FEBS Letters 444 (1999) 130–135 FEBS 21520

Regulation of human hsp90α gene expression

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Received 30 November 1998

Abstract Mammalian HSP90α and HSP90β are encoded by two individual genes. On the basis of the upstream sequences of the human hsp90α gene, GenBank accession number U25822, we have constructed CAT reporter plasmids driven by individual fragments of the hsp90α gene. We found that (1) the proximal heat shock element complex located at −96/−60 enhances hsp90α promoter expression; (2) heat shock induction depends upon the coexistence of distal heat shock element at −1031/−1022 and the proximal heat shock element complex of the hsp90α gene; (3) unlike hsp90β, downstream sequences of the transcription start site inhibit hsp90α expression. We conclude that the regulatory mechanisms for the expression of hsp90α and hsp90β genes are different.

Key words: Hsp90α gene; Heat shock element; Chloramphenicol acetyltransferase; Gene regulation

1. Introduction

Heat shock proteins have long been known to be stress related proteins that participate in an immediate, 'acute phase' cellular response. We have reported in our early work with human peripheral blood T lymphocytes that HSP70 is a typical stress protein. hsp70 mRNA can be induced not only at an elevated temperature but also effectively in the cold (4°C). HSP90, in contrast, is expressed constitutively and can be further activated by either heat shock or T cell mitogens [1,2]. The distinctive way of their expression in human lymphocytes suggested that the 90 kDa protein may have more cellular functions other than a stress related one. It is now clear that HSP90 existed abundantly in the cytosol even without heat or other stress stimulation, and as a member of the cellular molecular chaperones [3,4]. HSP90 mostly participates in the folding of nuclear receptor family and signal transduction molecules and has thus been named a specific molecular chaperone [5–9].

Mammalian HSP90α and HSP90β are two major cellular proteins of the HSP90 subfamily and are highly homologous (86%) to each other at the protein level. The two HSP90s are found to be encoded by two individual genes, α and β [10,11]. Both copies of the hsp90 genes contain introns. It is also known that the first exon of the hsp90α and β gene is untranslated. The translational initiations of both genes start from the very beginning of the second exon. The existence of an intron in a heat shock gene may indicate that some relatively complicated regulation mechanisms are involved or required for certain sophisticated functions of the HSP90 in vivo. We have reported elsewhere that the first intron of the hsp90β gene is essential in the high constitutive expression and is critical for heat shock induction [12]. In addition, only the antisense cDNA of hsp90β plays a similar role as the HSP90 inhibitory drug geldanamycin (GA) in the induction of hsp90α, hsp70, as well as the autoregulation of the hsp90β gene in Jurkat cells [13]. In order to answer such questions as whether the two hsp90 genes are regulated differently, and whether the two genes function complementarily to each other or work cooperatively, we have to explore first whether the two genes are controlled by similar mechanisms and are expressed simultaneously in vivo or not. Although the cloned 5′ flanking region of the hsp90α gene has been shown to induce heterologous target gene expression in vitro by Hickey et al. [10], little is known about its control mechanism. We have successfully subcloned and sequenced the 5′ flanking region of the hsp90α gene up to −1757 bp for the first time [14] (GenBank accession number U25822). In this paper we examine how the hsp90α gene expression is regulated. Our results will be able to shed light on the understanding of any differential expression and the possibility of a close cooperation of the hsp90 genes in mammalian cells.

2. Materials and methods

2.1. Preparation of whole cell extracts (WCE)

The WCE was prepared according to Mosser's method [15] with slight modifications. 2×10^7 Jurkat cells, cultured under normal condition (37°C) or heat shocked at 42°C for 60 min, were suspended in 50 μl of PBS and stored at −70°C overnight. 300 μl of lysis buffer (20 mM HEPES pH 7.9, 420 mM NaCl, 1.5 mM $MgCl_2$, 100 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 25% glycerol) was first added into the frozen pellets. After gentle mixing, it was then centrifuged at $12\,000\times g$ for 40 min at 4°C. The resulting supernatants were stored at −70°C until use. The concentration of protein in the supernatants was determined by the method of Bradford [16].

