Volume 17 Number 11 1989 Nucleic Acids Research

In situ detection of a heat-shock regulatory element binding protein using a soluble short synthetic enhancer sequence

Annick Harel-Bellan*, Anna T.Brini, Douglas K.Ferris[1], Philippe Robin[2] and William L.Farrar

Laboratory of Molecular Immunoregulation, Biological Response Modifier Program, Building 567, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, [1]Biological Carcinogenesis Development Program, Program Resources, Inc., Frederick Cancer Research Facility, Frederick, MD 21701, USA and [2]Laboratoire d'Immunologie, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif, France

Received March 1, 1989; Revised and Accepted May 3, 1989

ABSTRACT

In various studies, enhancer binding proteins have been successfully absorbed out by competing sequences inserted into plasmids, resulting in the inhibition of the plasmid expression. Theoretically, such a result could be achieved using synthetic enhancer sequences not inserted into plasmids. In this study, a double stranded DNA sequence corresponding to the human heat shock regulatory element was chemically synthesized. By *in vitro* retardation assays, the synthetic sequence was shown to bind specifically a protein in extracts from the human T cell line Jurkat. When the synthetic enhancer was electroporated into Jurkat cells, not only the enhancer was shown to remain undegraded into the cells for up to 2 days, but also it was shown to bind intracellularly a protein. The binding was specific and was modulated upon heat shock. Furthermore, the binding protein was shown to be of the expected molecular weight by UV crosslinking. However, when the synthetic enhancer element was co-electroporated with an HSP 70-CAT reporter construct, the expression of the reporter plasmid was consistently enhanced in the presence of the exogenous synthetic enhancer.

INTRODUCTION

HSP 70, a member of the heat shock protein family, is an ubiquitous protein, which expression is regulated by stress as well as by growth factors (1−3), suggesting an important function for cell survival and cell proliferation. The heat shock family is highly evolutionary conserved, at the level of the genes and gene products, as well as at the level of the sequences involved in gene regulation. Sequencing and deletion studies of a cloned human HSP 70 5′ regulatory region have indicated that human HSP 70 expression was regulated by (at least) two regulatory sequences (4, 5) (Fig. 1). The Heat Shock Element (HSE) is highly homologous to sequences similarly located in the regulatory region of the Yeast or Drosophila HSP 70 gene (6−8). Furthermore, the heat shock element from one species confers heat shock inducibility to an heterologous promoter in cells from distinct species, indicating a high level of conservation in the regulatory sequences and proteins (9, 10). Finally, the heat shock element is present in the regulatory region of most of the heat induced genes, suggesting that it is responsible for coordinate induction of these genes by heat shock (11). The heat shock element confers heat shock inducibility to an HSP 70 -CAT construct (see Fig. 1), whereas the Serum Regulatory Element (SRE), which regulates HSP 70 expression in Hela cells, confers serum inducibility to the gene (4, 5).

Although it has been demonstrated that enhancers are cis-acting elements (12−14), enhancers specifically bind trans-acting proteins (15−21), which facilitate the transcription of the regulated gene by the RNA polymerase complex. The HSE binding protein, HSF, has been described alternatively as a 70 Kd protein in yeast and drosophila (8), as a 140 Kd protein, which gives rise to a 70 Kd species upon mild proteolysis in yeast (22) and

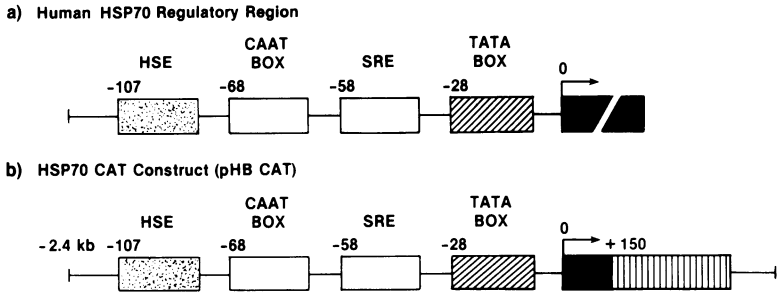


Figure 1. Map of the human HSP 70 5′ regulatory region, including the position of the Heat Shock Element (HSE), the serum regulatory element (SRE), the CAAT box and the TATA box (a) and of the HSP 70-CAT construct (b) used in this study, as derived from Wu et al. (4). Numbers refer to the position of each regulatory sequence, with respect to the transcription start site (arrow). Solid boxes: HSP 70 transcribed sequences; vertical stripped box: CAT sequence.

as a 110 Kd protein in drosophila (23). In human, HSF has been recently characterized as an 83 Kd molecule (24).

