Sequence and Regulation of a Gene Encoding a Human 89-Kilodalton Heat Shock Protein

EILEEN HICKEY,¹ SUSAN E. BRANDON,¹ GEORGEANN SMALE,² DAVID LLOYD,^{1†} and LEE A. WEBER^{1,2*}

Biology Department¹ and Department of Biochemistry and Molecular Biology,² University of South Florida, Tampa, Florida 33620

Received 13 January 1989/Accepted 20 March 1989

Vertebrate cells synthesize two forms of the 82- to 90-kilodalton heat shock protein that are encoded by distinct gene families. In HeLa cells, both proteins (hsp89a and hsp89B) are abundant under normal growth conditions and are synthesized at increased rates in response to heat stress. Only the larger form, hsp89 α , is induced by the adenovirus E1A gene product (M. C. Simon, K. Kitchener, H. T. Kao, E. Hickey, L. Weber, R. Voellmy, N. Heintz, and J. R. Nevins, Mol. Cell. Biol. 7:2884-2890, 1987). We have isolated a human hsp89a gene that shows complete sequence identity with heat- and E1A-inducible cDNA used as a hybridization probe. The 5'-flanking region contained overlapping and inverted consensus heat shock control elements that can confer heat-inducible expression on a β-globin reporter gene. The gene contained 10 intervening sequences. The first intron was located adjacent to the translation start codon, an arrangement also found in the Drosophila hsp82 gene. The spliced mRNA sequence contained a single open reading frame encoding an 84,564-dalton polypeptide showing high homology with the hsp82 to hsp90 proteins of other organisms. The deduced hsp89\alpha protein sequence differed from the human hsp89\beta sequence reported elsewhere (N. F. Rebbe, J. Ware, R. M. Bertina, P. Modrich, and D. W. Stafford (Gene 53:235-245, 1987) in at least 99 out of the 732 amino acids. Transcription of the $hsp89\alpha$ gene was induced by serum during normal cell growth, but expression did not appear to be restricted to a particular stage of the cell cycle. hsp89a mRNA was considerably more stable than the mRNA encoding hsp70, which can account for the higher constitutive rate of hsp89 synthesis in unstressed cells.

The 82- to 90-kilodalton (kDa) class of heat shock proteins (HSPs) have long been recognized as cytoplasmic proteins that are abundant in the absence of stress (40, 42, 78) and which are induced to higher levels of synthesis by heat shock. In avian and mammalian cells and tissues, these proteins (hereafter referred to as hsp89) have been found in association with several different regulatory and structural proteins. hsp89 has been shown to interact with several viral oncogene products that possess tyrosine kinase activity. including pp60src (10, 55), and the yes (46), fps (55), fes, and fgr (85) gene products. In rabbit reticulocytes, hsp89 has been identified as the 90-kDa component of highly purified preparations of the hemin-controlled translational repressor, an eIF-2 α -specific protein kinase (63). hsp89 appears to stimulate the activity of this enzyme. In avian (3, 85) and calf (60) cells, hsp89 has been identified as the non-steroidbinding subunit of the estrogen receptor complex and has since been shown to be a common component of other steroid hormone receptors (33). The steroid-binding component of these receptors appears to be inactive with respect to DNA binding when complexed with hsp89 (30, 58, 66). Specific association of murine hsp89 with tubulin has been reported (67), and calmodulin-sensitive actin binding has been demonstrated in vitro (37, 53). A form of hsp89 is a tumor-specific transplantation antigen in methylcholanthrene-induced tumors in mice (74).

Two forms of hsp89 that differ slightly in molecular mass have been identified in murine (2, 51, 74), human (25), and sea urchin (4) cells. In mouse cells, the proteins are known as hsp84 and hsp86, reflecting their different sizes as determined by gel electrophoresis. Teratocarcinoma cells constitutively express both hsp84 and hsp86 at high levels during proliferation, but upon induction of differentiation, synthesis of hsp86 is specifically down regulated (2). Synthesis of hsp86 and/or hsp84 is induced independently of the other HSPs by estrogen treatment in the murine uterus but not in murine liver or spleen (59). Partial protein sequencing has identified the hsp86 form as the mouse tumor-specific transplantation antigen (74). However, the studies cited above reporting associations between hsp89 and other cellular proteins do not distinguish between the two forms of the HSP.

We have previously isolated several plasmid clones containing inserts homologous to hsp89 mRNA from cDNA libraries prepared from both heat-shocked (28) and control (65) HeLa cell mRNA. The clones represent two sequence families that do not show cross-hybridization under standard conditions. Two different size classes of hsp89 mRNA have been demonstrated, which correspond to each family of cDNA (28). The mRNAs are referred to as $hsp89\alpha$ (2.95 kilobases) and hsp89ß (2.7 kilobases) (70). Both mRNAs are coordinately induced by heat shock in HeLa cells (28). The complete sequence of a human hsp89 cDNA has recently been reported (61). This sequence encodes a protein very similar to mouse hsp84 (51) and corresponds exactly in sequence with the human partial cDNA clone pHS811 (hsp89ß) isolated by our laboratory (28). This form of hsp89 mRNA is not induced by the adenovirus E1A gene product, whereas the $hsp89\alpha$ form represented by clone pHS801 is strongly induced along with at least one member of the hsp70 gene family (70). This hsp70 gene is also induced by serum in the absence of heat shock (80, 81).

In this paper, we report the isolation and characterization of a complete human $hsp89\alpha$ structural gene and flanking

^{*} Corresponding author.

⁺ Present address: Department of Molecular Genetics. Pfizer Central Research, Groton, CT 06340.

sequences. The transcript from this gene is interrupted by 10 intervening sequences, including one located in the 5' mRNA leader that is spliced directly at the AUG start codon. The promoter sequence drives heat-inducible expression of a reporter gene, indicating that this $hsp89\alpha$ gene encodes a bona fide heat shock protein. The same gene is also expressed constitutively. Quantitative primer extension experiments using a gene-specific synthetic oligonucleotide demonstrated that transcription is enhanced by heat stress and adenovirus infection, while constitutive expression is dependent upon serum. Serum-induced expression of hsp89 α is apparently not restricted to a specific stage of the cell cycle in HeLa cells. The spliced $hsp89\alpha$ mRNA encodes a protein of 84,564 daltons that differs from the hsp89ß sequence (61) in 99 of 732 residues. Comparison of hsp89 protein and derived amino acid sequences from several species suggests that two forms of the gene diverged early during vertebrate evolution and have been conserved. They encode distinct proteins that can be induced independently and may carry out different functions.

MATERIALS AND METHODS

Nucleic acid isolation and Southern blot analysis. Highmolecular-weight human DNA was isolated from placenta by the method of Blin and Stafford (7). Total cytoplasmic RNA was isolated from HeLa cells by using a detergent lysis procedure described by Sadis et al. (65). $Poly(A)^+$ RNA was prepared by using standard methods (49). For primer extension experiments, the RNA was precipitated with ethanol an additional time after being adjusted to contain 1 M LiCl. For Southern blot analysis, DNA was digested with restriction enzymes by using conditions recommended by the manufacturer (Boehringer Mannheim Biochemicals). Samples (10 µg) were electrophoresed on 0.7% agarose gels and blotted onto nitrocellulose (72). The blots were hybridized with nicktranslated pHS801 or pHS811 plasmid probes as previously described (27, 62) with a final stringent wash for 15 min at 68°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.01% sodium dodecyl sulfate.

Isolation of the human hsp89 α gene. An AluI-HaeIII human genomic library in phage Charon 4A (44) was screened by hybridization with the hsp98 α -specific cDNA clone pHS801 (28) under conditions described in detail elsewhere (27). From 1,000,000 plaques, seven hybridizing phage were isolated and plaque purified. DNA was prepared from plate lysates (49) and mapped by restriction digestion and Southern blot analysis with the pHS801 probe.

Mapping of transcribed sequences and exon-intron boundaries. Regions of the phage DNA encoding heat-inducible RNA were mapped by differential hybridization of duplicate Southern blots with $poly(A)^+$ RNA isolated either from HeLa cells after 3 h of heat shock at 42°C or from control cells. The RNA was partially degraded with alkali and was ³²P labeled by using polynucleotide kinase (49). EcoRI, EcoRI-BamHI, and HindIII fragments (see Fig. 2) were subcloned into M13 phage (50) or Bluescribe KS and SK plasmid vectors (Stratagene). S1 nuclease protection analysis was done with end-labeled double-stranded probes (49) or single-stranded probes obtained from fragments cloned in M13 (12) by using the hybridization procedure described by Favaloro et al. (21). Probes were denatured at 90°C for 10 min and hybridized with 10 µg of total cytoplasmic RNA for 3 h at temperatures between 50 and 60°C, which were determined to be optimal for each probe. The boundaries of the six exons located at the 3' end of the gene were verified by comparison with overlapping cDNA sequences derived from the cDNA plasmids pHS801 and pHS808 (28) and pCP75 (65), which extend from the poly(A) sequence toward the 5' end of the gene. The transcription start site and the junction of exons 1 and 2 were determined by directly sequencing poly(A)⁺ RNA isolated from heat-shocked cells as described below.

Sequencing. Restriction fragments cloned into the Bluescript phagemid vectors were used to construct nested deletions with exonuclease III and mung bean nuclease under conditions recommended by Stratagene with the following modification. After the deleted end was made flush by mung bean nuclease digestion, the deleted fragments were excised from the vector by cleavage with EcoRI, fractionated on low-melting-temperature agarose gels (54), and subcloned into Smal-EcoRI-cleaved M13 vectors for sequencing by the dideoxy-chain termination method (50). This modification avoids the frequent problem of deletion of the sequencing primer site within the phagemid vector. The programs of Schwindiger and Warner (68) were used for sequence collation, and DNA and protein sequence homologies were determined with the PCS program (39). The 5'-untranslated leader sequence of hsp89a mRNA was sequenced with reverse transcriptase at 50°C as described by Geliebter (22) by using an end-labeled synthetic oligonucleotide primer with the sequence GTCTGGGTTTCCTCAG GCAT.

Analysis of hsp89a gene expression. Quantitative primer extension with the end-labeled synthetic oligonucleotide described above was carried out by the same method used for RNA sequencing, except that all four deoxynucleotides were present at 400 µM and dideoxynucleotides were omitted. Primer annealing times was reduced to 15 min, which was found to be optimal in preliminary experiments. This assay gave a linear increase in the primer extension product with inputs of 0.5 to 10 µg of total cytoplasmic RNA. Northern (RNA) blot and slot blot analyses were carried out as described previously (28, 49). Conditions for heat shock at 42°C have also been described previously (29). Serum starvation and stimulation was accomplished by maintaining cells for 48 h in Joklik medium (GIBCO Laboratories) containing 0.5% calf serum (81) and then by suspending them in medium with 10% fresh serum. Cultures were synchronized by double thymidine block as previously described (77). Total cytoplasmic RNA was isolated for analysis at 3-h intervals for 24 h after serum replenishment or refeeding with thymidine-free medium containing 5% fresh serum. Isolation of nuclei and run-on transcription assays were done as described previously (14, 65). RNA from HeLa cells infected with either wild-type adenovirus or the E1A deletion mutant dl312 was generously provided by C. Simon (Rockefeller University, New York, N.Y.) (70). The RNA was isolated 6 h postinfection. The human H4 histone cDNA probe pF0108A was a gift from G. Stein (University of Massachusetts Medical School, Worcester).

Fusion gene construction and transfection. A chimeric gene was constructed by standard procedures; the gene contained the 2,100-base-pair (bp) NcoI-PstI fragment of the human β -globin (43) gene fused by blunt-end ligation to the 5' untranslated leader region of the $hsp89\alpha$ gene (see Fig. 4). The fusion was made at the filled-in AvaII site located 40 bp 3' from the *cap* site of the $hsp89\alpha$ gene. The fusion gene contained the heat shock control element (HSE) and approximately 1,700 bp of additional upstream sequences. The globin gene fragment provided the normal globin initiation codon in the filled NcoI site and also contained the entire



FIG. 1. The genes encoding both forms of human hsp89 are each present in multiple copies. Human DNA was digested with *Bam*HI (lanes 3 and 4) or *PstI* (lanes 5 and 6). Ten micrograms of each digest was resolved by electrophoresis on a 1% agarose gel and analyzed by Southern blotting. Lane 1, *Hind*III-digested lambda DNA. Lane 2 contains an amount of linearized pHS801 (*hsp89a*) plasmid DNA equivalent to a single gene copy per 10 μ g of DNA. Lanes 2, 3, and 5 show hybridization with the *hsp89a*-specific probe pHS801. Arrowheads indicate the sizes of fragments expected from the gene cloned in lambda 86. Lanes 4 and 6 have been hybridized with the *hsp89* β -specific probe pHS811.

remaining 3'-transcribed sequences as well as about 500 bp of the 3'-flanking sequences. HeLa cells were transfected with CsCl-purified supercoiled plasmid containing the chimeric gene by the calcium phosphate method (24). The cells were put into surface culture at 2×10^6 cells per 75-cm² flask in Eagle minimal essential medium with 10% fetal calf serum and were fed again after 16 h of culture. Transfection was carried out by incubating each flask with a precipitate containing 20 µg of the plasmid carrying the chimeric gene and 20 µg of salmon sperm DNA for 16 h. Cells were washed and fed with complete medium as described above and cultured at 37°C. After 30 h, cells were heat shocked at 42°C for 2 h and returned to 37°C for 1 h of recovery. Total cellular RNA was isolated by guanidinium isothiocyanate extraction (49). Samples of RNA (10 μ g) were analyzed by quantitative primer extension as described above, with an end-labeled synthetic oligonucleotide specific for the globin mRNA sequence (CAGACTTCTCCTCAGGAGCT).