2.2. Polymerase chain reaction (PCR)

In each PCR experiment, 0.5 μg of template genomic DNA, 10 pmol of each primer, 2.5 mM each of dATP, dGTP, dCTP and TTP, 0.6 U of Taq DNA polymerase, 2.5 μl of the 10×PCR reaction buffer (500 mM KCl, 100 mM Tris-HCl pH 8.5, 15 mM $MgCl_2$, 0.1% Triton X-100), and 1 μl of DMSO were first added, bidistilled water was then added to make up a final reaction volume of 20 μl. Each PCR cycle was 94°C for 1 min, 57°C for 1 min, 72°C for 3 min; in the second experiment it was 94°C for 1 min, 45°C for 1 min and 72°C for 4 min. After 30 cycles, the reaction mixture was further incubated at 72°C for another 10 min.

2.3. Construction of the chloramphenicol acetyltransferase (CAT) reporter plasmids

A DNA fragment covering −218/+37 bp of the hsp90α gene was a PCR amplification product from KS∝LCAT (a gift from Dr. Lee A. Weber, University of Nevada) which contains the 5′ flanking fragment of the human hsp90α gene [10]. The PCR product was obtained

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PII: S0014-5793(99)00044-7

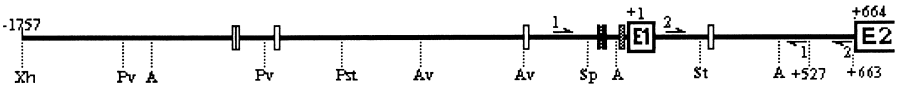

Fig. 1. Schematic diagram of the regulatory regions of the human hsp90α gene. Diagram corresponds to sequences (−1757 bp) from within the 5′ upstream sequence of the human hsp90α gene through the second exon (E2, +664). Arrows indicate the locations and directions of primers for PCR. The endonuclease restriction sites used in the cloning of the reporter plasmids are indicated (A: *Apa*I (−1377, −36, +443); Av: *Ava*II (−607, −305); E: *Eco*RV (−1034); Pst: *Pst*I (−847); Pv: *Pvu*II (−1462, −1050); Sp: *Sph*I (−125); St: *Stu*I (+206); Xh: *Xho*I (−1756)). Locations of some major elements within the 'full length' region are labeled from upstream downward: CAAT box at −1144; HSE at −1032, −303, −96; TATA box: −30; HSE +228. E1: exon 1; E2: exon 2.

with a pair of synthesized oligonucleotide primers (primer pair 1 shown in Fig. 1: 5′ primer: 5′-GATTGAGGGAAGGTTGCC-3′; 3′ primer with an *Bam*HI site, 5′-AGGGATCCAACGGCACAG-3′). Following double digestions at the *Bam*HI site within the primer and the *Sph*I site at −125 bp, a PCR fragment was recovered and inserted into the corresponding sites of a plasmid containing hsp90α upstream sequences up to −1757 bp. The pSKH90-A which runs from bp −1756 to +37 of the hsp90α gene was thus constructed. The upstream fragment was then restriction digested and inserted into the pBLCAT3 vector according to the techniques described by Sambrook et al. [17] and designated the hsp90α1 reporter construct. Based on hsp90α1, five subcloned CAT reporter plasmids (α1 series) truncated at different sites 5′ upstream of the hsp90α gene were obtained (Fig. 2).

The DNA fragment of the hsp90α α4 series was constructed by (1) PCR amplification of a +147/+663 fragment from genomic DNA of human peripheral blood leukocytes with a second pair of primers (primer pair 2 shown in Fig. 1: 5′ primer: 5′-GACCAGATCCCTGA-AGCAGCCTTT-3′; 3′ primer: 5′-CTGGAACGACACGCGCCGG-TTT-3′); (2) digesting the PCR product with *Stu*I at +206, and ligating it with the *Stu*I site of another PCR fragment of −218/+527 (α3[1]) to construct a transit fragment of −218/+663 bp; (3) after proper digestion and ligation with corresponding sites in the α1 series constructs to form a new series of reporter constructs ending at +663. In this paper, we designated the 2420 bp fragment of −1756/+663 the 'full length' regulatory region of hsp90α (Fig. 3).