Attempts to modulate gene expression in eukaryotic cells include mutagenesis, directed mutagenesis and antisense technology (25, 26). Likely due to the slow division rate of mammalian cells, mutagenesis is a highly inefficient process. The antisense technology has been more succesful so far (27−29). A specially promising approach uses short synthetic oligonucleotides complementary to crucial regions of the messenger RNA (30−36). These short sequences readily penetrate into the cells, and therefore therapeutic applications can be envisionned. However, despite efforts to stabilize them (37, 38), single stranded oligonucleotides are highly sensitive to nuclease degradation. Furthermore, high intracellular concentrations need to be achieved in order to saturate the majority of the intracellular messages with the complementary sequence and obtain significant blockade of gene expression. Theoretically, a more efficient way of blocking the expression of a gene could be the use of double stranded enhancer sequences, which would compete with the endogenous enhancer sequences for binding to the cognate protein. This strategy would have two major advantages. First, a double stranded sequence would be far more stable intracellularly than a single stranded sequence. Second, the intracellular concentration necessary for gene inhibition could be far lower, the number of sequences to compete with in the endogenous genome being lower than the number of cytoplasmic messages. This study is an attempt to modulate the expression of an HSP-70 reporter construct, using a synthetic double stranded sequence corresponding to the Heat Shock Element.

MATERIAL AND METHODS

Cell culture and reagents

The human T cell leukemia cell line Jurkat was grown in RPMI complemented with 10 % fetal calf serum and standard concentrations of L-Glutamine and antibiotics.

The complementary oligonucleotide sequences corresponding to HSE (5′CTGGAATATTCCCG-3′ and complementary strand) were synthesized on a Biosearch multiple column DNA synthetizer model 8700, purified on acrylamide-urea gels followed by electroelution and sequential ethanol precipitation or alternatively purchased from Midland Certified Reagent Company, (Midland, Texas). The two strands were annealed

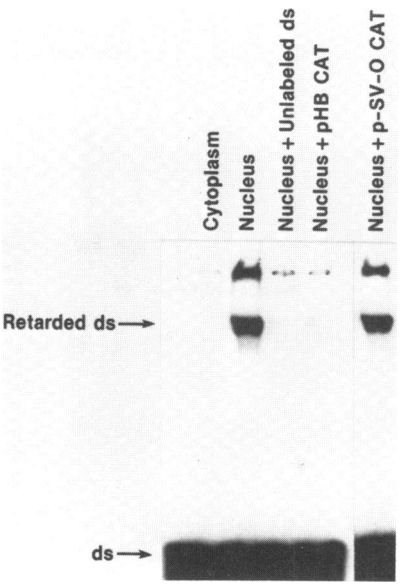


Figure 2. Autoradiograph of a gel shift assay of synthetic HSE, using protein extracts from Jurkat (JK) cells, in the presence or absence of the indicated unlabeled inhibitors.

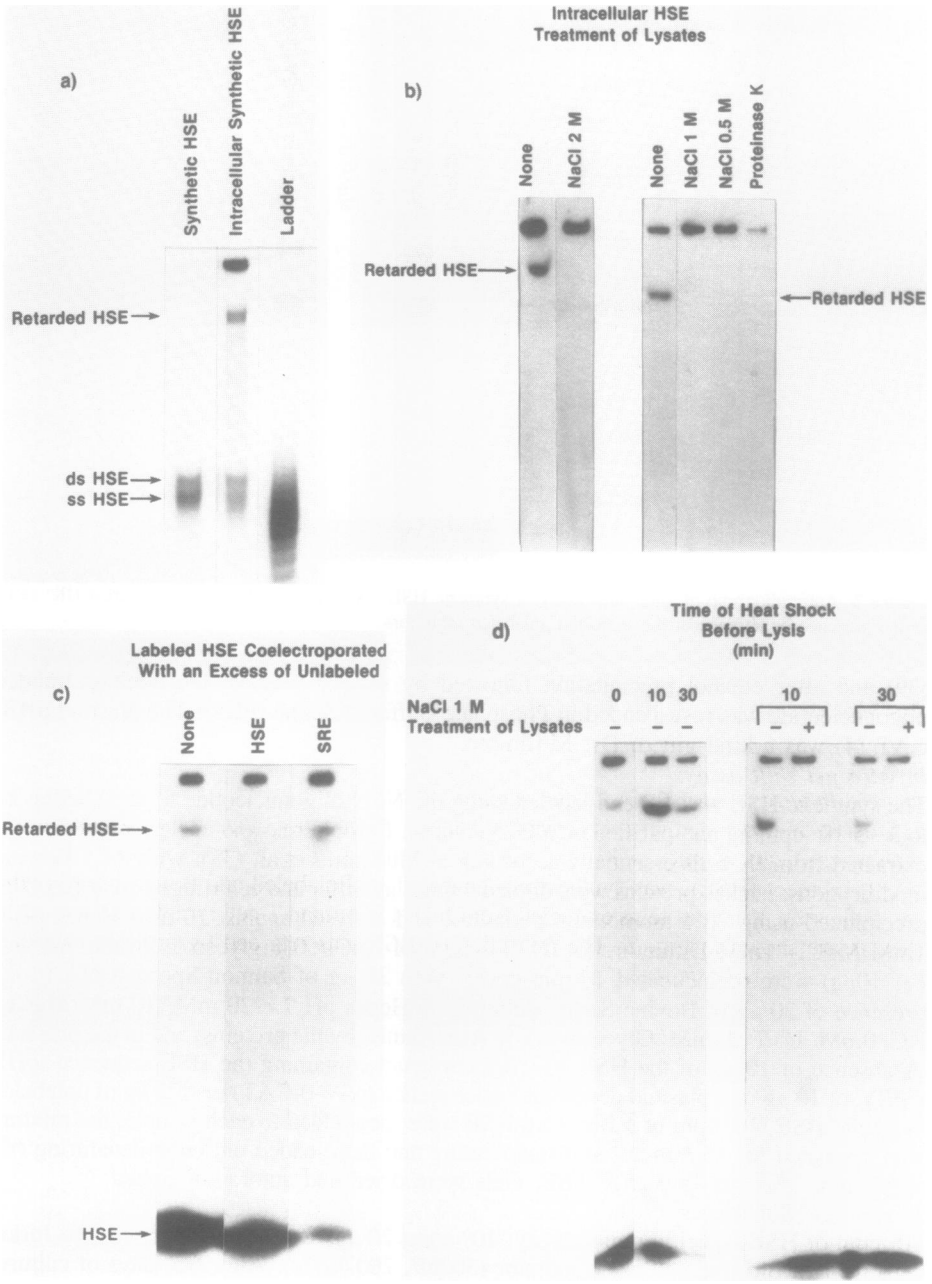
(39) and after ethanol precipitation followed by several washes, the double stranded oligonucleotide was resuspended in Phosphate Buffered Saline (PBS). The plasmid pHB-CAT (4) was a kind gift of Dr. Morimoto.