RESULTS

hsp89α and *hsp89β* mRNAs are encoded by different gene families. The plasmids pHS801 and pHS811 contain partial cDNA inserts that do not hybridize with each other under standard conditions and specifically identify *hsp89α* and *hsp89β* mRNA, respectively (28). A comparison of the hybridization pattern in Southern blot experiments with digests of genomic DNA shows that each probe hybridized to an entirely different set of restriction fragments (Fig. 1). In addition to fragments corresponding to the *hsp89α* gene subsequently characterized in this report (Fig. 1, arrowheads), there were other hybridizing sequences corresponding to about two to four additional genes or pseudogenes in the human genome. The fragments hybridizing to the *hsp89β* probe indicated that there are also a similar number of copies of this gene sequence.

Isolation and characterization of a human $hsp89\alpha$ gene. A human genomic library in Charon 4A (44) was screened by hybridization with the $hsp89\alpha$ -specific cDNA probe pHS801.



FIG. 2. The structural map of the human hsp89 gene cloned in phage lambda 86. The restriction map of the genomic fragment cloned in lambda 86 is shown at the top. The expanded section below shows the structure of the region that was sequenced. Exons (\blacksquare) and the location of the 5' consensus heat control element (\square) are indicated. The arrow marks the transcription start site. The positions of the initiator ATG and the polyadenylation site are also shown. The broken line above the 3' region indicates the extent of the overlapping cDNA clones that were sequenced to delineate the boundaries of intervening sequences. Indicated under the expanded map are the S1 probes used to define other exons. The precise size and location of the 5' exon was determined by direct dideoxy sequencing of the mRNA with a synthetic oligonucleotide primer complementary to the coding region at the beginning of exon 2. Restriction sites are abbreviated as follows: A, AccI; B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; Ps, PstI; Pv, PvuII; X, XhoI; Xb, Xbal.

Seven positive phage isolates were further analyzed by restriction digestion and Southern blot hybridization with the same probe. Two phage showing the strongest hybridization signals were analyzed by restriction mapping. The two phage were found to contain overlapping segments of the same genomic region (data not shown). One phage, lambda 86, was selected for further structural and nucleotide sequence analysis as described in Materials and Methods. The structural map of lambda 86 is shown in Fig. 2. The boundaries of the region that hybridized with mRNA sequences that increase in abundance after heat shock were localized by differential hybridization. Appropriate restriction fragments were subcloned into M13 and phagemid vectors, and the region shown expanded below the map of the phage in Fig. 2 was analyzed by S1 nuclease hybridization procedures with the indicated uniformly labeled and end-labeled probes. The complete nucleotide sequence of this 7,394-bp segment was also determined. The gene encoded a 5,998-bp primary transcript which contained 10 intervening sequences. In order to precisely localize each exon within the 3' region of the gene, the sequences of three overlapping cDNA clones isolated independently from heatshocked (28) and control (65) HeLa cell cDNA libraries were also determined. All three cDNA inserts showed complete sequence identity with the cloned $hsp89\alpha$ gene and permitted the identification of the exons covered by the cDNA regions shown in Fig. 2. We were unable to identify the transcription start site and the 3' boundary of the small first exon by S1

-836 Basticists conceptions and there are areas and the second and the second and the constants areas and the constants areas areas and the constants areas CECCOECAES AASSCECESS SECESSES CECESSES CECESSEARCE SCECALEORT ALECECECES COECESSES SECTESSEAR SETTIMAR AGACTUCES -728 AAAAAAABAACBOO BOBOBCHEBE CEBEBOOCBHE BOTATATAAB BOABBOBCES SEELEBOBOE CAGTTOCTT CAGCOTCCCCG GTOTGGCTOT GCCGTTGGTC CTOTGGCGGTC -616 ACTIAGCAA Getencest settetses scotetses stasantess sanossent sessesses secosses seasedtets sesses - 506 cogggoggec gogetteta tteoggagge ctogggacog etgoggttte egeacoocge -396 aagcagccag ggccgcgctc ttoasgaats saccassoss coscassoto cccgaggete tgeageageg coagagetgs egecgeaege gaacagageg geccegeege gggteeeers cceggeeees etgegggege aggregagga ggecgeegtg -286 sossagoogo agooogogog ssagsgoaco oggegetetto stigeggaco soggoggege actgegooos ssotgogege soctooogga agogogoaca ogotogtget -176 agtigoogog stoogaaatg aggtoatoot tigtoagoog sostistatt ttogettigs tigageticg saagtotoca attogestit agggagatetti - 66 ATG CCT GAG GAA ACC CAG ACC CAA GAC CAA CCG ATG Met Fro Glu Glu Thr Gln Thr Gln Asp Gln Fro Met stresstoto stattacoto tatagacato otsosanatt ttasacorgo gogatatogt tocan 36 GAG GAG GAG GAG GAT GAG ACG TTC GCC TTT CAG GCA GAA ATT GCC CAG TTG ATG TCA TTG ATC ATC AAT ACT TTC TAC TCG AAC AAA GAG ATC TTT Glu Glu Glu Glu Glu Val Glu Thr Phe Ala Phe Gln Ala Glu Ile Ala Gln Leu Met Ser Leu Ile Ile Asn Thr Phe Tyr Ser Asn Lys Glu Ile Phe 132 CTG AGA GAG CTC ATT TCA AAT TCA TCA GAT gtaagt cacttattaa cccagaatog gatttiggtt tcagtgtgaa cttottgggg gtgctgtatg 228 Leu Arg Glu Leu Ile Ser Asn Ser Ser Asp cttaaattaa tatttttgt taacag GCA THG GAC AAA ATC CGG FAT GAA AGC THG ACA GAT CCC AGT AAA THA GAC TCT GGG AAA GAG CHG CAT ATT Ala Leu Asp Lys Ile Arg Tyr Glu Ser Leu Thr Asp Pro Ser Lys Leu Asp Ser Gly Lys Glu Leu His Ile 326 AAC CTT AIA CCG AAC AAA GAT CGA ACT CTC ACT ATT GTG GAT ACT GGA ATT GGA ATG ACC AAG GCT GAC TTG ATC AAT AAC CTT GGT ACT ATC Asn Leu Ile Pro Asn Lys Gin Asp Arg Thr Leu Thr Ile Val Asp Thr Gly Ile Gly Met Thr Lys Als Asp Leu Ile Asn Asn Leu Gly Thr Ile 422 GCC AAG TCT GGG ACC AAA GCG TTC ATG GAA GCT TTG CAG GCT GGT GCA GAT ATC TCT ATG ATT GGC CAG TTC GGT GTT GGT TTT TAT TCT GCT TAT Ale Lys Ser Gly Thr Lys Ale Phe Met Glu Ale Leu Gln Ale Gly Ale Asp Ile Ser Met Ile Gly Gln Phe Gly Vel Gly Phe Tyr Ser Ale Tyr 518 TTG GTT GCT GAG AAA GTA ACT GTG ATC ACC AAA CAT AAC GAT GAG GAG CAG TAC GCT TGG GAG TCC TCA GCA GGG GGA TCA TTC ACA GTG AGG ACA Leu Val Ala Glu Lys Val Thr Val Ile Thr Lys His Asn Asp Asp Glu Gln Tyr Ala Trp Glu Ser Ser Ala Gly Gly Ser Fhe Thr Val Arg Thr 614 GAC ACA G gtaggence tganeatten etgettangt gagegstegen aggetgeget getgenneet tegenteet geggatgege tgangettae aganttigtg Asp Thr 720 tttgeccase gatcaggaac angetagage acttaattag tgtaatggea tgacttggtg ggeggteett ttgggegagg cacettgeee anaaggttet getgtggtgg 830 cottigeate integration generalized the second activities and states at a second and the second at th 940 tctcttgcag GT GAA CCT ATG GGT CGT GGA ACA AAA GTT ATC CTA CAC CTG AAA GAA GAC CAA ACT GAG TAC TTG GAG GAA CGA AGA AGA AAA GTG GAG Gly Glu Pro Het Gly Arg Gly Thr Lys Val Ile Leu His Leu Lys Glu Asp Gln Thr Glu Tyr Leu Glu Glu Arg Arg Ile Lys Glu 1036 ATT GTG AAG AAA CAT TCT CAG TTT ATT GGA TAT CCC ATT ACT CTT TTT gtaagttt ttatgtaatt gcagagtgaa tttctgtctg taggtgattg Ile Val Lys Lys His Ser Gin Phe Ile Gly Tyr Pro Ile Thr Leu Phe 1132 gggtgatigt actacateet tagteettag atetgttean etagtetgan geetggggan eetaagetet actacetane tetgtanaag tteeteggge tattangtgg 1242 atgtatcama tattgatgam magctgggtg cttctcmagt tggctmattt mgacttgggc cttmggttgm ammtagtggt gcmgcgctgt cgcttagttc tggmgtgcmg 1352 Castsatsts attestst teettesaa s GTG GAG AAG GAA CGT GAT AAA GAA GTA AGC GAT GAT GAG GCT GAA GAA AAG GAA GAC AAA GAA GAA 1449 Val Glu Lys Glu Arg Asp Lys Glu Val Ser Asp Asp Glu Ala Glu Glu Lys Glu Asp Lys Glu Glu 1545 AAG AAG AAG AAG ATT AAG GAA AAG TAC ATC GAT CAA GAA GAG GTC AAC AAA ACA AAG CCC ATC TGG ACC AGA AAT CCC GAC GAT ATT ACT AAT GAG Lys Lys Lys Lys Ile Lys Glu Lys Tyr Ile Asp Glu Glu Glu Leu Asn Lys Thr Lys Pro Ile Trp Thr Arg Asn Pro Asp Asp Ile Thr Asn Glu 1641 GAG TAC GGA GAA TTC TAT AAG AGC TTG ACC AAT GAC TGG GAA GAT CAC TTG GCA GTG AAG g tgagtgactg atgggtgott caagcttgtc cttagattat Glu Tyr Gly Glu Phe Tyr Lys Ser Leu Thr Asn Asp Trp Glu Asp His Leu Ala Val Lys 1742 catctttctc caccacccca aatatcttct ataatgcatt gttcattggt acttagtgta tctgttttat tacag CAT TIT TCA GTI GAA GGA CAG TIG GAA TTC His Phe Ser Val Glu Gly Gln Leu Glu Phe 1847 AGA GCC CTT CTA TTT GTC CCA CGA CGT GCT CCT TTT GAI CTG TTT GAA AAC AGA AAG AAA AAG AAC AAC ATC AAA TTG TAI GTA CGC AGA GTT TTC Arg Ala Leu Leu Fne Val Pro Arg Arg Ala Pro Phe Asp Leu Phe Glu Asn Arg Lys Lys Asn Asn Ile Lys Leu Tyr Val Arg Arg Val Phe 1943 ATC ATG GAT AAC TGT GAG GAG CTA ATC CCT GAA TAT CTG A gtaagtata gataggaasa atastcactg tcactgatta aagaagtact ttctgggtgg Ile Met Asp Asm Cys Glu Glu Leu Ile Pro Glu Tyr Leu 2042 gcatggtggt tracasectat astoctages ettigggagg cogggtggg cagateactt gaggtcagga gttcaagaec agectgggea acatggtgaa accoratete 2152 tectesette casesetter occostate tercarecte areceteses acaecttese occoccore arecerent tecestest grantigor ocactecott 2262 ccagoctagag cascagagta agactatoto assasatacat cccatoatot toagattato titastitia agaatttaca tagtaccagt titatotita gaatgactca 2372 gtgesttigg titstatitt titleg AC TTC ATT AGA GGG GTG GTA GAC TCG GAG GAT CTC CCT CTA AAC ATA TCC CGT GAG ATG TTG CAA CAA AGC Asp Phe Ile Arg Gly Val Val Asp Ser Glu Asp Leu Pro Leu Asn Ile Ser Arg Glu Met Leu Gin Gin Ser 2469 AAA ATT TTG AAA GTT ATC AGG AAG AAT TTG GTC AAA AAA TGC TTA GAA CTC TTI ACT GAA CTG GCG GAA GAT AAA GAG AAC TAC AAG AAA TTC TAT Lys Ile Leu Lys Vel Ile Arg Lys Asn Leu Vel Lys Lys Cys Leu Glu Leu Phe Thr Glu Leu Ale Glu Asp Lys Glu Asn Tyr Lys Lys Phe Tyr 2565

FIG. 3. Nucleotide sequence of the human $hsp89\alpha$ gene and deduced amino acid sequence of the polypeptide. The gene contains consensus heat shock control elements (Pelham consensus [56] is in upper case, underlined, and marked by stars; Xiao and Lis consensus [83] is marked with circles below the sequence), and a typical TATA (underlined, upper case). Exons are also shown in upper case; the introns and untranscribed sequences are shown in lower case. The 3' polyadenylation signal is underlined, and the polyadenylation site is indicated with an underline and a star above. Numbering of nucleotides is from +1 at the ATG.

nuclease methods. This information was obtained by directly sequencing the RNA with a synthetic oligonucleotide primer which had a sequence that terminated at the putative start codon of the protein-coding region. The fact that we were able to obtain a clear sequence with unfractionated $poly(A)^+$ RNA and the sequence identity with three different cDNA

clones strongly suggest that the gene cloned in lambda 86 produces the major $hsp89\alpha$ transcript in HeLa cells.