2.4. Cell culture and transfection

Jurkat cells were cultured in RPMI 1640 medium (Gibco-BRL) containing 10% fetal calf serum (FCS) at 37°C in a humidified atmosphere of 5% CO_2. Transfection was performed by using Lipofectamine (Gibco-BRL) following the manufacturer's instruction with slight modifications. A mixture of 3 μg of total DNA consists of reporter plasmid and pSV-β-galactosidase plasmid (Promega) at a ratio of 3:1 and 5 μl of lipofectamine in 200 μl reduced serum medium of Opti-MEM I (Gibco-BRL) was added to 800 μl of cell suspension at a density of $2–5\times10^6$ cell/ml in 35 mm plastic dishes. After incubation at 38°C for 5 h, 9 ml of RPMI 1640 medium with 12% FCS was added to the culture, cells were incubated for an additional 48 h and then heat shocked at 42°C for 60 min. For each reporter construct, three independent transfection experiments were performed.

For co-transfection, the ratio of reporter plasmid, sense or antisense eukaryotic expression plasmids (CMV-HSP90α^S, CMV-HSP90α^{AS}, CMV-HSP90β^S and CMV-HSP90β^{AS}) [13] and pSV-β-galactosidase was 1:1:1 respectively.

2.5. CAT and β-galactosidase activity assays

Transfected cell extracts were prepared by three cycles of freeze-thawing in 100 μl of 0.25 M Tris-HCl (pH7.8). Extracts were clarified by centrifugation at $12\,000\times g$ for 10 min at 4°C. For the CAT assay [17], to each 20 μl aliquot of cell extract, 30 μl of 0.1 M Tris-HCl (pH 7.8) was first added and incubated at 65°C for 15 min, and then 200 μl of CAT reaction buffer (0.125 M Tris-HCl (pH 7.8), 1.25 mM chloramphenicol, 0.1 μCi [^{3}H]acetyl CoA (10 Ci/mmol, Amersham)) was added, mixed and incubated at 37°C for 4–8 h. CAT activities were detected by transferring samples into individual counting vials followed by adding 5 ml of scintillation cocktail for tritium counting in a Beckman scintillation counter LS-6500. For β-galactosidase assay, 5 μl of cell extracts was mixed with 20 μl of LumiGal reaction buffer (Clontech Laboratories) and incubated at 37°C for 60 min followed by adding 30 μl of LumiGal accelerator, the fluorescence was then measured in the Monolight 2010 Luminometer. CAT activity was individually normalized by comparing the activity with that of β-galactosidase from the same cell extract.

2.6. Electrophoretic mobility shift assay (EMSA)

DNA fragments were labeled by filling-in reaction with [α-^{32}P]dCTP (3000 Ci/mmol) and used as probes at 5×10^4 cpm in each experiment in the presence of a 10^4-fold excess of sonicated salmon sperm DNA, with or without 10 μg of individual WCE. For competition analysis, unlabeled DNA fragment was added in excess as indicated. Binding reaction was carried out in DNA binding buffer (10 mM Tris-HCl pH 7.6, 50 mM NaCl, 1 mM EDTA and 50% glycerol) at 22°C for 45 min as described by Ausubel et al. [18] with minor modifications [19]. DNA-protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 19:1) run in Tris-borate/EDTA buffer, pH 8.3. Gels were then dried and autoradiographed.

3. Results

As reported in Section 2, we first constructed subclones of the 5′ flanking sequence of the hsp90α gene, and obtained five individual truncation fragments starting from −1756 (α1), −1377 (α1.1), −847 (α1.2), −125 (α1.3), −36 (α1.4) through +37 (Fig. 2). All of them are recovered and ligated to the upstream of CAT designated the 'α1' series of the hsp90α gene. All of the 3′ ends of the clones in the 'α4' series have been further extended to the 3′ end of the first intron (+663). There are nine individual clones of the series shown in Fig. 3 that starts from −1756 (α4), −1377 (α4.1), −1050 (α4.2), −1034 (α4.3), −847 (α4.4), −125 (α4.5), −36 (α4.6), +206 (α4.7) and +443 (α4.8).