In vitro gel shift assays

The synthetic HSE was ^{32}P end-labeled using the NEN oligonucleotide 5′ endlabeling kit to 3−5 10^7 cpm/μg and purified on a G25 column. Cytoplasmic and nuclear proteins were extracted from JK cells essentially according to Miskimins et al. (38), with the following modifications: nucleic proteins were diluted 4 times in buffer and centrifuged 1h at 10,000g, precipitated using 70% ammonium persulfate and dialysed against 10 mM Hepes pH8/ 1mM $MgCl_2$/ 1mM Dithiothreitol (DTT)/ 50 mM NaCl/50% glycerol. Protein extracts (~ 10μg) were preincubated 10 min on ice with 20 μg of Salmon Sperm DNA in the presence of 20 μg of Bovine Serum Albumin in Hepes pH 7.8 20 mM/ 10 mM $MgCl_2$/ KCl 0.6M/ DTT 10 mM/ Glycerol 2.5 % (contributed by the proteins) and in the presence or absence of 10 μg of the HSP 70 CAT construct containing the HSE sequence (pHB CAT), of 10 μg of a plasmid devoid of this sequence (pSV-0-CAT) or of 1 μg of unlabeled synthetic HSE. 10^5 cpm of ^{32}P-labeled HSE were then added to each sample, the mixture was incubated for 15 min at room temperature and then loaded onto non-denaturing 6% acrylamide gels run in 0.25× TBE. Gels were dried and autoradiographed.

In situ gel shift assays

10^6 cpm of HSE (specific radioactivity: 10^6 cpm/ 20 μg) were electroporated into Jurkat cells using a Bio-Rad cell electroporator (500uF, 200 Volts). After two days of culture, live cells were purified on a LSM (Organon Teknica Corporation, Durham, NC) gradient and, when indicated, heat shocked at 42°C for the indicated period of time. Cells were washed in PBS several times, resuspended in a small volume of TBE, lysed by 3 cycles


a)
Synthetic HSE
Intracellular Synthetic HSE
Ladder
Retarded HSE
ds HSE
ss HSE
b)
Intracellular HSE
Treatment of Lysates
None
NaCl 2 M
None
NaCl 1 M
NaCl 0.5 M
Proteinase K
Retarded HSE
Retarded HSE
c)
Labeled HSE Coelectroporated
With an Excess of Unlabeled
None
HSE
SRE
Retarded HSE
HSE
d)
Time of Heat Shock
Before Lysis
(min)
NaCl 1 M
Treatment of Lysates
0
10
30
10
30
– – – – + – +

of freeze/thawing, and centrifuged 5 min in a microfuge to eliminate particulate debris. When indicated, NaCl was added to the final concentrations indicated or, alternatively, lysates were treated with proteinase K (50μg/ml, 15 min at 37°C; similar treatment of ^{32}P-labeled enhancer alone showed that the proteinase K used in this study was totally devoid of nuclease activity). Lysates were then loaded onto non denaturing acrylamide gels as indicated. Gels were dried and autoradiographed.