Sequence and organization of the hsp89 α gene. The nucleotide sequence of the hsp89 α gene and surrounding regions and the deduced amino acid sequence of the protein is shown in Fig. 3. The A of the translation start codon is designated

Glu Gin Phe	Ser Lys Asn	Ile Lys			LICCARL LES	CCLCLLL AGE	tittet tit	tititt aat	itcagaaa gtci	tttteee 266	59
gee catactt	tgtttcag C L	IT GGA ATA C. Bu Gly Ile H	AC GAA GAC TO is Glu Asp S	CT CAA AAT C er Gin Asn A	GG AAG AAG C rg Lys Lys L	TT TCA GAG C eu Ser Glu L	TG TTA AGG T ou Lou Arg T	AC IAC ACA I yr Tyr Thr S	ICT GCC TCT GG Mer Ala Ser Gi	FT GAT GAG 276 Ly Asp Glu	38
ATG GTT TCT Met Val Ser	CTC AAG GAC Lou Lys Asp	TAC TGC ACC Tyr Cys Thr	AGA ATG AAG Arg Met Lys	GAG AAC CAG Glu Asn Gln	AAA CAT ATC Lys His Ile	TAT TAT ATC Tyr Tyr Ile	ACA G sta Thr	aga gaacact	atg ttacagto	eat 286	31
acacgtogtt	cttacaacct	tstassctct	stessistet	tttctactca	sstageacts	ttacaactgg	tattgatcta	sscaasataa	ttescatges	ctaggtcatt 297	11
ttctgtctta	ssttctscct	aggtatotgg	ctagcaagaa	aagtoagago	tagatgaaac	cattettaac	tgtteeeegg	tctesegte	acttigtaat	acctcag 307	18
GT GAG ACC Gly Glu Thr	AAG GAC CAG Lys Asp Gln	GTA GCT AAC Val Ala Asn	TCA GCC TTT Ser Ala Phe	GTG GAA CGT Val Glu Arg	CTT CGG AAA Leu Arg Lys	CAT GGC TTA His Gly Lou	GAA GTG ATC Glu Val Ile	TAT ATG ATT Tyr Met Ile	GAG CCC ATT Glu Pro Ile	GAT GAG 317 Asp Glu	/3
TAC TGT GTC Tyr Cys Val	CAA CAG CTG Gin Gin Leu	AAG GAA TIT Lys Glu Phe	GAG GGG AAG Glu Gly Lys	ACT TTA GTG Thr Lou Val	TCA GTC ACC Ser Val Thr	AAA GAA GGC Lys Glu Gly	CTG GAA CTT Leu Glu Leu	CCA GAG GAT Pro Glu Asp	GAA GAA GAG Glu Glu Glu	AAA AAG 326 Lys Lys	J9
AAG CAG GAA Lys Gln Glu	GAG AAA AAA Glu Lys Lys	ACA AAG TIT Thr Lys Phe	GAG AAC CTC Glu Asn Leu	TGC AAA ATC Cys Lys Ile	ATG AAA GAC Met Lys Asp	ATA TTG GAG Ile Leu Glu	AAA AAA GTT Lys Lys Val	GAA AAG gt Glu Lys	at gtgaataca	16 336	11
catttcctga	tcattgatac	ttctaaggtg	ctttcaagct	tagtcataca	tagcccattt	tcgcatgttt	tcaacttaaa	acagaaaact	atstoststs	tssctssscs 347	11
csstssctca	cgcctgcaat	cccagcactt	tessassets		tcacaaggtc	aggagatoga	gaccatcotg	sciascacss	tgaaactcag	tototactaa 358	11
aaatagaaaa	aaataaacca	ssestsstes	cacggootgt	aatoctagoo	acttessass	CTEASECASE	agaatcgcct	SAACCCASSA	sscssaggtt	scastgages 369	11
aagatogoac	cactgcactc	cascotasst	gatssascsa	gactctatct		attgtgcatg	tessecatge	aattataacc	tgtgctcttt	ggatacctaa 380)1
tgcgacattt	aagttgtatt	tgacagtaga	tagtattttg	gatctattga	aatttesstt	ctacagattt	catttcacaa	tgaaagttta	ggattastct	ttctaggttc 391	11
ctagtcatca	ctttt gga t	tacag GTG C Val V	FTT GTG TCA / Val Val Ser /	AC CGA TTG (Asn Arg Leu)	STG ACA TCT (Val Thr Ser)	CCA TGC TGT / Pro Cys Cys 1	ATT GTC ACA . 11e Val Thr	AGC ACA TAT (Ser Thr Tyr (GGC TGG ACA G Gly Trp Thr A	CA AAC 400	8(
ATG GAG AGA Met Glu Arg	ATC ATG AAA Ile Met Lys	GCT CAA GCC Ala Gln Ala	CTA AGA GAC Leu Arg Asp	AAC TCA ACA Asn Ser Thr	ATG GGT TAC Met Gly Tyr	ATG GCA GCA Met Ala Ala	AAG AAA CAC Lys Lys His	CTG GAG ATA Leu Glu Ile	AAC CCT GAC Asn Pro Asp	CAT TCC 410 His Ser)4
ATT ATT GAG Ile Ile Glu	ACC TTA AGG Thr Lou Arg	CAA AAG GCA Gln Lys Ala	GAG GCT GAT Glu Ala Asp	AAG AAC GAC Lys Asn Asp	AAG TCT GTG Lys Ser Val	AAG GAT CTG Lys Asp Leu	GTC ATC TTG Val Ile Leu	CTT TAT GAA Leu Tyr Glu	ACT GCG CTC Thr Ala Lou	CTG TCT 420 Leu Ser)0
TCT GGC TTC Ser Gly Phe	AGT CTG GAA Ser Leu Glu	GAT CCC CAG Asp Pro Gin	ACA CAT GCT Thr His Ala	AAC AGG ATC Asn Arg Ile	TAC AGG ATG Tyr Arg Met	ATC AMA CTT Ile Lys Leu	GGT CTG G (Gly Leu	staascett a	tectetgte et	gttaaaa 429)9
gaaaataaac	acacgtgaca	ttgaagaaaa	tgggtasact	ttoagttato	casacttess	scaccttstc	tgettgetge	ttggaggtat	teestatgt	ttttttagg 440	9
gaaaataaac gataagtaag	acacgigaca gictiacaag	ttgaagaaaa agcaaagaaa	tgggtasact tgasettgag	ttoagttato actoatatgt	caaacttgga cctgtaatac	gcaccttgtc tgtcttgaaa	tgettgetge gengntagnn	ttggaggtat accaagagta	teesstatst tteccctest	ttttttagg 440 agctggettt 451)9 .9
gatagtag gatagtag aggasstett	acacgigaca gictiacaag igiaataiga	ttgaagaaaa agcaaagaaa ggatttatt	tgggtaaact tgaaattgag ttggaaacag	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp	casactigga ccigtasiac GAA GAI GAC Glu Asp Asp	gcacctigic tgictigaaa CCT ACT GCT Pro Thr Ala	tgottgotgo gcagatagaa GAT GAT ACC Asp Asp Thr	ttssasstat accaasasta AGT GCT GCT Ser Ala Ala	teaagtaigt ttaccctaat GTA ACT GAA Val Thr Glu	tttttttagg 440 agctggcttt 451 GAA ATG 461 Glu Met)9 19 18
gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou	acacgigaca giotiacaag igiaataiga GAA GGA GAT Glu Gly Asp	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Asp Asp Thr	tgggtasact tgasattgag ttggasacag TCA COC ATG Ber Arg Het	ttoagttato actoatatgt GT ATT GAT GLy Ile Asp GAA GAA GTA Glu Glu Val	casacttgga cctgtaatac GAA GAT GAC Glu Asp Asp GAC <u>TAA</u> TCTC Asp *	gcacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGG	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr SATGACT TAC	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTTCA GTA	tasagtatgt ttaccctaat GTA ACT GAA Val Thr Glu CTCTACA ATTC	ttttttagg 440 agctggcttt 451 GAA ATG 481 Glu Met XTCTGA 471)9 19 18 19
gataagtaag aagaaatott CCA CCC CTT Pro Pro Leu TAATATATTT	acacgtgaca gtottacaag tgtaatatga GAA GGA GAT Glu Gly Asp TCAAGGATGT	ttgaagaaa agcaaagaaa ggatttatt GAC GAC ACA Asp Asp Thr TITICTITAT	tgssettgsg tgssettgsg ttgssecog TCA COC ATG Ber Arg Het TITTGTTAAT	ttoagttato actoatatgt GT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAGT	casactigga cotgtaatac GAA GAT GAC Glu Asp Asp GAC <u>TAA</u> TCTC Asp * CTGTATGGCA	gcacctigte tgtctigaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGG TGACAACTAC	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr BATGACT TAC TITAAGGGGA	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTTCA GTA AGATAAGATT	taaagtafgt ttaccotaat GTA ACT GAA Val Thr Glu CTCTACA ATTC TCTGTCTACT	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Het 451 XTCTGA 471 AAGTGATGCT 482)9 19 18 19
gaaaataaac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT	acacgtgaca gtottacaag tgtaatatga GAA GGA GAT Glu Gly Asp TCAAGGAIGT AGGCACTAAA	ttgaagaaa agcaaagaaa ggatttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG	tgsstaact tgaaattgag ttggaaacag TCA CGC ATG Ber Arg Met TITTGTTAAT TAATGCTITT	ttoagttato actoatatgt GT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTTCAT	casactigga cotgtastac GAA GAT GAC Glu Asp Asp GAC IAA TCTC Asp " CTGTATGGCA GTTGGTTTAT	gcacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGGG TGACAACTAC TTTCACAGAT	tgettgetge gengatagan GAT GAT ACC Asp Asp Thr SATGACT TAC TITAAGGGGA TGGGGTAACG	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTTCA GTA AGATAAGATT TGCACTGTAA	taaagtafgt ttaccotaat GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA	tttttttags 440 agctggettt 451 GAA ATG 461 Glu Met XTCTGA 471 AAGTGATGCT 482 ACATGATGTT 493)9 19 18 19 19
gaaaataaac gataagtaag aagaaatott OCA COC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTTIGTGG	acaegtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGGCACTAAA TCTAAAGTGT	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Aap Aap Thr TITICTITAT GCAGAGCTAG TTAGCTGTCA	tggstasect tgassttgag ttggssscag TCA CGC ATG Ser Arg Met TITIGITAAT TAATGCTITI AGCCGGATGC	ttoagttato actostatgt of ATT GAT Gly 11. Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTTCAT CTAAGTAGAC	caaacttgga ectgtaatac GAA GAT GAC GIu Aap Aap GAC <u>TAA</u> TCTC Aap * CTGTATGGCA GTTGGTTTAT CAAATCTTGT	gcacettgte tgtettgaaa OCT ACT GCT Pro Thr Ala CTGGCTG AGG TGACAACTAC TTTCACAGAT TATTGAAGTG	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr MIGACT TAC TITAAGGGGA TGGGGTAACG TICTGAGCTG	ttegaggtat accaegagta AGT GCT GCT Ser Ale Ale CTGTTCA GTA AGATAAGATT TGCACTGTAA TATCTTGATG	tasagtafgt ttaccotaat GTA ACT GAA Val Thr Glu CTCTACA ATTC TCTGTCTACT GACGTATGTA TTTAGAAAAG	tttttttags 440 agetggettt 451 GAA ATG 481 Glu Not 471 AAGTGATGCT 482 ACATGATGTT 493 TATTCGTTAC 504)9 19 18 19 29 39
дааассаас дааассаас аадааасса ССА ССС СТТ Рто Рто Leu ТААТАТАТТТ GTGATACCTT ААСТТТОТОG АТСТТОТАGO	acaegtgaca gtottaesaag tgtaatatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGGCACTAAA TCTAAAGTGT ATCTACTITT	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG TTAGCTGTCA CGAACTITIC	tggstasect tgaasttggg ttggssscag TCA CGC ATG Ser Arg Het TITIGTTAAT TAATGCTTTT AGCCGGATGC ATTCCCTGTA	ttoagttato actoatatgt of ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTTCAT CTAAGTAGAC GTTGACAATT	caaacttgga cctgtaatac GAA GAT GAC GIU AAP AAP GAC TAA TCTK AAP * CTGTATGGCA GTTGGTITAT CAAATCTTGT CTGCATGTAC	gcaccttgtc tgtcttgaaa CCT ACT GCT Pro Thr Ala CTOGCTG AGOO TGACAACTAC TITCACAGAT TATTGAAGTG TAGTOCTCTA	tgettgetge geagstagas GAT GAT ACC AAP APP Thr BATGACT TAC TITTAAGGGGA TGGGGTAACG TICTGAGCTG GAAATAGGTT	ttggaggtat accasgagta AGT GCT GCT Ser Ala Ala CTGTTCA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC	teanagtaigt ttaccoteat GTA ACT GAA Val Thr Glu CTCTACA ATTC TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG	tttttttags 440 agetggettt 451 GAA ATG 481 GU Het 481 XTCTGA 471 AAGTGATGCT 482 ACATGATGTT 493 TATTCGTTAC 504 AAGGATCTCT 515	09 19 18 19 29 39 39 39
gaaaattaac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTTIGTAGG CCACAGGGCT	acaegtgaca gtottacaag tgtaatatga GAA GGA GAT Glu Gly Asp TCAAGGATOT AGGCACTAAA TCTAAGTOT ATCTACTTTT TGTTTTCCAA	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG TTAGCTGTCA CGAACTITIC AGAAAAGTAT	tggstasset tggssseag ttggssseag TCA CGC ATG Ber Arg Het TITIGTIAAT TAATGCTITI AGCCGGATGC ATTCCCTGTA TGTTTGGAGG	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAGT TGAGTTTCAT CTAAGTAGAC GTTGACAATT AGCAAAGTTA	CREASECTERS COLUMNIC AND AND COLUMNIC AND AND CAR IAN TOTA AND TOTATOGCA CITOGATTAT CAMATCITOF CITOCATOTAC AMAGCCIACC	geacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGGG TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC	tgettgetge geagatagaa GAT GAT ACC Anp Anp Thr DATGACT TAC TITIAAGGOGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT	ttegesgtat accessegta AGT GCT GCT Ser Als Als CTGTTCA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AMACTGAAGC TCAAAATAA	teanagtaigt ttaccoteat GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA	tttttttags 440 agetggettt 451 GAA ATG 451 GIL HAE 451 CTETGA 471 AAGTGATGET 482 ACATGATGET 493 TATTEGTIAE 504 AAGGATEET 515 GTETIGTGGA 526)9 19 18 19 39 39 39 39