3.1. The constitutive expression of hsp90α gene

Studying the constitutive expression of the α1 series of the hsp90α gene, we found that the longest construct (−1756/+37) gives the highest activity of CAT, which is 15.5-fold higher than the basal level of activity driven by the core promoter −36/+37 (α1.4). With 5′ truncation down to −847 bp, the construct gives a similar reporter activity to that of the longest construct. However, we unexpectedly found that the reporter activity of −1377/+37 was very low, only next to the lowest value of construct α1.4 in the α1 series. Results shown in Fig. 2 indicate that (1) a 89 bp upstream promoter element (UPE) between −125 bp and −37 bp confers a 10-fold elevated constitutive expression relative to the core promoter construct (−36/+37); (2) the presence of a fragment of −1377/−848 confers a negative effect to the CAT activity driven by −847/+37; (3) −1756/−1377 is a positive regulatory fragment that readily overcomes the inhibitory effect of −1377/−848 and increases the level of constitutive expression to 2.3-fold of construct −1377/+37 of the gene (Fig. 2).

We then constructed some more reporter plasmids of the α1 series such as α1.6–1.10 starting from −305, −607, −1034, −1050 and −1462 through +37 and found that the activities

[1] 'α3' was amplified by PCR with the 5′ primer of primer pair 1 and another 3′ primer, 5′-GCTGACAAAGGATGACCTCAT-3′.

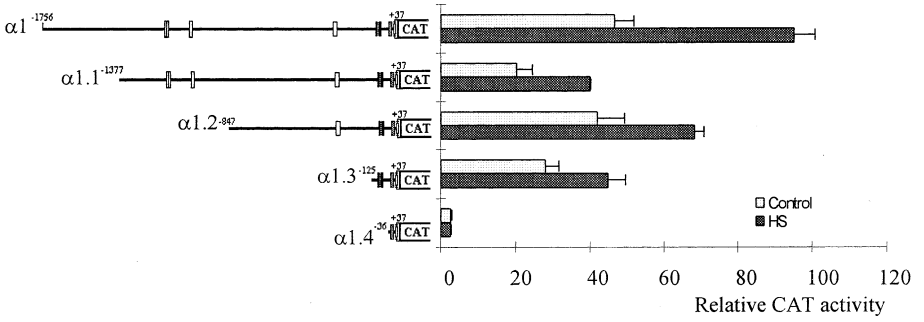

Fig. 2. Constitutive and heat shock inducible expression levels from the 5′ regulatory region of hsp90α-CAT fusion constructs (α1 series). The relative transcription unit is defined by the ratio of the CAT activity of the hsp90α fusion constructs relative to the activity of a cotransfected β-galactosidase control. Locations of some major elements within the 5′ upstream region are labeled from upstream downward: CAAT box at −1144; HSE at −1032, −303, −96; TATA box: −30; fragment ended at +37 relative to the initiation site at +1 of hsp90α gene; E1: exon 1. The filled and open bars in the right panel indicate the constitutive and heat inducible levels of CAT activities driven by the individual deletion constructs. Standard deviation of each experiment is shown at the top of each bar.

of the new constructs starting from −305 and −607 are similar to that of −125/+37 (data not shown); −1034 and −1050 through +37 are comparable with that of −847/+37; and −1462/+37 is almost the same as −1377/+37 (partly shown in Fig. 4a). We were thus able to narrow down the afore mentioned positive and negative regulatory regions of the hsp90α gene to −1756/−1463 and −1377/−1051 respectively.

Upon transfection of the α4 series constructs into Jurkat cells, we found that the CAT activities driven by the regulatory sequences in the series are completely 5′ fragment length dependent (Fig. 3). Comparing constructs of α1 with the α4 series (Fig. 4a), we found that the constitutive activities of the α4 series are obviously lower than those of the 5′ flanking region alone which indicates that the coexistence of the first exon and the first intron plays an inhibitory role to the expression of the gene. In the presence of the above mentioned inhibitory fragment (−1377/−1051), construct α1.1 covering −1377/+37 still shows higher activity than α4.1 covering −1377/+663.