UV cross-linking experiments

For *in vitro* cross-linking experiments, 20 μg of total proteins extracted from heat shocked cells were incubated at 4°C with 2 ng (10^5 cpm) of ^{32}P-labeled HSE, in the presence of an excess of poly dI:dC or of unlabeled HSE, as indicated. For *in situ* crosslinking experiments, cells were transfected with ^{32}P-labeled HSE (10^6 cpm/10^6 cells) in the presence of an excess of poly dI:dC or of unlabeled HSE, as indicated. At day 1 after transfection, cells were heat shocked, washed, lysed by freeze-thawing and debris were eliminated by centrifugation at 4°C. Cell lysates (*in situ* crosslinking) or incubation mixtures (*in vitro* crosslinking) were UV irradiated on ice (41). Samples were then boiled in standard loading buffer and loaded onto a 7.5% acrylamide SDS PAGE standard gel. Autoradiography of the dried gel was performed using standard procedures.

Chlorampenicol Transacetylase (CAT) assays

Jurkat cells were electroporated with 10 μg of pHB CAT. After 24 h of culture in RPMI supplemented with 5% Fetal Calf Serum, live cells were purified and divided into two aliquots, one of which being heat shocked (30 min, 42°C). After 3 hours of culture at 37°C, cells were washed and lysed by freeze-thawing. Equal amounts of cellular extracts were assayed for CAT activity using standard procedures (40). Radioactivity in spots corresponding to acetylated and non acetylated forms was counted and % of conversion were calculated.

RESULTS

In vitro binding of a protein to the synthetic HSE

In order to demonstrate that a short synthetic enhancer sequence binds to the cognate protein with a significant affinity, a 14 bp double stranded sequence, corresponding to the Heat Shock Element (HSE), was synthesized, annealed, radiolabeled and tested in an *in vitro* gel shift assay, using proteins extracted from Jurkat (a human T cell lymphoma line) cells. Results are shown in Fig. 2. The migration of HSE was retarded by proteins extracted from nuclei and not by equivalent amounts of cytoplasmic proteins, indicating that the

Figure 3. Autoradiographs of *in situ* retardation assays of electroporated synthetic HSE in JK cells, showing the intracellular formation of an HSE-protein complex.
(a): non-denaturing 20% gel electrophoresis of radio-labeled synthetic HSE (10^3 cpm) and of JK cell lysates electroporated with radio-labeled synthetic HSE; ladder: 4bp-22bp oligo dT ladder (BRL, MD). Note that in this particular experiment the synthetic HSE had not been completely annealed. (b) non-denaturing 6% gel electrophoresis of JK cell lysates electroporated with radio-labeled synthetic HSE. Lysates have been treated with 1M Na Cl or with 50 μg/ml of proteinase K when indicated. (c) non-denaturing 6% gel electrophoresis of JK cell lysates electroporated with radio-labeled synthetic HSE in the presence or absence, as indicated, of a 5 time excess of unlabeled HSE or of an unrelated sequence (SRE, see Fig. 1). (d) non-denaturing 6% gel electrophoresis of lysates of JK cell electroporated with radio-labeled synthetic HSE and heat shocked for indicated periods of time previous to lysis. Lysates have been treated with 1M Na Cl (+) or not (−) as indicated.

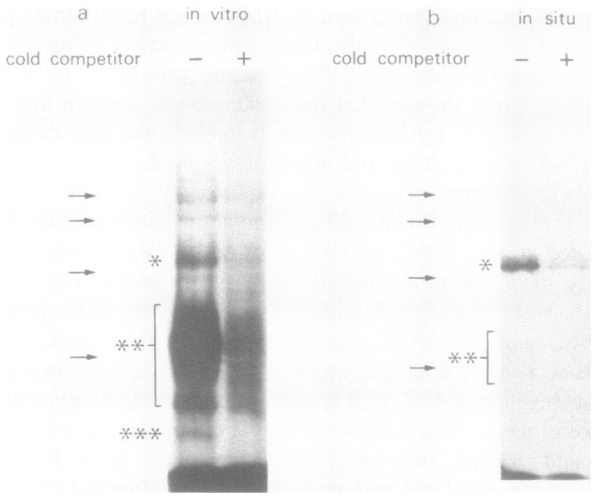


Figure 4. Characterization of the HSE binding protein by UV crosslinking. Incubation mixture of radiolabeled HSE and proteins extracted from Jurkat cells (a) or lysates from cells electroporated with radiolabeled HSE (b) were UV irradiated and submitted to SDS-PAGE analysis. Arrows indicate the position of the molecular weight markers (116, 92, 66 and 45 Kd). Stars indicate the position of the HSE-specific bands.

binding protein was restricted to the nuclear compartment. The binding was specific, since it was inhibited by an excess of unlabeled HSE or by an excess of the plasmid, pHB CAT, containing the regulatory sequence of human HSP 70 including the HSE (see Fig. 1) and not by an unrelated plasmid in which only the sequence CAT was inserted (pSV-0CAT). These results indicate that the 14 bp sequence binds the cognate protein with an affinity sufficient for detection in gel shift assays.