gaaaataaac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTTTGTAGG ATCTTGTAGG CCACAGGGCT TGGAAATGTA	acaegtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATOT AGGCACTAAA TCTAAGTOT ATCTACTTTT TOTTTCCAA GTGCTCGAOT	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Aap Aap Thr TITICTITAT GCAGAGCTAG TTAGCTGICA CGAACTITIC AGAAAAGTAT CACATICTOC	tggstasset tggssettggg ttggsseseg TCA COC ATG Ber Arg Met TITIGTTAAT TAATGCTTTT AGCCOGATGC ATTCCCTGTA TGTTTGGAGG TTAAAGTTGT	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTTCAT CTAAGTAGAAC GTTGACAATT AGCAAAGTAA AACAAATACA	Casacttgga ectgtastac GAA GAT GAC Glu Asp Asp GAC IAA TCTC Asp " CTGTATGGCA GTTGGTITAT CAAATCTTGT CTGCATGTAC AAAGCCTAAC GATGAGTIAA	geacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTOGCTG AGGO TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTOCTCTA TAAGCATATC AAGetattgt	tgettgetge geagstagas GAT GAT ACC Asp Asp Thr MATGACT TAC TITAAGGGGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt	ttegesgtat accasgegta AGT GCT GCT Ser Ale Ale CTGTTCA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AMACTGAAGC TCAAAAATAA cttetttegg	teasegtatgt ttaccoteat GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA EES===EES	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Mat CTCTGA 471 AAGTGATGCT 462 ACATGATGCT 463 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetgga 537	29 19 18 19 29 39 19 59 59 79
gaaaataac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTITOTOG ATCTTGTAGG CCACAGGCT TOGAAATGTA tgecagttag	acaegtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGOCACTAAA TCTAAGTGT ATCTACTITI TGTTTTCCAA GTGCTCGAGT toceasatgt	ttgaagaaaa agcaaagaaa gsatttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG TIAGCTGICA CGAACTITIC AGAAAAGTAT CACATICTOC asascactag	tggstasset tggssstagg ttggssstagg ttggssstagg ttggssstagg ttggssstagg ttggsstagg ttggstagg ttggstagg ttggttgggagg	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTICAT CTAAGTAGAC GTTGACAAGTTA AACAAATACA ggagatggtt	савасttgga есtgtastac GAA GAT GAC Glu Asp Asp GAC IAA TCTA Asp * СТОТАТОССА СТОСАТОТАТ САААТСТТОГ СТОСАТОТАС АААССТАСС GATGAOTTAA вавсесtago	geacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGGG TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC AAGetattgt tgetceaags	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr MIGACT TACC TITTAAGGGGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gttgacatgg	ttegeegstat acceaegegta AGT GCT GCT Ser Ale Ale CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAAATAA cttatttegg tcttccceegc	tesengteigt ttaccoteat GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SES===EESS atgtactcag	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Met 471 AAGTGATGCT 482 ACATGATGCT 483 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetggs 548	29 19 18 19 29 39 39 39 39 39 39
gaaaataac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTITGTGG ATCTTGTAGG CCACAGGGCT TGGAAATGTA tgacagttag gtggagcaca	acaegtgaca gtottaeaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATOT AGGCACTAAA TCTAAAGTOT ATCTAACTITT TOTITTCCAA GTQCTCGAOT toesaaatgt tgtaggeaca	ttgaagaaaa agcaaagaaa gattttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG TTAGCTGTCA CGAACTITIC AGAAAAGTAT CACATICTGC aasaacactag gaasacagga	tggstasset tggssstagg ttggssstagg ttggssstagg ttaggssstagg transcrift AGCCGGATGC ATTCCCTGTA TGTTTGGAGG TTAAAGTTGT gogttaggeca	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTICAT CTAAGTAGAC GTTGACAAGTTA AGCAAAGTACA gsagatggtt catgcatecc	CREASECTERS CONTRACTOR CAA GAT GAC Clu Asp Asp GAC IAA TOTA Asp a CTGTATGGCA GTTGGTTTAT CAAATCTTGT CTGCATGTAC AAAGCCTACC GATGAGTTAA assesses ctgcgtcost	geacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala TGACAACTAC TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC AAgetattgt tgstecaagg gagttacatg	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr NATGACT TACC TITTAAGGOGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgaeagtgt gttgaeatgg tgttetta	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAATAA cttatttagg tcttcccagc gtgtccccgt	teanagtaigt ttaccoteat GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SES===SESS atgtactcag tgttttgatg	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Met 471 AAGTGATGCT 482 ACATGATGCT 483 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetggs 559 ttattecatgs 559	09 19 18 19 29 39 19 39 59 59 79 39
gaaaataac gataagtaag aagaaatott OCA COC CTT Pro Pro Leu TAATATATTT GTGATACCTT AACTITGTGG ATCTTGTAGG CCACAGGCT TGGAAATGTA tgacagttag gtggagcaca aatacottot	acaegtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGGCACTAAA TCTAAGTGT AGTACTTTT TGTTTTCCAA GTGCTCGAGT toccasastgt tgtaggcaca gtgctasata	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Aap Aap Thr TITICTITAT GCAGAGCTAG TIAGCTGTCA CGAACTITIC AGAAAAGTAT CACATICTOC aaaacactag gaaaacagga cagtocotta	tggstasset tggssstag ttggssseag TCA CGC ATG Ber Arg Het TITIGTIAAT TAATGCTITI AGCCGGATGC ATTCCCTGTA TGTTTGGAGG TTAAAGTTGT gegeteagee atgeoggees atteettgge	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu OLU Val ATTAAAAGT TGAGTTTCAT CTAAGTAGAC GTTGACAATT AGCAAAGTTA AGCAAATACA ggagatggtt catgcatccc cttacagtgt	casacttgga octgtaatac GAA GAT GAC Glu Asp Asp GAC IAA TCTA Asp " CTOTATOGCA GTTOGTITAT CAAATCTTOT CTOCATOTAC AAAGCCTACC GATGAGTTAA asacsctago ctgcgtocat otosaagtto	scacettgte tgeettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGGG TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCCTCTA TAAGCCATATC AAGetattgt tgetceaags sagttacatg tttacaaatc	tgettgetge geagatagaa GAT GAT ACC Anp Anp Thr DATGACT TACC TITIAAGGOGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gttgacatgg tgttetetta tacttaaage	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AMACTGAAGC TCAAAATAA cttatttagg tcttcccagc gtgtccacgt catcctggct	tessegtatgt ttaccotest GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SESSSESS atgtactcag tgttttgatg gasscagsag	tttttttagg 440 agetggettt 451 GAA ATG 451 GIL HAE 451 CTCTGA 471 AAGTGATGCT 482 ACATGATGTT 493 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetgg 537 caggtgtggg 548 ttatteatgg 559 aategettga 570)9 19 18 19 29 39 39 39 39 39 39 39 39
gaaaataac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTITGTAGG ATCTTGTAGG CCACAGGGCT TGGAAATGTA tgacagttag gtggagcaca aatoottot acctggagg	acaegtgaca stottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGGCACTAAA TCTAAGTGT ATCTACTTTT TGTTTTCCAA GTGCTCGAGT toceasaatgt tgtaggoaca stgctagattgo	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Aap Aap Thr TITICTITAT GCAGAGCTAG CGAGAGCTAG CGAACTITIC AGAAAAGTAT CACATICTOC aasaacactag gaaaacagga cagtoacta	tggstasset tggssstggg ttggsssegg ttggsssegg ttggsssegg ber Arg Het TITIGTIAAT TAANGCTITI AGCCGGATGC ATTCCCTGTA TGTTTGGAGG TTAAAGTTGT gegetesgs atgesgsess atgesgsess atgestgeses	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTITCAT CTAAGTAGAC GTTGACAATT AGCAAAGTTA AACAAATACA gsagatggt ttgoactgoa ttgoactgoa	casacttgga octgtaatao GAA GAT GAC Glu Asp Asp GAC IAA TCTC Asp " CTOTATOGCA GTTGGTTTAT CAAATCTTGT CTGCATGTAC AAAGCCTACC GATGAGTTAA assocatago ctgogtocat otocaggtoog	scacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGGO TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC AAGetattgt tgetecaags sagttacatg ttacaagetgaa	tgettgetge geagstagas GAT GAT ACC Asp Asp Thr BATGACT TAC TITAAGGOGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gttgacatgg tgtteetta tgtteetta tacttaage actocatete	ttggaggtat accaagagta AGT GCT GCT Ser Als Als CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAATAA cttatttagg tcttcccagc gtgtccacgt catcetggct assassaga	tasagtatgt ttaccotaat GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SSS==\$SSS atgtactcag tgttttgatg gaggcaggag tgaaaaatc	tttttttags 440 agetggettt 451 GAA ATG 451 GU Met 451 CTCTGA 471 AAGTGATGCT 482 ACATGATGCT 493 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetgg 537 caggtgtggs 548 ttattcatgg 559 aatgeetgg 551	09 19 18 19 29 39 59 59 59 59 59 59 59 59 59 59 59 59 59
gaaaataac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTTTOTAGG ATCTTOTAGG CCACAGGCT TOGAAATGTA tgacagttag gtggagcaca aatacottot acctgggags	acaegtgaca gtottaeeaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATOT AGGCACTAAA TCTAAGGTOT AICTACTITI TOITITICAA GTGCTCGAGT toesaaatgt tgtaggeaeaa gggtasaata cggaggttgo ototgggact	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG CGAGAGCTAG CGAACTGTCA CGAACTGTCA CGAACTGTCC aasaccactag gaaaacagta cagtoactta sgtsssctag gassstaga	tggstasset tggssstag ttggsssong TCA COC ATG Ber Arg Met TITIGTTAAT TAATGCITIT AGCCOGATGC AITCCCTGTA TGTTTGGAGG TIAAAGTIGT gogotoagac atgoagacaa attoottggo gattgoacaa atgotsaat	ttoagttato actoatatgt of ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTITCAT CTAAGTAGAC GTTGACAATT AGCAAAGTTA AGCAAAGTAA ggagatggtt catgoatcoc cttacagtgt ttgcactgca acagtotto	casacttgga ectgtaatac GAA GAT GAC Glu Asp Asp GAC IAA TCTC Asp ** CTGTATGGCA GTTGGTTTAT CAAATCTTGT CTGCATGTAC AAAGCCTACC GATGAGTTAA assesstagc ctgsgtcost ctcssgtco gootgagoag acttotgass	geacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTOGCTG AGGO TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTOCTCTA TAAGCATATC AAGetattgt tgetecaags gagttacatg tttacaaatc caagagegaa tttagtagtt	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr MIGACT TACC TITAAGGGGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gttgacatgg tgttgetetta tacttaaagc actocatete ctagagacaa	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTTCA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAATAA cttatttagg tcttcccagc gtgtccacgt catcetggct assessaga ascttggtta	teasagtaigt ttaccoteat GTA ACT GAA Val Thr Glu CTCTACA ATTO ACTTGATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SEEssagess atgtactcag tgttttgatg Sagscagsag tgaasaaatc gatagcatat	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Mat 451 CTETGA 471 AAGTGATGET 482 ACATGATGET 483 TATTEGTTAC 504 AAGGATETET 515 GTETTGTGGA 526 agtatetgga 537 caggtggggs 548 ttatteatgg 559 astegettga 570 aggettgga 581	09 19 18 19 29 39 39 39 39 39 39 39 39 39 39 39 39 39