3.2. The heat shock regulation of the hsp90α gene

In terms of the length of 5′ flanking sequences, the profiles of heat shock inducibility of the individual constructs in the α1 and α4 series are mostly comparable. Constructs with longer length of the 5′ flanking region (>1 kb) generally confer over 2-fold heat shock induction of the reporter activity. Both constructs starting from −1050 in the two series confer a markedly higher level of induction than those starting from its downstream (Fig. 4b).

3.3. The binding activities of WCE to the heat shock element (HSE) of the hsp90α gene

We recovered a 78 bp proximal fragment (−114/−37) and used it as a probe or specific competitor for EMSA. Specific binding complex only showed up in nuclear extract from heat shocked cells (Fig. 5, lane 2), and became faint in 20–80-fold molar excess of specific competitor (lanes 3–5). With ≥100-fold molar excess of the above competitor (lanes 6–8), or a distal (−1158/−975) upstream fragment of 184 bp (lanes 9

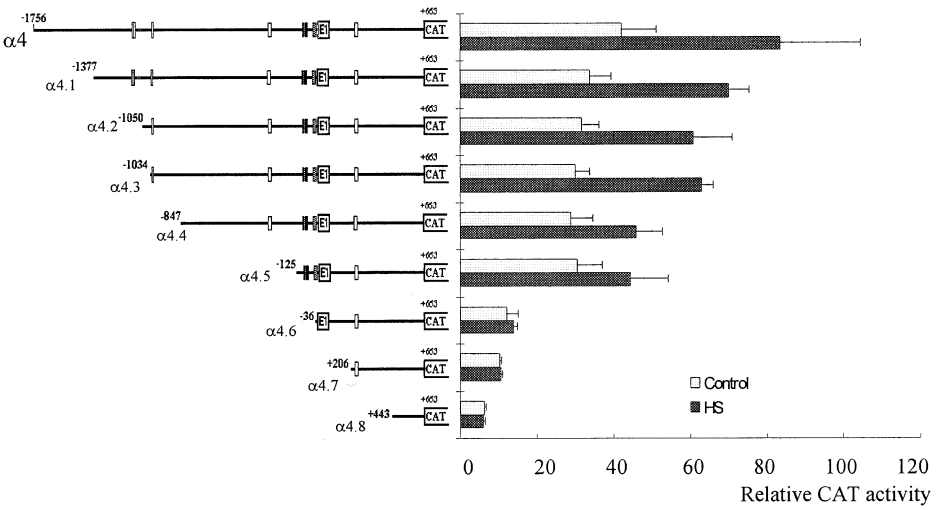

Fig. 3. Constitutive and heat shock inducible expression levels from the 'full length' regulatory region of hsp90α-CAT fusion constructs (α4 series). The relative transcription unit is defined by the ratio of the CAT activity of the hsp90α fusion constructs relative to the activity of a cotransfected β-galactosidase control. Locations of some major elements within the 'full length' region are labeled from upstream downward: CAAT box at −1144; HSE at −1032, −303, −96; TATA box: −30; HSE +228. E1: exon 1; E2: exon 2. The filled and open bars in the right panel indicate the constitutive and heat inducible levels of CAT activities driven by the individual deletion constructs. Standard deviation of each experiment is shown at the top of each bar.

and 10), the binding complexes were abolished. However, the intron HSE containing fragment −36/+442 is inefficient in the competition at the same molar ratio (lanes 11 and 12).

4. Discussion

4.1. Control of the constitutive expression of the hsp90α gene

Considering our previous finding that the 5′ flanking sequences is inefficient in heat shock induction of the human hsp90β gene [12], we have constructed and examined the 'full length' activity of hsp90α gene (−1756/+663) in this paper.