In situ gel shift assays

In order to assess the intracellular stability of the synthetic HSE, the ^{32}P labeled double stranded synthetic HSE was electroporated into JK cells and, following lysis, the cell extracts were analysed by electrophoresis on a native acrylamide gel (Fig 3a). Results indicated that a significant amount of the synthetic HSE remained undegraded in the cells, up to 2 days after electroporation. Furthermore, a portion of the ^{32}P-labeled HSE was 'retarded', essentially as it is in an *in vitro* gel shift assay, suggesting an interaction with a protein(s) *in situ*. This observation was further investigated. Lysates from cells transfected with ^{32}P-labeled HSE were treated with a high concentration of NaCl, in order to disrupt DNA-protein complexes, or with proteinase K, in order to degrade proteins. Both treatments were able to abolish the retarded migration of the synthetic HSE (Fig. 3b and 3d). This results strongly suggest that the 'retarded' migration of radioactivity was not due to the incorporation of free ^{32}P, provided by the intracellular degradation of labeled HSE, into other cellular components, or to the intracellular ligation of the double stranded sequence, but rather to the binding of a protein to the synthetic HSE. Furthermore, the intracellular binding of the protein to the synthetic HSE was shown to be sequence specific, since it was inhibited when an excess of unlabeled synthetic HSE was coelectroporated, whereas it was not inhibited by an excess of an unrelated double stranded sequence (Fig 3 c). Since HSE is the 5′ regulatory sequence responsible for the heat-shock induction of HSP 70,

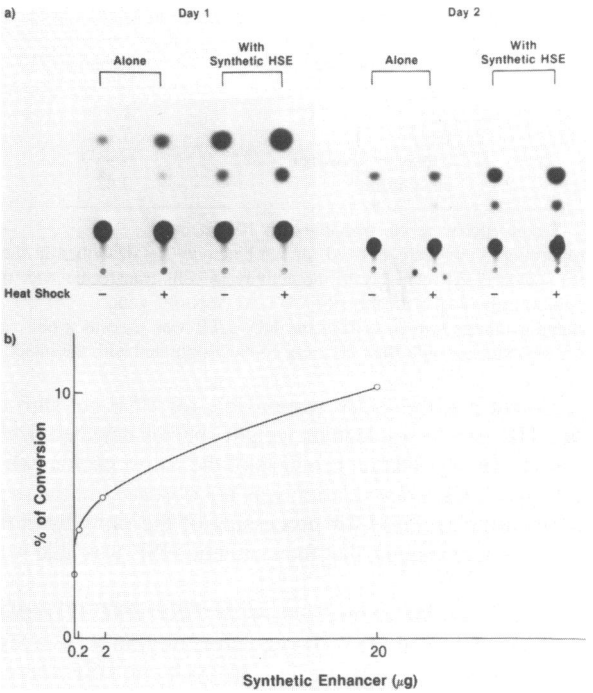


Figure 5. Effect of an intracellular excess of HSE on the expression of an HSP 70−CAT construct (pHB CAT) in JK cells. (a) autoradiograph of a CAT assay, representative of 5 experiments. (b) Dose curve (% of conversion), showing the effect of the synthetic HSE on the basal level of expression of pHB CAT (representative of 2 experiments).

we also studied the effect of heat shock on the intracellular binding of protein(s) on the synthetic enhancer (Fig. 3 d). Heat-shocking the electroporated JK cells, before lysis and loading onto the gel, consistently resulted in an increase in the proportion of the 'retarded' species (3−5 fold).

Characterization of the 'in situ' binding protein

In order to characterize the binding protein(s), lysates from cells electroporated with radiolabeled HSE were submitted to UV irradiation and loaded onto standard SDS-PAGE acrylamide gels, together with UV irradiated *in vitro* incubation mixtures of extracted proteins and radiolabeled oligonucleotide, used as a control (Fig. 4). *In vitro*, three major sets of binding species were detected, corresponding to sequence specific binding as shown by inhibition with unlabeled HSE sequence (Fig. 4 a). One of these bands (labeled * in the figure) displayed an apparent molecular weight of 70 Kd, which is the size described for HSE binding protein from yeast and drosophila in some reports (8). Furthermore, this size is not very different from the molecular weight of the human HSF, 83 Kd (24). The slight discrepancy between the two studies could be due to the gel system or the cell type used, or alternatively, to a mild proteolysis in our case. When cellular lysates from cells electroporated with radiolabeled HSE were similarly processed, the major species detected was the 70 Kd protein (Fig. 4b). Our interpretation of these results is that the protein which migrates in our gel system with an apparent molecular weight of 70 Kd is the HSE binding

Table 1: Enhancement of CAT expression is not observed using an irrelevant sequence or the entire promoter region.