gaaactaac gatagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTITOTOG ATCTTOTAGG CCACAGGCT TOGAAATOTA tgacagttag gtggagcaca aatacottot acctgggagg gtagagcoc ttotgagoot	acacgtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGOCACTAAA TCTAAGTGT ATCTACTITI IGTITICCAA GTGCTCGAGT tocsasatgt tgtaggoaca gtgotasata cggaggttgo ctotgggact	ttgaagaaaa agcaaagaaa gsatttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAGCTAG GTAGCTGTCA CGAACTITIC AGAAAAGTAT CACATICTGC aaascactag gaasacoagga cagtoactta ggtaggotgg gaasacoagga cagtoactta ggtaggotgg	tggstassot tgssstigg ttgsssong TCA COC ATG Ser Arg Met TITIOTIAAT TAATGCITIT AGCCGGATGC ATTCCCTGTA TGTITIGAAGG TTAAAGTTGT gogtcageca stgoagecas sttoottggo gsttgcacca atggstasst taggsstss	ttoagttato actoatatgt OT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTTICAT CTAAGTAGAC GTTGACAATT AGCAAAGTTA AACAAATACA ggagatggt catgcatccc cttacagtgt ttgcactgca aaagtotto otgggacttg	casacttgga ectgtastac GAA GAT GAC Glu Asp Asp GAC IAA TCTA Asp * TCTA CTGTATGGCA GTTGGTTTAT CAAATCTTGT CTGCATGTAC AAAGCCTACC GATGAGTTAA assoctago ctgcgtcost ctosasgtto gcotgagoag acttctgasa gatstgtgeo	gcaccttgto tgtottgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGG TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC AAGetattgt tgotocaags gagttacatg tttaccasato caagagogaa tttagtggtt agtgtotaag	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr MIGACT TACC TITAAGGOGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gttgacagtg tgttetetta tacttaaage actocatoto ctagagacaa ggggcagtge	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAAATAA cttatttagg tottoccagc gtgtccacgt catcctggot aasaasaga aacttggtta accaggcaggg	teanagtaigt ttaccoteant GTA ACT GAA Val Thr Glu CTCTACA ATTO ACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SES===EEEE atgtactcag tgttttgatg gaggcaggag tgaaaaaatc gatagcatat agagacottg	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Met 451 XTTCTGA 471 AAGTGATGCT 482 ACATGATGCT 483 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetgge 537 cagstgtggs 548 ttatteatgg 559 aategettga 570 ageceaatag 581 aggatttgea 592 teteagtete 603	09 19 18 19 29 39 59 59 59 59 59 59 59 59 59 59 59 59 59
gaaaataac gataagtaag aagaaatott CCA CCC CTT Pro Pro Lou TAATATATIT GTGATACCTT AACTITGTGG ATCTTGTAGG CCACAGGGCT TGGAAATGTA tgacagtag gtggagcaca aatacottot acctgggagg gtaagagtoo ttotgagoct gtgctaagca	acacgtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGGCACTAAA TCTAAGTGT ATCTACTITT TOTITTCCAA GTGCTCGAGT tocsasatgt tgtaggoaca gtgctasata cggaggttgo ctotgggact aacgocatat ggtattgaat	ttgaagaaaa agcaaagaaa gattttatt GAC GAC ACA Aap Aap Thr TITICTITAT GCAGAGCTAG TTAGCTGTCA CGAACTITIC AGAAAAGTAT CACATICTOC aasaacactag gaaaacagga cagtoactta gstsgsctgg aasaacagga gstsgstsgtaa	tggstasset tggssasseg ttggssseeg TCA CGC ATG Ser Arg Met TITIGTTAAT TAATGCTTTT AATGCTTTT AATGCTTAT GCCGGATGC ATTCCCTGTA TGTTTGGAGG TTAAAGTTGT gogstegsee atgosgsees atgosgsees atgosgsees atgosgsees ttggssstge tggssstge	ttoagttato actoatatgt of ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTITCAT CTAAGTAGAC GTTGACAAGTTA AGCAAAGTACA Ssegatggt catgcatccc cttacagtgt ttgcactgca acaagtottc ctgggacttg gtttagtoct	casacttgga octgtastac GAA GAT GAC Glu Asp Asp GAC IAA TOTA Asp a CTGTATGGCA GTTGGTITAT CAAATCTTGT CTGCATGTAC AAAGCCTACC GATGAGTTAA ascactago ctgogtoost otosaagtto gootgagoag acttotgasa gstatgtgao ttasattoto	geacettgte tgtettgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGG TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC AAGetattgt tgeteeag gagttacatg tttacaaato caagagegaa tttagtggtt agtgtetaag tgaaaagtto	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr NIGACT TACC TITIAAGGOGA TGGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacatgg gtgacatgg tggtetetta tacttaaagc actocatete ctagagacaa ggggacaa ggggacaa ggggacaa	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAATAA cttatttagg tcttcccagc gtgtccacgt catcctggct aacttggtta acctggtta	teanagtaigt ttaccotaat GTA ACT GAA Val Thr Glu CTCTACA ATTO ACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SESaaasses atgtattgatg tgttttgatg gatagcatat agagacottg acaggaggaa	tttttttags 440 agetggettt 451 GAA ATG 461 Glu Met 471 AAGTGATGCT 482 ACATGATGCT 483 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetgga 537 caggtgtggs 548 ttatteatgg 559 aategettga 570 ageecaatag 581 aggatttgea 592 teteggtagat 614	09 19 18 19 29 39 59 59 59 59 59 59 59 59 59 59 59 59 59
gaaaataas gaaaatatt CCA CCC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTITGIGG ATCTTGIAGG CCACAGGCT TGGAAATGTA tgacgttag gtgsgcoca satacottot acctgsgags gtasgagtoot ttotgagoot gtgctaagca tgaggtctta	acacgtgaca gtottacaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATGT AGGCACTAAA TCTAAGTGT ATCTACTITT TGTTTTCCAA GTQCTCGAGT tossaasgat tgtaggoaca gtgctasata cggaggttgc ctotgggact gstattgat gtattgatg	ttgaagaaaa agcaaagaaa ggatttatt GAC GAC ACA Asp Asp Thr TITICTITAT GCAGAOCTAG TIAGCTOTCA CGAACTITIC AGAAAAGTAT CGACATICTOC agaaaacagg gaaacaga gaaacaga gaaacaga gaaacaga gaaaacaga gaacaga gaacaga gaacaga gaacaga gaacagaacaga g	tggstasset tggssattggg ttggssattgg ttggssattgg ttggssattg ser Arg Het TITIGTAAT TAATGCTITI AGCCGGATGC ATTCCCTGTA TGTTTGGAGG TTAAAGTTGT gogtcagscas attcottggo gattgoacea atggstsatt taggsattgo tggcastcas tsactoceas	ttoagttato actoatatgt GT ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTITCAT CTAAGTAGAC GTTGACAAGTTA AGCAAAGTACA gsagatggt catgoatoco cttacagtgt ttgoactgoa aaaagtotto otgggacttg gtttagtoct gcccoatttt	casacttgga octgtastac GAA GAT GAC Glu Asp Asp GAC IAA TOTA Asp a CTGTATGGCA GTTGGTTTAT CAAATCTTGT CTGCATGTAC AAAGCCTACC GATGAGTTAA assoctago ctgcgtoost ctossagtto gootgagoag acttotgasa gstatgtgao ttoasattoto attotgotaa	gcaccttgto tgtottgaaa CCT ACT GCT Pro Thr Ala CTGGCTG AGG TGACAACTAC TTTCACAGAT TATTGAAGTG TAGTCCTCTA TAAGCATATC AAGetattgt tgstotaag gagttacatg tttagtagtt agtgtotag tttggtagtt agtgtotag gttttgttgo	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr NIGACT TACC TITIAAGGOGA TGGGGTAACG TICIGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gtgacatgg tgttetetta tacttaaagc actocatete ctagagacaa gsgscagtge ttaateceet gtactgaaag	ttggaggtat accaagagta AGT GCT GCT Ser Als Als CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AAACTGAAGC TCAAAATAA ettatttagg tetteceage gtgtccaegt catcetgget assassaga acttggtta acaggcaggg tcgatastat gtgttagaag	teanagtatigt ttaccoteant GTA ACT GAA Val Thr Glu CTCTACA ATTO TCTGTCTACT GACGTATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA ESSaagses atgtactcag tgttttgatg gasgcagga tgasaaastc gatagcatat agagacctg acaggagga aaaggtttac	tttttttags 440 agetggettt 451 GAA ATG 451 GU Met 451 CTCTGA 471 AAGTGATGCT 482 ACATGATGCT 483 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetggs 548 ttatteatgg 559 aategettgs 548 ttatteatgg 559 aategettge 592 teteagtete 603 ttaegtaaat 614 ggtgttaaac 625	
gaaaataac gataagtaag aagaaatott OCA COC CTT Pro Pro Lou TAATATATTT GTGATACCTT AACTITOTOG ATCTTOTAGG CCACAGOGCT TGGAAATGTA tgacagttag gtggggcaca aatooottot acctgggagtag gtaagagtoo ttotgagoot gtgctaagoa tgaggtotta taggaagtga	acaegtgaca gtottaceaag tgtastatga GAA GGA GAT Glu Gly Asp TCAAGGATOT AGOCACTAAA TCTAAGGATOT ATCTAAGTOT ATCTAAGTOT ATCTAAGTOT ATCTAAGTOT COCAEGAAG GTOCTCGAOT toceasaatgt tgtaggocasat sggaggttgo ototgggact acogocatat ggtattgaat gtagtgocasaa	ttgaagaaaa agcaaagaaa ggattttatt GAC GAC ACA Aap Aap Thr TITICTITAT GCAGAGCTAG CGAGAGCTAG CGAGAGCTAG CGAGACTATIC CGAGACTATIC CACATICTOC aaaacaactag gaaaacaaga gatagaacta ggatagaata astssactga gottggtaaa aatttaaatt gggtaaaaag	tggstassot tggssttgg ttggsssog TCA COC ATG Ber Arg Met TITIGTTAAT TAATGCTTTT AATGCTTTT AATGCTGTA TGTTTGGAGG TTAAAGTTGT gggotoagsos stgostgssos stgostgssos stgotgssos stggotssst taggssstgo tggossos tggossos tggossos stggossos tggossos tggossos tggossos tggossos tggossos tggossos tggossos tggossos tggossos tggossos	ttoagttato actoatatgt of ATT GAT Gly Ile Asp GAA GAA GTA Glu Glu Val ATTAAAAAGT TGAGTITCAT CTAAGTAGAC GTTGACAATT AGCAAAGTTA AGCAAAGTTA AGCAAAGTAC SEAgatggt catgcatccc cttacagtgt ttgcactgca aasagtottc ctgggactgg gtttagtoct gcccatttt tttttcccag	casacttgga octgtaatao GAA GAT GAC Glu Asp Asp GAC IAA TCTC Asp " CTGTATGGCA GTTGGTITAT CAAATCTTGT CTGCATGTAC GATGACTIAC GATGACTIAC GATGACTIAC GATGACTIAC GATGACTIAC GATGACTIAC GATGACTIAC Soctgageag acttotgaga gatatgtgac ttaaattoto attotgotaa gtotagoagg	gcacottgto tgtottgaaa CCT ACT GCT Pro Thr Ala CTOGCTG ACCA TGACAACTAC TTACACAACTAC TTTCACAGAT TATTGAAGTG TAGTOCTCTA TAGGCATATC AAGetattgt tgctccaagg gagttacatg tttacaagetgaa tttggtggtt agtgtotaag tgaaaagtto gtttgttgo agaetattaa	tgettgetge geagatagaa GAT GAT ACC Asp Asp Thr TATGACT TAC TITAAGGGGA GGGGTAACG TICTGAGCTG GAAATAGGTT GTAAAGCTGT gtgacagtgt gttgacatgg tgttetetta taettaage actecatete ctagagacaa gssgeagtge ttaatcocct gatetgaaga	ttggaggtat accaagagta AGT GCT GCT Ser Ala Ala CTGTICA GTA AGATAAGATT TGCACTGTAA TATCTTGATG AMACTGAAGC TCAMAATAA ettatttagg tetteccaage gtgtccacgt catcatggeta aacttggtta acatggetagg tegataatat gtgttagaag ttetttcaat	tasagtatgt ttaccotast GTA ACT GAA Val Thr Glu CTCTACA ATTO ACTTGATGTA TTTAGAAAAG AACTTGATGG CTCAGACCCA SSSsesSSS atgtactcag tgttttgatg gatggcatat agagaccttg acaggaggaa aaggtttac tasasacata	tttttttagg 440 agetggettt 451 GAA ATG 451 GU Mat CTTCTGA 471 AAGTGATGCT 482 ACATGATGCT 483 TATTCGTTAC 504 AAGGATCTCT 515 GTCTTGTGGA 526 agtatetgga 537 caggtgtggg 548 ttatteatgg 559 aategetaga 559 aategetaga 581 aggatttgea 592 teteagtete 603 ttaegtaaat 614 ggtgttaaee 625 aggeettga 636	