4.1.1. The promoter of the hsp90α gene. The promoter region of eukaryotic genes consists of the core promoter and the upstream promoter elements (UPE). Most of the UPEs for RNA polymerase II transcription include the GC box and CAAT box located immediately upstream of the core promoter within −50 to −200 bp from the RNA start site [20]. In most of the hsp genes, other than the HSEs, both the CAAT box and Sp1 site are important elements for an efficient promoter [21,22]. Mammalian hsp70 and hsp90β genes carry Sp1 and the CAAT box while human hsp90α lacks the CAAT box in the promoter region. The core promoter of the hsp90α gene only consists of a TATA box at −30 bp, and a transcription initiation site at +1 [10,12,13,21,22]. The two Sp1 binding sites in the UPE are located at −45/−40 and −153/−148 bp [10,13]. In this paper, a construct covering bp −36/+37 functions as the core promoter of the hsp90α gene, which confers a relatively low level of CAT activity. The basal activity of the hsp90α gene is only comparable with the strength of a weak promoter such as the thymidine kinase gene (tk) in Jurkat cells (data not shown).

Within the 3′ of the first intron of the hsp90α gene, there is a region located between +534 and +643 where the A/T content is over 60%. We checked the sequences around it and found that there are some promoter-like elements such as the pyrimidine rich sequences of TCTCCAATTC at +563/+572 bp, and another less typical one of CCCTTCTATTTTC at +529/+541 bp, that are similar to those within the first intron of the hsp90β gene [12]. Although the activity is low, construct α4.6 (−36/+663) is the only one of the α4 series that presents higher activity than its corresponding construct of the α1 series in Fig. 4a. In addition, its activity interestingly corresponds to the additive effect of the upstream core promoter (α1.4, −36/+37) and intron promoter-like fragments α4.8 (+448/+663). These results indicate that intronic promoter-like sequences, although very inefficient, may participate in the constitutive expression of the hsp90α gene in the absence of upstream elements.

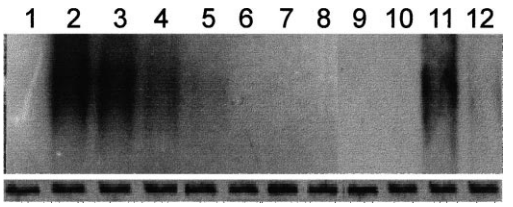

Fig. 5. Electrophoretic mobility shift assays of protein complex binding to the 5′ proximal HSE complex of the hsp90α gene. An upstream DNA fragment of −114/−37 carrying the proximal HSE complex was used as the probe in EMSA. 4% PAGE profile of the DNA-protein complex from untreated cells (lane 1), heat shocked cell extracts (lanes 2–12). The binding was competed by the addition of a 20-, 40-, 80-, 100-, 200- and 400-fold molar excess of a specific competitor (unlabeled fragment of −114/−37) (lanes 3–8). EMSAs carried out with the same of −114/−37 with 100- and 200-fold molar excess of each of the competitors: a fragment (−1158/−975) from the 5′ distal region with HSE motifs (lanes 9 and 10); a fragment from −36/+442 carrying the intronic HSE motifs of a tail-to-tail alignment (lanes 11 and 12). The same amount of WCE is dotted onto the nitrocellulose filter, stained with amido black and shown at the bottom of each lane.

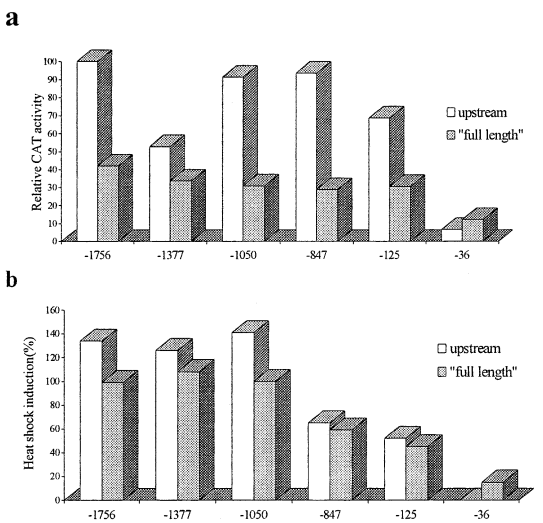

Fig. 4. Comparison of the expression levels between corresponding constructs of the α1 and α4 series. a: The constitutive level of CAT expression from the α1 series fusing to CAT at +37 of the hsp90α gene; and that from α4 series fusing to CAT at +663 as the 'full length' hsp90α gene. Numbers labeled at the bottom of each group show the positions of the 5′ end of each deletion construct. b: The increased percentage of CAT expression upon heat shock from the α1 series fusing to CAT at +37; and that from the α4 series fusing to CAT at +633 as the 'full length' hsp90α gene. Numbers labeled at the bottom of each group show the positions of the 5′ end of each deletion construct.