Reporter plasmid[a]	Co-electroporated with	Relative CAT activity [b]
HSP-70 CAT	–	1
	HSE[c]	5.1
	irrelevant sequence[d]	0.95
	cut plasmid[e]	0.42

[a] 10 μg of reporter CAT construct were electroporated in 10^7 JK cells.
[b] At day 1 after electroporation, cells were assayed for CAT activity, as described in the legend to Figure 4. % of conversion were standardized relatively to the activity in cells transfected with the plasmid alone.
[c] 20 μg of HSE were coelectroporated with the HSP-70 CAT reporter gene.
[d] A 12 bp double stranded synthetic sequence from the HIV LTR was used as a non relevant sequence.
[e] 20 μg of the pHB-CAT plasmid, cut with B*am* H1, was co-electroporated with the intact pHB-CAT construct.

protein in human, and that it is the factor responsible for HSE gel shift observed *in situ*, after electroporation. The smaller molecular weight species detected *in vitro* (** and *** in the figure) are likely to be degradation products of the native protein which have retained the DNA binding activity. Such a degradation was not observed in the *in situ* assay, likely due to the much shorter time required for processing of the samples in this assay. Taken together, these results strongly suggest that the synthetic HSE was able to bind the cognate protein *in situ*.

Effect of the synthetic HSE on the expression of an HSP 70-CAT reporter construct

The effect of the synthetic HSE on HSP-70 regulatory region was investigated using an HSP 70-CAT reporter construct (See Figure 1). The synthetic HSE was co-electroporated together with the HSP 70-CAT reporter construct (Fig. 5). The HSP 70-CAT construct had a significant basal level of expression, which was enhanced ~ 2–3 times upon heat shock. Since in '*in situ* gel shift assays', we have observed a similar fold increase in protein binding to the synthetic HSE, it appears likely that enhanced binding of protein to the corresponding sequence in the plasmid results in the increased HSP-70 CAT expression. Paradoxically, the co-electroporation of the synthetic HSE consistently enhanced the basal, as well as the heat shock induced, level of expression of the plasmid (5.4 ± 1.7 fold increase, mean of 5 experiments), in a dose dependent manner. Such an effect was not observed using an irrelevant double stranded sequence (Table 1). However, when the reporter plasmid was cut using a restriction enzyme between the promoter and the CAT gene, and used as a competitor for the native plasmid, a significant inihibition was observed (Table 1).

DISCUSSION

The synthetic HSE was shown to bind specifically a nuclear protein from Jurkat cell extracts *in vitro*. When electroporated into Jurkat cells, a significant amount of the synthetic HSE remained undegraded intracellularly. Furthermore, a portion of the intracellular HSE showed a retarded electrophoretic migration. This retarded migration could be attributed to protein binding since it was inhibited by high salt or protein hydrolysis. Furthermore, the retarded migration was increased upon heat shock. The increase was observed after 10 min of heat shock and was transient since the proportion of 'retarded' HSE returned to the basal level after 30 min of heat-shock. This confirmed the previously observed background level of binding to the HSE even in the absence of Heat Shock (22). This background level of binding is enhanced by Heat shock, due to some unknown intracellular signal resulting

in increased affinity of the binding protein for the HSE sequence. However, the return to basal level after 30 min is not consistent with previous studies (43, 44). This discrepancy could be due to increased fragility of the intracellular synthetic oligonucleotide upon heat shock.

The sequence specificity of the binding is suggested by several considerations: 1) The intracellular concentration of the synthetic enhancer, calculated from the total radioactivity associated with the intracellular HSE, was $10^{-6}-10^{-7}$M. This concentration is likely to be too low to saturate non sequence specific DNA binding proteins, which usually display a Kd of about 10^{-4}M. 2) The binding was displayed by the unlabeled relevant sequence, and not by an irrelevant sequence. 3) The binding was specifically modulated by the inducer of the gene, in that case the heat shock. When the amount of ^{32}P-labeled HSE bound to protein was estimated by measuring the radioactivity associated solely with the 'retarded' band, the number of complexed copies of synthetic HSE was estimated to be $\sim 10^4-10^5$ copies/cell. Given a one to one molecular stochiometry, this would suggest that JK cells contain at least 10^4 HSE-binding protein molecules per cell, consistent with previous estimations (9, 45). Therefore, our data suggest that '*in situ* gel shift assays' allowed the detection of a sequence specific DNA binding protein. However, it has to be noted that a high variability was observed in the amount of free oligonucleotide from one sample to another. The free oligonucleotide is likely to be more sensitive to nuclease degradation than the protein bound oligonucleotide, which may result in the observed variability. Finally, the *in situ* binding protein was shown to be of the expected molecular weight by UV crosslinking, suggesting that the intracellular synthetic HSE is able to bind *in situ* the cognate protein.