FIG. 3-Continued

as +1. Underlined sequences between -740 to -725 in the 5'-flanking region correspond to HSEs. A sequence similar to the HSE described by Pelham (56) is indicated in uppercase letters. Small circles beneath the sequence indicate two perfect and one imperfect repeats of the more simple consensus HSE sequence suggested by Xiao and Lis (83). A single TATA element was found 30 bp downstream from the HSE and 22 bp upstream from the transcription initiation site identified by RNA sequencing. Overlapping the 3' end of the underlined HSE region was the sequence TGGAAAAAG, which is similar to the serum control element (SRE) identified in a human hsp70 gene (82). This sequence was located between the HSE and the TATA in both genes. The $hsp89\alpha$ promoter contained a GGCGGG sequence corresponding to a putative SP1-binding site located at position -814.

The transcribed region contained 11 exons that ranged in size from 58 to 729 bp. All of the introns were flanked by typical splice consensus sequences that followed the GT-AG rule. The 58-bp exon 1 encoded the entire 5'-untranslated mRNA leader and was separated from exon 2 by the longest

H≪ HB M D Y	MP Ma	EET V V A S	QTQC	DQPME - HHC - HHC	EEEN	/ETF/	AF QAE	T AQLI	MSLI	INTF' V	YSNR	EIFL	REL I	A A A A A	ALD	KIRY	ESLT K S	DPSK KQ	ETI	ike L Epd	HINL K Di K D Y K F Ri	L IPN L S L S	KQDR P E TAG PEQK	ALTI TL TL TL TL	VDTG I R S	1 GMTK	ADLI SV E	ENNL:	GTIA	KSGTK	AF H E/	s	ADI V	SM1GQF	GVGFY	LF	D D DR	T V V V Q	150
Hex Hß M D Y	ITI R TS S	(HNI N S	DDEQ	YAWE G V I	SSAG N	GSF1	VRTD A F G F A F TL F	IG-EI 1 - 1 - 1 - 8 - 8 -	PHGR IM I L RI	GTKVI IV ILF	ILHL / YI R F	D D	D L	ERRI V SK K	KE 1 V V V V	nkikih! N I R	R R E V/	SYPI	TLFV YL YL K L Q V	EKE I G T	RDKE E E VE	VSDC I I PIF	DEAE	EKED EKG EKG DEKK K D	-KEEI E E I Egd I KD	EKE-KI DKDD GDKEG K ME EKKD	TDE - DDK	SED#	(PEIE - K - K - K - KL	dvgs i E E dei	DEEEE DD DD DAD	KKDG SGKDI SGKDI K PK	Dikuku (A T V	CKKKIK T T EEVQ	EKYIDI Tel	QEEL D I	NKTKP.	LWTRN	300
H∝ HB M D Y	PD S	DITI S	NEEY Q Q Q Q	GEFY	KSLT IS	NDWE	i dhla n P y	VKHF	SVEG	QLEFI	RALL F I	FVPR I I I I K	T	OLFEN S	RKKI K Q K	KNN I I	(LYVI	RRVF	imidin S S T E	CEEL D SD D A D	IPE)	YLNF W S	IRGN MK VK	/VDSI	DLPL	.NISRE	:MLQ(QSKI N V N	LKVI N M	RKNLV I I L I	TM Li	LFTE S IE A N	T I	dkeny s Qfe	KFYEQ A D SA)FSKI	NIKLGI L L L V V	H	450
Hex HB M D Y	ED	SQN T T N T	RKKL RR RR A AA	ADF AK	RYY1 H H FH NS	Q Q Q STK	GDEMV T T DFC / LT	SLKD SE SE A T	YCTR VS VS VS VS V	MKENI T D Ph	QKHI S S N	4 ¥ I T F	GETKL S E S E S LI	P S A EK	ISAF	VERL V E V	rkhg R R (Ar (Ar)	LEVI FV FV FV FL	YMIE T T Fltc	(P1D	VI AF	QQLK H T	EFE D D YK	GKTL S S Q	VSVTI D1	KEGLEI DFELE	L PED	EEEK S KA	KKQE M M R R	EKKTI SA SA DA IEN	S EPLT	CK IM L L L (AL -	E E S E	EKKVEI D D DN GDQ	INTER CONTRACT OF CONTRACTON OF CONTRACT OF CONTRACTON OF CONTRACT OF CONTRACT OF CONTRACTON OF CONTRACTON OF CONTRACTON OF CONTRACT OF CONTRACT.	NRLV	T SPCC S S D DA AA	R	600
H∝ Hß M D Y	TS G	TYG QF QF	NTAN S S	MER 1	(MKAC)ALRI M	TA S MS	GYMA M M S S	AKKH G Q S T	F S	PDHS P P KSP		L RQK J	EAD - - D - /DEGG	KND AQ	A A A T	DLVI V V TK	F F F F Y	TAL L	SSGI V T	FSLE D D	DPQT S V E TS	HAN S S F S F S	RIYR	MIKLO L S	GLGIDI N	EDOP EV EV EV E	TADE A EE EE MTT ETA	DAQS	PD PD PD Agdai - Taai	IPPLE PSLV PVEEV	GDDD E E DTE -PA	ISRM A A A E-	IEEVD					732

FIG. 4. Comparison of the amino acid sequence of human hsp89 α with that of hsp89 β and hsp90 proteins from other species. The upper sequence (H α) is that of the protein derived from the human *hsp89\alpha* gene. Aligned below it are the human hsp89 β (H β) (61), mouse hsp84 (M) (51), *Drosophila* (D) (6), and *S. cerevisiae* hsp83 (Y) (20) protein sequences. Blank spaces indicate identity with the human hsp89 α sequence, letters indicate amino acid substitutions, and dashes represent gaps introduced to maximize alignment.

intron of the gene (572 bp). The splice junction was unusual in that it was immediately adjacent to the initiator ATG. Excision of the first intron would create the sequence CCAA GATG, which is similar to the CCA/GCCATG sequence determined by Kozak (38) to be important for translation in eucaryotes. The adenosine at position -3 is thought to be particularly important for high translational efficiency (38). Excision of the single intron of the Drosophila hsp82 gene also would bring an adenosine into the -3 position relative to the ATG. An additional feature common to both the human and Drosophila genes is the presence of one perfect and several imperfect Xiao and Lis HSE sequences (83) located within intron 1 (indicated by circles under the sequence in Fig. 3). The 5'-flanking region and the first intron are the only locations within the 7,324-bp fragment containing the human $hsp89\alpha$ gene in which the TTCNNGAA sequence was found.

The rather long 3'-untranslated region of $hsp89\alpha$ mRNA contained 663 nucleotides and was AT rich (63% overall). The 100 nucleotides immediately after the stop codon contained over 70% A+T. There was an atypical polyadenylation signal, AACAAA (underlined in Fig. 3), located 17 nucleotides upstream from the polyadenylation site. The cDNA clone pHS801 was identical in sequence to the 3' end of the gene, including the AACAAA sequence, and terminated with a poly(A) stretch of over 20 nucleotides. This identified the site of polyadenylation indicated in Fig. 3 with a star and an underline.

Human hsp89 α protein sequence. The deduced amino acid sequence contained 732 residues with a predicted unmodified molecular mass of 84,564. As is the case for the other heat shock proteins, the hsp89 sequence has been highly conserved evolutionarily. A comparison of the human hsp89 α and hsp89 β protein sequences with mouse hsp84, *Drosophila* hsp82, and *Saccharomyces cerevisiae* hsp90 is shown in Fig. 4. The amino acid sequence of hsp89 α differs at 99 residues from the hsp89 β sequence. A major difference between the two human proteins is the presence of an additional block of 5 amino acids (QTQDQ), located near the amino terminus of hsp89 α , that was absent in hsp89 β . The human hsp89 β protein differs from mouse hsp84, which also lacks the QTQDQ sequence, at only 22 residues. Most of the substitutions that differentiate the hsp84-hsp89ß proteins are conservative. Thus, the human hsp89ß protein is clearly more similar to the mouse hsp84 sequence that to human hsp89 α . The carboxy-terminal sequence, EEVD, is conserved not only among the hsp89 proteins of several species (6, 20, 51, 61) but is also found at the carboxy terminus of hsp70 (5, 23, 31, 32, 45, 47, 48) and the hsp70 cognate (19, 71). Human hsp 89α protein shows 76% amino acid sequence homology with Drosophila hsp82 and 58% homology with the heat-inducible form of S. cerevisiae hsp90 (20). The three vertebrate hsp89 proteins have 7 to 12 additional amino acid residues at the amino terminus that are absent in the Drosophila and S. cerevisiae proteins. Frequent stretches of interspersed acidic and basic amino acids are characteristic of all the hsp89 proteins. The longest region in hsp89 α falls between residues 222 and 294. Despite the differences in amino acid sequence, hydropathy plots (data not shown) of the human hsp89 α and hsp89 β proteins are virtually identical, which would indicate a high degree of structural similarity.