4.1.2. 5′ Upstream sequences involved in the regulation of the hsp90α gene. In the presence of UPE, it is reasonable to find that the constitutive expression of α1.3 (−125/+37) is over 10-fold more efficient than the core promoter alone. However, the same fragment only elicits a 2.5-fold induction on the core promoter construct of α4.6 (Fig. 4a). This result indicates when the upstream sequences existed, the intron only showed an inhibitory role that was dominant over the weak intronic promoter activity. Interestingly, the fragment −1377/−1051 shows a ∼40% inhibition of the CAT activity on the α1 series (Fig. 4a), while it shows no effect on the α4 series. In addition, the CAT activity of −1377/+663 is only 64% of its corresponding construct (−1377/+37) in Jurkat cells. These discrepancies may be explained by the stronger inhibitory role that the downstream sequences exert to most of the constructs in α4 series. We thus conclude that, as represented by the 2240 bp 'full length' construct, the intron elements do not play any important role in the constitutive expression of the hsp90α gene when the upstream fragments coexisted in vivo.

As shown in Figs. 3 and 4a, the efficiency of constructs of the α4 series is 5′ length dependent. Activities of the α4 constructs are always lower than their corresponding constructs in the α1 series, which indicates that sequences downstream of the RNA initiation site play an inhibitory role to the constitutive expression of the hsp90α gene. Meanwhile, the inhibition of fragment −1377/−1050 shown in Fig. 4a is eliminated

in the presence of the downstream sequences. We thus suggest that the inhibitory effect conducted by the 5′ sequences alone is invalid in vivo. Compared with the essential role of the first intron of the hsp90β gene, the intron of the hsp90α gene is almost inefficient in constitutive expression. The 5′ flanking sequence per se is sufficient to confer on the gene a relatively high level of constitutive expression.

4.2. Non-contiguous HSEs synergistically confer higher heat shock inducibility to the hsp90α gene

4.2.1. The HSEs of the hsp90α gene. The HSE is the most critical element in hsp gene transcription. HSEs are characterized by the five nucleotide motifs of nGAAn in a 'head-to-head' or 'tail-to-tail' alignment [22]. As we sequenced the upstream region of the hsp90α gene, we found two of the five nucleotide motifs at −1031/−1022, 5′-c**GAA**a a**TTC**c-3′; and another array of four 5 bp motifs: 5′-g**G***G***A**c c**TTC**c c**GA***G*a a**TT***T*c-3′ located at −307/−288 with three mutations. The UPE of the hsp90α gene contains HSE from bp −96 to −60: 5′-GGAGGGTTCTTCCGGAAGTTCAAGAGGCTT-CTGGAAA-3′. Within an array of five contiguous 5 bp motifs (5′-g**GA***G*g g**TTC**t-3′; 5′c**TTC**c g**GAA**g-3′ and 5′g**TTC**a a**GA***G*g c**TTC**T g**GAA**a-3′), there clearly are some overlapping sequences, such as the underlined CT at −88/−87 and the G at −79, and substitutions of *G* for A at −93 and −71. We designated these HSE sequences the 5′ 'proximal HSE complex'. Within the first intron, there is another HSE without mutations, 5′-c**TTC**a g**GAA**t-3′, located at +238/+247. Therefore only two HSEs of the hsp90α gene (−307/−288 and −96/−60) match the criterion of a mammalian 'typical HSE' that consists of at least three adjacent 5 bp modules; however, the two HSEs contain either mutations in the 2–4 positions of the 5 bp module or with some sequences overlapping.