However, instead of inhibiting the expression of an homologous CAT reporter plasmid by absorbing out the cognate protein, the synthetic HSE consistently enhanced the basal level as well as the heat shocked induced expression of the plasmid. This activation of transcription is in opposition with the inhibition observed when exogenous enhancer sequences inserted into plasmids are introduced into cells together with the relevant reporter plasmid (16, 21). In particular, HSE containing plasmids have been shown to inhibit the expression of cotransfected HSP-reporter constructs (46) and, when integrated into the host genome, the expression of endogeneous heat shock genes (47). Indeed, in our study, when an excess of the HSP-70 CAT construct, in which the CAT sequence had been separated from the promoter using a restriction enzyme (cut plasmid), was coelectroporated with the the intact reporter plasmid, an inhibition was also observed. This suggests that the sequence is able to absorb out the cognate protein solely when it is integrated into a longer DNA fragment and not when it is in the soluble form. Heat shock induced transcriptional activation of HSP 70 gene seems to involve several major steps. First, a translocation of the HSE binding factor from the cytoplasm to the nucleus is suggested by various studies (48, 49). Second, heat increases the affinity of the protein for the enhancer sequence, increase which occurs *in vitro*, when cytoplasmic proteins from non heat shocked cells are heated to the appropriate temperature (48). In a third step, the protein is phosphorylated, phosphorylation which seems to involve nuclear proteins since it cannot be reconstituted in heated cytoplasmic extracts (48, 49). Finally, the HSE-bound protein interacts with surrounding proteins, bound to distinct sequences, and increases the rate of transcription initiations (50). Plasmids as well as synthetic enhancers used as competitors, can interact with any of these activation steps and several hypothesis may account for the discrepancy between the effects of the two types of polynucleotides. First it is conceivable

that the excess of short synthetic cognate sequence is so high, as compared to the excess obtained with plasmids used as inhibitors, that it induces the synthesis of an excess of the binding protein. Second, plasmids and synthetic enhancer could have distinct effects on the translocation step. For example, the translocation of the protein could be facilitated when it is bound to the synthetic enhancer, and not when it is bound to the same sequence inserted into a plasmid. Third, the binding of the synthetic oligonucleotide could facilitate the phosphorylation of the protein, which would not be observed when the same sequence is inserted into a plasmid. Finally, the difference could be related to the last step of the activation process, which involves the interaction between the HSE bound protein and the surrounding proteins. Several lines of evidence suggest that protein-protein interactions, which can occur at a large distance, involve the looping of DNA (51–53). In that regard, synthetic oligonucleotides might behave as 'infinite loops', meaning that the oligonucleotide would not prevent the bound protein to interact with surrounding proteins on the gene regulatory region, and in fact, by some allosteric mechanism, could facilitate this interaction. Alternative explanations can also be envisioned. For example, it is conceivable that two proteins bind to the HSE. One would have an inhibiting effect on the transcription and would not require the presence of the surrounding protein binding sites, whereas the other one would have an enhancing effect and would require an interaction with the surrounding proteins in order to display a sufficient affinity. In that case, the soluble enhancer would bind solely to the inhibiting factor therefore facilitating the expression of the plasmid. Similar studies on the effect of other 'soluble' synthetic enhancer elements on gene transcription, *in vitro* as well as *in situ*, should allow the clarification of this issue.

ACKNOWLEDGEMENTS

We are grateful to Leo Lee and Don Simms for the preparation of the oligonucleotides, to Dr. Richard Morimoto for the kind gift of the pHB-CAT plasmid, and to Dr. Joost Oppenheim for helpful comments on the manuscript. A. H-B is a Chargee de recherche in CNRS (France), on leave in the LMI.

*Present address: Laboratoire d'Immunologie, CNRS UA1156, Institut Gustave Roussy, Pavillon de Recherche 1, 39 rue Camille Desmoulins, 94805 Villejuif, France

REFERENCES

1. Lindquist, S. (1986) Ann. Rev. Bioch. **55**, 1151.
2. Wu, B. and Morimoto, R. (1985) Proc. Natl. Acad. Sci. USA **82**, 6070–6074.
3. Ferris, D., Harel-Bellan, A., Morimoto, R. and Farrar, W. L. (1988) Proc. Natl. Acad. Sci. USA **85**, 3850–3854.
4. Wu, B., Kingston, R. E. and Morimoto, R. (1986) Proc. Natl. Acad. Sci. USA **83**, 629–633.
5. Morgan, W. D., Williams, G. T., Morimoto, R. I., Greene, J., Kingston, R. E. and Tjian, R. (1987) Mol. Cel. Biol. **7**, 1129–1138.
6. Pelham, H. R. B. (1982) Cell **30**, 517–528.
7. Topol, J., Ruden, D. M. and Parker, C. S. (1985) Cell **42**, 527–537
8. Wiederrecht, G., Shuey, D. J., Kibbe, W. A. and Parker, C. S. (1987) Cell **48**, 507–515
9. Bienz, M. and Pelham, H. R. B. (1986) Cell **45**, 753–760.
10. Mirault, M. E., Southgate, R. and Delwart, E. (1982) EMBO J. **1**, 12791287
11. Pelham, H. (1985) Trends in Genet. **1**, 31–35
12. Moreau, P., Hen, R., Wasylick, B., Everett, R., Gaub, M. P. and Chambon, P. (1981) Nuc. Ac. Res. **9**, 6047–6068.
13. Banerji, J., Rusconi, S. and Shaffner, W. (1981) Cell **27**, 299–308.
14. Banerji, J., Olson. L. and Shaffner, W. (1983) Cell **33**, 729–740.