The $hsp89\alpha$ gene contains a functional heat shock promoter. In order to test whether the gene we isolated encodes a heat-inducible form of hsp89 or a constitutively expressed heat shock cognate protein, we constructed a β-globin fusion gene containing the hsp89 promoter region which is depicted in Fig. 5A. Figure 5B shows the response of this gene to heat stress after transfection into HeLa cells. Transfected and untransfected cells were brought to 42°C for 2 h to initiate heat shock gene transcription and then returned to 37°C for 1 h to allow accumulation of spliced mRNA transcripts (65, 84). RNA was prepared and assayed by quantitative primer extension with a globin mRNA-specific oligonucleotide. Induction of $hsp89\alpha$ mRNA was also measured with the specific oligonucleotide used to determine the 5' leader sequence of the mRNA. In Fig. 5B it can be seen that no transcripts were detected with the globin-specific probe in untransfected cells (lanes 1 and 2). A low level of a transcript giving the expected 88-nucleotide primer extension product was present in cells transfected with the fusion gene at 37°C (lane 3), and the amount of this transcript increased by



FIG. 5. The promoter of the cloned $hsp89\alpha$ gene can drive heat-induced transcription of a globin reporter gene. (A) Structure of the chimeric $hsp89\alpha$ - β -globin gene. Construction of the fusion gene is described in Materials and Methods. Restriction sites are abbreviated as follows: X, Xho1: E, EcoRI: A, AvaII: N. Nco1: P. Pstl. Wavy lines indicate vector sequences: solid lines are the inserted fragments. Transcribed sequences of the $hsp89\alpha$ gene (**1**) and the exons of the globin gene (**2**) are indicated. The expanded map shows the details of the fusion site, the position of the oligonucleotide primer, and the expected primer extension product from the fusion transcript. (B) Total cytoplasmic RNA was isolated from untransfected HeLa cells (lanes 1 and 2) or cells transfected with the $hsp89\alpha$ - β -globin fusion gene shown above (lanes 3 to 6). Cells were incubated at 37°C (lanes 1, 3, and 5) or were heat shocked for 2 h at 42°C and allowed to recover at 37°C for an additional hour (lanes 2, 4, and 6). Samples (3.6 μ g) of RNA were analyzed by primer extension with end-labeled synthetic oligonucleotides complementary to either the globin transcript (lanes 1 to 4), or specific for $hsp89\alpha$ (lanes 5 and 6). Primer extension products were analyzed by electrophoresis on denaturing polyacrylamide gels and autoradiographed. The sizes in nucleotides of end-labeled *Hae*III fragments of ϕ X174 phage DNA (lane M) as well as the primer extension products are indicated.

approximately threefold after heat shock (lane 4). The same RNA preparations from the transfected cells showed similar changes in the level of endogenous $hsp89\alpha$ transcripts (lanes 5 and 6). Thus, the transfected globin fusion gene appears to be regulated in parallel with the natural $hsp89\alpha$ transcript and is expressed at normal temperature but at a higher level in response to heat stress.

Induction of the cloned $hsp89\alpha$ gene by adenovirus infection and serum. Transcription of human $hsp89\alpha$ but not $hsp89\beta$ is directly or indirectly activated in concert with one form of human hsp70 by the adenovirus E1A gene product (70). To determine whether the $hsp89\alpha$ gene that was isolated is responsive to E1A, we assayed RNA preparations from HeLa cells 6 h after infection with wild-type adenovirus or with the E1A-deficient mutant adenovirus dl312 (Fig. 6A). Lanes 1 and 2 show the primer extension products obtained



FIG. 6. Induction of the $hsp89\alpha$ gene transcript by the adenovirus E1A gene product and by serum. (A) Samples of total cytoplasmic RNA were analyzed by quantitative primer extension with the hsp89a-specific oligonucleotide primer as described for Fig. 5. Lanes: 1, 2 µg of RNA from uninfected HeLa cells heat shocked at 42°C for 3 h; 2, 2 µg of RNA from uninfected cells heat shocked at 42°C for 5 h; 3, 5 µg of RNA from cells 6 h after infection with the E1A-deficient adenovirus mutant dl312; 4, 5 µg of RNA from cells 6 h after infection with wild-type adenovirus. Unstressed cells were infected at 37°C. (B) HeLa cells were serum starved and suspended in fresh medium containing 10% fresh serum as described in Materials and Methods. Cytoplasmic RNA was isolated at time zero (lane 6) and at 3-h intervals for a period of 24 h (lanes 7 to 14). Ten micrograms of each RNA sample was analyzed by primer extension as described above. Lane 5 is the primer extension product obtained from 2 µg of RNA from cells incubated for 3 h at 42°C.

from RNA isolated from cells that had been incubated at 42°C for 3 h (lane 1) or 5 h (lane 2) with the $hsp89\alpha$ gene-specific oligonucleotide primer. In lanes 3 and 4, the levels of $hsp89\alpha$ mRNA in parallel cultures infected with wild-type and mutant adenovirus are compared. The level of $hsp89\alpha$ transcript in cells infected with the E1A-deficient adenovirus mutant (lane 3) was typical of that found in uninfected cells. The abundance of the $hsp89\alpha$ transcript was increased three- to fivefold by infection with wild-type adenovirus (lane 4), indicating that the $hsp89\alpha$ gene is responsive to the E1A gene product.

The human *hsp70* gene that is activated by the adenovirus E1A product is also regulated by serum (70, 81). Since the hsp89a promoter region contains a similar SRE-like sequence, we investigated whether this gene is also serum responsive. HeLa cells were serum starved for 48 h in medium containing 0.5% calf serum and then fed with medium containing 10% serum. The level of the $hsp89\alpha$ gene-specific transcripts was then measured by primer extension over the following 24 h (Fig. 6, right panel). Serumstarved cells before refeeding contained a considerably lower amount of hsp89a mRNA than was found in exponentially growing cultures (lane 6). After addition of 10% fresh serum, induction of the $hsp89\alpha$ transcript was evident beginning at hour 6 (lane 8). The mRNA increased in abundance by approximately fourfold through hours 12 to 15 (lanes 10 and 11) and then declined to near the original level. Other experiments (data not shown) in which fresh serum alone was added to depleted cultures showed a similar induction of $hsp89\alpha$. Feeding serum-depleted cells with fresh medium containing 0.5% serum produced less than a 50% increase in the mRNA. Thus, a serum constituent rather than some other component of the medium controlled the level of hsp89a mRNA in unstressed cells.

Serum induction of $hsp89\alpha$ in HeLa cells is not restricted to a specific stage of the cell cycle. When deprived of serum, HeLa cells, like many transformed cell lines (18), arrest cell division at random points within the cell cycle. Parallel experiments were conducted in which cells were either starved for serum as described above or synchronized by a double thymidine block. $hsp89\alpha$ mRNA levels were then

					A									B					
HR	0	3	6	9	12	15	18	21	24	0	3	6	9	12	15	18	21	24	mRNA
		-		•	s. 84		-	-			-	-		-	-	-	-	-	HISTONE H4
	-		-		•		-								-		-	• • • •	HSP 89%

FIG. 7. Induction of the $hsp89\alpha$ gene by serum is not strictly dependent upon the stage of the cell cycle. In parallel, HeLa cell cultures were either synchronized by double thymidine block (A) or starved for serum and refed as described for Fig. 6 (B). Cytoplasmic RNA was prepared from each culture at 3-h intervals starting immediately before suspending the cells in fresh medium, which contained 5% serum in the case of the synchronized culture and 10% serum in the case of the serum-depleted cells. Samples (10 µg) of RNA were analyzed for H4 histone and $hsp89\alpha$ mRNA levels by Northern blot hybridization with nick-translated pF0108A and pHS801 plasmid DNAs, respectively, as probes.

determined by Northern blot analysis with the pHS801 probe. hsp89a mRNA accumulation in synchronized cells released from a double thymidine block (Fig. 7A) was compared with that in asynchronous, serum-stimulated cultures (Fig. 7B). The level of H4 histone mRNA, which is abundant only during S phase (16, 26, 57), was also measured as an indicator of cell synchrony. The cells released from the thymidine block showed a rapid synchronous entry into S phase, as was indicated by accumulation and decline of histone mRNA. The serum-starved cells refed with fresh serum showed little synchrony by this criterion. The temporal pattern and intensity of the increase and decrease of hsp89a mRNA, however, was similar in both cultures and did not appear to be related to the stage of the cell cycle. Since the thymidine block was released by suspending the cells in thymidine-free medium containing fresh serum 16 h after the previous feeding, it is likely that the observed stimulation of hsp89a mRNA accumulation in this experiment was a serum effect.

The induction and slow decline of the $hsp89\alpha$ mRNA level after feeding of serum-starved cells differed from the transient serum induction of hsp70 mRNA reported by Wu and Morimoto (81). We therefore compared transcription of both genes after serum stimulation by using a nuclear run-on assay and also measured the relative abundance of each mRNA by slot blot hybridization (Fig. 8). The intensities of the hybridization signals were measured by densitometry and displayed graphically. Transcription of both the $hsp89\alpha$ and hsp70 genes was activated concurrently by 6 h after serum addition and returned to basal levels by 12 h after feeding. hsp70 mRNA accumulation followed the pattern of transcription very closely, reaching a maximal level between 6 and 9 h after feeding and declining rapidly thereafter. In contrast, the levels of hsp89a mRNA remained elevated long after the period of highest transcription had passed. $hsp89\alpha$ mRNA levels were highest 15 h after feeding and declined much more slowly. Despite the decline in the transcription rate, hsp89a mRNA levels remained elevated 24 h after serum addition. This indicated that hsp89a mRNA does not share the characteristically short half-life of hsp70 mRNA (73). The longer half-life of $hsp89\alpha$ mRNA appears to be responsible for the higher constitutive synthesis of the protein in unstressed cells.

DISCUSSION

Three lines of evidence indicated that the gene described in this report encodes the major species of $hsp89\alpha$ mRNA expressed in HeLa cells both in response to heat shock and during normal growth conditions. First, the sequences of the 3' half of the protein-coding region and the entire 622nucleotide 3'-untranslated region are identical to sequences contained in three independently isolated cDNA clones prepared from RNA isolated from heat-shocked (28) and normally growing cells (65). Also, the gene encoded an mRNA with the same 5'-untranslated leader sequence that



FIG. 8. Transcription and accumulation of hsp70 and $hsp89\alpha$ mRNA after feeding of serum-depleted HeLa cells. Nuclei were prepared at 3-h intervals from the serum-depleted and serum-stimulated HeLa cells described for Fig. 7. (a) Labeled nuclear run-on transcripts were hybridized to filters containing duplicate 10-µg dots of the hsp70-specific cDNA plasmid pHS709 (hsp70) (\odot) or the $hsp89\alpha$ -specific cDNA pHS801 (hsp89 α) (\bigcirc). The autoradiograms shown below were analyzed by densitometry, and the results are presented graphically. (b) Samples (5 µg) of cytoplasmic RNA were analyzed by slot blot hybridization with the same hsp70-(\odot) or $hsp89\alpha$ -specific (\bigcirc cDNA plasmids as probes. The results obtained by autoradiogram that is depicted was exposed for a longer period of time than the $hsp89\alpha$ slot blot.

was obtained by direct RNA sequencing. Finally, primer extension experiments with a synthetic oligonucleotide complementary to the predicted mRNA sequence identified a single RNA species that is both expressed constitutively at 37° C and present at elevated levels in response to heat shock. Other products were occasionally detected when much less stringent conditions were used for primer extension (data not shown). Whether these products represent different *hsp89* mRNAs encoded by the other members of the gene family detected by Southern blot analysis (Fig. 1) is not clear. However, greater than 0.5% of total poly(A)⁺ mRNA from control cells and greater than 1.5% of total poly(A)⁺ mRNA from heat-shocked cells can form hybrids with the pHS801 partial cDNA sequence (D. Lloyd, unpublished observation).

The presence of intervening sequences in a heat shock protein gene can no longer be considered an unusual phenomenon in higher organisms. The heat shock genes were first characterized for S. cerevisiae and Drosophila melanogaster, two organisms with concise genomes which in general contain few interrupted genes. The first heat shock gene shown to contain an intervening sequence was hsp82 of D. melanogaster (6). To date, a human hsp27 gene (27), the Caenorhabditis small heat shock protein genes (64), and a gene for a chicken 108-kilodalton heat shock protein (36) have also been shown to encode spliced mRNA molecules. The chicken ubiquitin gene that is induced by heat shock also contains an intron (9). The absence of intervening sequences may be characteristic only of genes that encode the heat-inducible forms of hsp70, which appear to lack introns in all organisms including humans (45).

Despite the presence of intervening sequences, newly synthesized $hsp89\alpha$ mRNA accumulates rapidly in the cytoplasm of HeLa cells heat shocked at 42°C, while processing or transport of several non-heat shock mRNAs is inhibited or delayed (65). The intron-exon junctions of the heat shock gene do not appear to be remarkable except in that the splice site sequences match the primate consensus (69) very closely. It is possible that the heat shock gene transcripts may have a kinetic advantage over non-heat shock mRNA precursors in cells undergoing heat stress when RNAsplicing capacity is reduced (8, 34, 84).