4.2.2. Synergistic role between HSEs. In Fig. 4b, we show that the activity increased upon heat shock as the percentage augmentation of reporter activity in comparison with that of corresponding constitutive expression of the construct. It has been reported in *Drosophila* that multiple arrays of the 5 bp module without any mutations in the 2–4 positions do not necessarily produce higher heat shock induction [23]. This is comparable to our results in human cells. Since we were unable to identify any important role of the HSE at around −300 bp (see Section 3.1), we suggest that a point mutation in the third or fourth position of the nGAAn motifs may cause serious damage in the heat shock inducibility.

HSEs located far upstream (−1031/−1022) or in the first intron (+238/+247) contain only two modules without mutation. The coexistence of the proximal HSE complex with these HSEs occurs in the following cases: (1) the α1 series constructs longer than −1050/+37 form the most efficient combination that confers higher induction synergistically; (2) construct α4.5 (−125/+663) with the coexistence of the proximal HSE complex and an intronic HSE is slightly induced by heat shock. Therefore, the HSE complex in the proximal region of the hsp90α gene plays a central role in conferring heat shock induction synergistically. Moreover, the distance between the two non-contiguous HSEs and the way of alignment, that is, head-to-head or tail-to-tail, may also be important for the induction efficiency.

Two major 5′ length dependent elevations in heat shock induced hsp90α expression are shown in profile in Fig. 4b. The first one depends on the addition of the 5′ proximal HSE complex within −125/−37 (Fig. 4b, fifth column from the left) and the second one related to the existence of distal HSE in the fragment of −1050/−847 (third column from the left in Fig. 4b). These results indicate that a synergistic effect of non-contiguous HSEs takes a great part in the heat shock inducibility of the hsp90α gene.

The result in Fig. 5 show that nuclear extract of heat shocked Jurkat cells binds to the 5′ distal and proximal HSEs with higher affinity than to the intronic one, which indicates that at equal molar excess, the distal HSE at −1031/−1022 (lanes 9 and 10) and the proximal HSE at −96/−60 (lanes 7 and 8) bind to heat shock factor (HSF) much more efficiently than the intron HSE at +238/+247 (lanes 11 and 12). Activated HSFs of the heat shocked cells may simultaneously bind to any two available sites, and may help DNA loop formation between the two HSEs at a proper distance apart [24]. The tertiary complex thus formed may provide the structural basis for the synergistic effect of higher heat shock inducibility to the gene. We thus confirm our suggestion that the 5′ proximal HSE complex participates in heat shock induction of the hsp90α gene through its cooperation with either the preferred distal HSE or the less effective intronic HSE. Upon loop formation between the proximal HSE and the upstream HSE at around −1030, the CAAT box of hsp90α located at −1144/−1136 may also be drawn closer to the proximal region. The important effect of the CAAT box and its binding factors are thus validated to promote the transcription initiation of the hsp90α gene [21,22].

In summary, although the two copies of HSP90 are highly homologous at the protein level, the regulatory regions of their encoding genes are by no means similar. We report in this paper that the 5′ flanking sequences play a dominant role in both constitutive and heat shock induced expression of the human hsp90α gene in Jurkat cells. In contrast, the first intron of the hsp90β gene is extremely important [12]. Based on our findings here and reported elsewhere [14], we conclude that the transcription elements and the activation mechanisms of the two genes are quite different. We suggest that the apparent cellular HSP90 in certain cell types or in certain stages of development may probably be the expression product of only one copy of the hsp90 genes activated specifically. With a competitive PCR detection (H.F. Li, X.K. Cheng and Y.F. Shen, to be published), we were able to show that the two copies of hsp90 are differentially expressed in most of the human tissues examined. Therefore, the major functional dimer form of cellular HSP90 in certain cell types may very likely be a homodimer in vivo. To find evidence at the protein level and the signal pathway involved is our next aim.

Acknowledgements: We thank Dr. Lee A. Weber of the University of Nevada for providing us with the plasmid KS∝ LCAT. The work was supported by the National Natural Science Foundation Grant 39570779, the Pandeng project and the '863' project of the Ministry of Science and Technology in China.

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