15. Wilderman, A. G., Sassone-Corsi, P., Grundstom, T., Zenke, M. and Chambon, P. (1984) EMBO J. **3**, 3129–3133.
16. Sassone-Corsi, P., Wildeman, A. and Chambon, P. (1985) Nature **313**, 458–463.
17. Xhiao, J. H., Davidson, I., Ferrandon, D., Rosales, R.,Vigneron, M., Macchi, M., Ruffenach, F. and Chambon, P. (1987) EMBO J. **6** 3005–3013 (1987)
18. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. and Karin, M. (1987) Cell **49**, 729–739.
19. Sen, R. and Baltimore, D. (1986) Cell **47**, 921–928.
20. Augereau, P. and Chambon, P. (1986) EMBO J. **5**, 1791–1797.
21. Mercola, M., Goverman, J., Mirell, C. and Calame, K. (1985) Science **227**, 266–270.
22. Sorger, P. K. and Pelham, H. R. B. (1987) EMBO J. **6**, 3035–3041.
23. Wu, K., Wilson, S., Walker, B., Dawid, I., Paisley, T., Zimarino, V. and Ueda, H. (1987) Science **238**, 1247–1253
24. Goldenberg, C. J., Luo, Y., Fenna, M., Baler, R., Weinman, R. and Voellmy, R. (1988) J. Biol. Chem. **263**, 19734–19739
25. Izant, J. C. and Weintraub, H. (1984) Cell **36**, 1007.
26. Izant, J. C. and Weintraub, H. (1985) Science **229**, 345.
27. De Benedetti, A., Pytel, B. A. and Baglioni, C. (1987) Proc. Natl. Acad. Sci. USA **84**, 658.
28. Knecht, D. A. and Loomis, W. L. (1987) Science **236**, 1081
29. Holt, J. T., Venkat, G. T., Moulton, A. D. and Nienhuis , A. W. (1986) Proc. Natl. Acad. Sci. USA **83**, 4794.
30. Amini, S., Deseau, V., Reddy, S., Shalloway, D. and Bollen, J. B. (1986) Mol. Cel. Biol. **6**, 2305.
31. Zamecnick, P. C. and Stephenson, M. L. (1978) Proc. Natl. Acad. Sci. USA **75**, 280.
32. Stephenson, M. L. and Zamecnick, P. C. (1978) Proc. Natl. Acad. Sci. USA **75**, 285.
33. Zamecnick, P. C., Goodchild, J., Tagushi, Y. and Sarin, P. S. (1986) Proc. Natl. Acad. Sci. USA **83**, 4143.
34. Heikkila, R., Schwab, G., Wickstrom, E., Loke, S. L. Pluznick, D. H., Watt, R. and Neckers, L. M. (1987) Nature **328**, 445.
35. Holt, J. T., Redner, R. L. and Nienhuis, A. W. (1988) Mol. Cel. Biol. **8**, 963.
36. Harel-Bellan, A., Ferris, D., Vinocour, M., Holt, J. T. and Farrar, W. L. (1988) J. Immunol. **140**, 2431.
37. Jayaraman, J., Mc Parland, K., Miller, P. and T'so, P. O. P. (1981) Proc. Natl. Acad. Sci. USA, **78**, 1537.
38. Lemaitre, M., Bayard, B. and Lebleu, B. (1987) Proc. Natl. Acad. Sci. USA **84**, 648.
39. Piette, J. and Yaniv, M. (1987) EMBO J. **6**, 1331–1337
40. Miskimins, W. K., Roberts. M. P., McCleland, A. and Ruddle, F. (1985) Proc. Natl. Acad. Sci. USA. **82**, 6741–6744.
41. Korner, M., Harel-Bellan, A., Kelvin, D., Brini, A. T. and Farrar, W. L. submitted.
42. Gorman, C. M., Moffat, L. F. and Howard, B. H. (1982) Mol. Cel. Biol. **2**, 1044–1051.
43. Kingston, R. E., Schuetz, T. J. and Larin, Z. (1987) Mol. Cel. Biol., **7**, 1530–1534
44. Zimarino, V. and Wu, K. (1987) Nature **327**, 727–730
45. Thomas, G. H. and Elgin, S. C. R. (1988) EMBO J. **7**, 2191
46. Xiao, H. and Lis, J. T. (1986) Mol. Cel. Biol. **6**, 3200–3206
47. Jonhston, R. N. and Lucey, B. L. (1988) Science **242**, 1551–1554
48. Larson, J. S., Shuetz, T. J. and Kingston, R. E. (1988) Nature **335**, 372–375
49. Sorger, P. K., Lewis, M. J. and Pelham, H. R. B. (1987) Nature **329**, 8284
50. Cohen, R. S. and Meselson, M. (1988) Nature **332**, 856–858
51. Schleif, R. (1988) Science **240**, 127–128
52. Wang, J. C. and Giaever, G. N. (1988) Science **240**, 300–304
53. Shuey, D. J. and Parker, C. S. (1986) Nature **323**, 459–461

This article, submitted on disc, has been automatically converted into this typeset format by the publisher.