, R. I. (1989) Mol. Cell. Biol. 9, 3166–3173
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Banerji, S. S., Theodorakis, N. G., and Morimoto, R. I. (1984) Mol. Cell. Biol. 4, 2437–2448
25. Wu, B., Hunt, C., and Morimoto, R. I. (1985) Mol. Cell. Biol. 5, 330–341
26. Hikage, E., Branden, S. E., Smale, G., Lloyd, D., and Weber, L. A. (1989) Mol. Cell. Biol. 9, 2615–2626
27. Gunnung, P., Ponte, P., Okayama, H., Engel, J., Blau, H., and Kedes, L. (1983) Mol. Cell. Biol. 3, 787–795
28. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–157
29. Cotto, J. J., Kline, M., and Morimoto, R. I. (1996) J. Biol. Chem. 271, 3355–3359
30. Gunnung, P., Leavitt, J., Muscat, G., Ng, S. Y., and Kedes, L. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4831–4835
31. Dean, A., Erard, F., Schneider, A. B., and Schechter, A. N. (1981) Science 212, 459–461
32. Haldeman, M. T., Finley, D., and Pickart, C. M. (1995) J. Biol. Chem. 270, 9507–9516
33. Fytlovich, S., Gervais, M., Agrimonti, C., and Guiard, B. (1993) EMBO J. 12, 1209–1212
34. Zhang, L., and Guarante, L. (1994) J. Biol. Chem. 269, 14643–14647
35. Zhang, L., and Guarante, L. (1995) EMBO J. 14, 313–320
36. Padmanabhan, G., Venkateswaran, V., and Rangarajan, P. N. (1989) Trends Biochem. Sci. 14, 492–496
37. Buettner, R., Kannan, P., Ihofh, A., Bauer, R., Yim, S. O., Glockshuber, R., Van Dyke, M. W., and Tainsky, M. A. (1993) Mol. Cell. Biol. 13, 4174–4185
38. Mumberg, D., Lucchello, F. C., Schuermann, M., and Muller, R. (1991) Genes Dev. 5, 1212–1223
39. Nakabeppu, Y., and Nathans, D. (1991) Cell 64, 751–759
40. Yan, J., Wilson, R. M., Tratner, I., and Verma, I. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5077–5081
41. Chen, H., Smit-McBride, Z., Lewis, S., Sharif, M., and Privalsky, M. L. (1993) Mol. Cell. Biol. 13, 2366–2376
42. Gigueré, V., Tii, M., Flock, G., Ong, E., Evans, R. M., and Oulakowski, G. (1994) Genes Dev. 8, 538–553
43. Singh, M. K., and Yu, J. (1984) Nature 309, 631–633