The position of the first intron has been conserved in the human $hsp89\alpha$ gene and in the hsp82 gene from four different Drosophila species (6). A single exon encoding the entire 5'-untranslated mRNA leader and the unusual location of the splice junction adjacent to the initiator ATG codon is retained in all cases. The nucleotide sequences surrounding both the 5' and 3' splice sites are also conserved. The Drosophila consensus sequence for the 12 nucleotides surrounding the 3' terminus of the first exon is ATACAAG/ GTRAG. The human sequence differs at only three positions, being AGCCAAG/GTGAC (differences are underlined). The Drosophila acceptor splice junction has the less specific consensus YYNTTNCAG/ATG. The equivalent human sequence, TCGTTCCAG/ATG, is completely consistent with the Drosophila consensus sequence. The sequences surrounding the ATG that are important for efficient translation are created by the splicing event. Evolutionary pressure for efficient translation of heat shock mRNA during stress may therefore have led to the conservation of sequences at this splice junction. These conserved sequences are not found in the other introns. An additional notable feature of the first intron of the $hsp89\alpha$ gene is the presence of HSE sequences that could potentially bind heat shock transcription factor (83). In each of the hsp82 gene se-

Human hsp89α	MPEETQTQDQPMEEEEVETFAFQAE
Mouse hsp86	MPEETQTQDQPMEEEEVETFAFQAE
Calf ERBP	MPEETQA
Chick hsp90	MPEAVQTQDQPM-EEEVETFAFQAE
Rabbit HCR	MPEEVQTQDQPMETFAVQTFAFQAE
Human hsp89ß	MPEEVHHGEEEVETFAFQAE
Mouse hsp84	MPEEVHHGEEEVETFAFQAE
Drosophila hsp82	MPEEAETFAFQAE
Yeast hsp83	MASETFEFQAE
T. cruzi	MTETFAFQAE
E. coli htpG	MKGQETRGFQSE

FIG. 9. Comparison of amino-terminal hsp89 α and hsp89 β protein sequences. Sequences of the following hsp89 homologs were taken from the indicated references: mouse hsp86 (74), mouse hsp84 (51), calf ERBP (estrogen receptor binding protein) (60), chick hsp90 (N. Binart, personal communication), rabbit HCR (hemin-controlled repressor) (63), human hsp89 β (61), *Drosophila* hsp89 (6), *S. cerevisiae* hsp83 (20), *Trypanosoma cruzi* heat shock protein (17), and *E. coli* htpG gene product (1).

quences published for the four *Drosophila* species, the intron also contains at least one partial or complete HSE consensus sequence (6). Heat-inducible transcription from the human $hsp89\alpha$ promoter does not require the first intron, as evidenced by expression of the $hsp89\alpha$ - β -globin gene reported here. Whether the internal HSE-like elements may nonetheless function to enhance transcription of the $hsp89\alpha$ gene is currently under investigation. It is possible that the location of the first intron could have been conserved through evolution as a consequence of the presence of HSE elements which may act in concert with those in the 5'-flanking region of the gene.

The deduced amino acid sequence of the human hsp 89α protein along with the human hsp89ß sequence determined by Rebbe et al. (61) allows for the classification of other vertebrate hsp89 homologs as either α -like or β -like. The N-terminal amino acid sequences that have been reported for hsp89 proteins from a variety of organisms are aligned in Fig. 9. The vertebrate hsp89 sequences fall into two clearly delineated classes based on the presence or absence of a glutamine-rich segment (T/VQTQDQ) separating two groups of acidic residues (PEEV/T and M/GEEE[E]V). Human hsp89 α contains the glutamine-rich sequence, while human hsp89ß does not. Conservation of the distinct amino acid sequences of the α and β forms across phylogenetic divisions is greater than the sequence conservation between the two forms within a single species. The differences between human hsp89 α and human hsp89 β (99 residues) are greater than the differences between human $hsp89\beta$ and mouse hsp84 (Fig. 4). hsp84 is 97% identical to human hsp89ß, differing at only 22 residues, all of which also differ from the sequence of human hsp89 α . Human hsp89 α , in contrast, is only 83% homologous with mouse hsp84 but contains the same N-terminal 30 amino acids as mouse hsp86 (74) (Fig. 4). The 200 C-terminal amino acids of mouse hsp86 recently deduced from a cDNA sequence are also identical to those of human hsp89 α (V. Legagneux, personal communication). Thus, murine hsp86 is clearly hsp89 α , and murine hsp84 is hsp89^β. The sequence of the chicken steroid receptorassociated hsp90 that has recently been completed by N. Binart (personal communication) indicates that this protein is an α form and differs from human hsp89 α at only 32 residues (96% identity). The N-terminal 7 amino acid residues of the calf steroid receptor-binding protein (60) contain the glutamine-rich sequence, which indicates that this hsp89

is also an α form. The remaining 78 residues that have been sequenced show only four differences from the corresponding regions of human hsp89 α but 14 differences from human hsp89 β . The rabbit hsp89 protein found in association with the hemin-controlled repressor protein kinase (63) is also an α form.

The functions of the hsp89 proteins are still unclear. hsp89 is an abundant protein that exists primarily as a dimer (37, 78). The two different forms of hsp89 may interact to form a heterodimer. However, differential regulation of the two genes during development (2) would argue that heterodimer assembly, if it occurs, is not obligatory. Most studies have suggested that hsp89 is a soluble protein that is localized within the cytoplasmic compartment (40, 42, 78). Immunofluorescence studies have shown a general distribution of hsp89 throughout the cytoplasm of murine and chicken cells (75) with particular accumulation in ruffled borders (37) and in regions containing microtubules (67). In D. melanogaster, hsp82 becomes more localized toward the periphery of the cell at elevated temperatures (13). One report which used immunogold labeling suggested that small amounts of hsp84 enter the nucleus of mouse cells upon heat shock (75), although the absolute level found in the cytoplasm does not decrease. Collier and Schlesinger report transport of hsp90 to the cell nucleus in chicken cells only after a second heat shock (15). Association of hsp89 with tubulin and actin (53, 67) as well as with a number of proteins having regulatory functions, such as tyrosine kinase oncogene products (10, 46, 55, 85), steroid receptors (33), and the eIF- 2α kinase (63), has been reported. The newly synthesized and as yet unphosphorylated tyrosine kinase oncogene products are selectively associated with hsp89 and a 50-kilodalton polypeptide in a complex having a half-life of about 15 min (11). Phosphorylation and membrane association of the oncogene products coincide with dissociation from the complex (11). The tyrosine kinase activity is suppressed in the complex with hsp89. The steroid receptors are also inactive in DNA binding while associated with hsp89 (30, 58, 66). These observations have led to the suggestion that hsp89 functions in transport of regulatory proteins to their final cellular locations on the membrane or in the nucleus (3, 67). It has also been suggested that hsp89 sequesters regulatory proteins in an inactive form after synthesis (3, 25) until they reach their destination within the cell or until the appropriate signal for their activity is received. Both the α and β forms of hsp89 are modified by phosphorylation at serine residues in vivo (35, 79), and these modifications may influence the interactions of the HSP with other cellular constituents. In vitro, casein kinase II phosphorylates threonine as well as serine in the eIF-2 α kinase-associated hsp89 (63). Both forms of human hsp89 have been shown in vivo to contain phosphorylated serine at two specific sites within the highly charged region located between residues 222 and 290 (44a). The same amino acids are also phosphorylated in vitro by casein kinase II. The serines flank one of two adjacent regions of the polypeptide with high α -helical potential which have been proposed to be the site of interaction of hsp89 with steroid receptors and specifically to interdigitate with the DNA-binding "fingers" (3). Phosphorylation of these sites could have a profound effect on the heat shock protein-receptor interaction. The specific amino acid sequence varies in this region of hsp89 among the different species, but the characteristic clustering of charged amino acids is present in all but the Escherichia coli hsp89 homolog p62.5. Phosphorylation of human hsp89 by a novel doublestranded DNA-dependent kinase has also been reported (76). This kinase phosphorylates only hsp89 α at one or more threonines in the amino-terminal third of the molecule (C. Anderson, personal communication). The phosphorylation site(s) is distinct both from the sites phosphorylated by casein kinase II described above and from the sites phosphorylated in the eIF-2 α kinase-associated hsp89 (63). Identification of phosphorylation sites that are unique to either the α or β form of hsp89 may provide clues toward understanding the possibly different biological roles of the two very similar proteins.

The genes encoding the hsp89 proteins have been shown to be under complex regulation, responding independently to nutritional status of the cells (41, 79), developmental events (2, 4, 52), hormone stimulation (59), and viral infection (70) as well as to the variety of stresses that induce the full complement of stress proteins. The $hsp89\alpha$ gene we have isolated has been shown specifically to be induced by heat stress, in response to the adenovirus E1A gene product, and after addition of fresh serum. The constitutive level of this transcript is controlled by growth factors contained in serum, as might be expected for an mRNA encoding an abundant cytoplasmic protein. Thus, the protein accumulates in concert with the general increase in cytoplasmic mass that occurs during proliferation. Although transcription of both hsp70 and $hsp89\alpha$ genes are induced by serum, the steady-state level of $hsp89\alpha$ mRNA found in exponentially growing cells is considerably higher. Consequently, hsp89 is synthesized in unstressed cells to a much greater extent than is hsp70. We have shown that this difference can be explained by the longer half-life of hsp89 mRNA during normal cell growth conditions in which hsp70 mRNA turns over rapidly (73). Our results do not rule out the possibility of other forms of the hsp89 gene that contribute to the mRNA pool during normal growth conditions or during stress. Such genes might be regulated differently than the $hsp89\alpha$ gene that was studied. However, unless the other genes contribute only minor amounts of transcript, they must be virtually identical to the lambda 86 gene or else completely fail to hybridize with any of the probes that were used. Isolation and analysis of the other members of the hsp89 gene family will be necessary to definitively determine whether they encode other types of hsp89 proteins distinct from the α and β forms or whether they are inactive pseudogenes.

ACKNOWLEDGMENTS

We thank Nancy Lowell and Gary Lewis for their technical assistance with DNA sequencing. We are indebted to Maria Catelli, Nadine Binart, Olivier Bensaude, Carl Anderson, and Neil Rebbe for helpful discussions and for sharing their results prior to publication.

This work was supported by Public Health Service grants GM32381 and CA36207 (to E.H. and L.A.W.) from the National Institutes of Health.

LITERATURE CITED

- Bardwell, J. C., and E. A. Craig. 1987. Eukaryotic M_r 83,000 heat shock protein has a homologue in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 84:5177-5181.
- Barnier, J. V., O. Bensaude, M. Morange, and C. Babinet. 1987. Mouse 89 kD heat shock protein. Two polypeptides with distinct developmental regulation. Exp. Cell Res. 170:186–194.
- Baulieu, E. E. 1987. Steroid hormone antagonists at the receptor level: a role for the heat-shock protein MW 90,000 (hsp 90). J. Cell Biochem. 35:161–174.
- Bedard, P. A., and B. P. Brandhorst. 1986. Translational activation of maternal mRNA encoding the heat-shock protein hsp90 during sea urchin embryogenesis. Dev. Biol. 117:286–293.

- Bienz, M. 1984. Xenopus hsp 70 genes are constitutively expressed in injected oocytes. EMBO J. 3:2477–2483.
- Blackman, R. K., M. Meselson. 1986. Interspecific nucleotide sequence comparisons used to identify regulatory and structural features of the *Drosophila* hsp82 gene. J. Mol. Biol. 188:499– 515.
- 7. Blin, N., and D. W. Stafford. 1976. A general method for isolation of high molecular weight DNA. Nucleic Acids Res. 3: 2303–2308.
- 8. Bond, U. 1988. Heat shock but not other stress inducers lead to the disruption of a sub-set of snRNPs and inhibition of *in vitro* splicing in HeLa cells. EMBO J. 7:3509–3518.
- Bond, U., and M. J. Schlesinger. 1986. The chicken ubiquitin gene contains a heat shock promoter and expresses an unstable mRNA in heat-shocked cells. Mol. Cell. Biol. 6:4602–4610.
- Brugge, J., E. Erikson, and R. L. Erikson. 1981. The specific interaction of the Rous sarcoma virus transforming protein, pp60src, with two cellular proteins. Cell 25:363–372.
- Brugge, J., W. Yonemoto, and D. Darrow. 1983. Rous sarcoma virus transforming protein and two cellular proteins: analysis of the turnover and distribution of this complex. Mol. Cell. Biol. 3: 9–19.
- 12. Calzone, F. J., R. J. Britten, and E. H. Davidson. 1987. Mapping gene transcripts by nuclease protection assays and cDNA primer extension. Methods Enzymol. 152:611-632.
- Carbajal, M. E., J. L. Duband, F. Lettre, J. P. Valet, and R. M. Tanguay. 1986. Cellular localization of *Drosophila* 83-kilodalton heat shock protein in normal, heat-shocked, and recovering cultured cells with a specific antibody. Biochem. Cell Biol. 64: 816-825.
- Clayton, D. F., and J. E. Darnell. 1983. Changes in liver specific compared to common gene transcription during primary culture of mouse hepatocytes. Mol. Cell. Biol. 3:1552–1561.
- Collier, N. C., and M. J. Schlesinger. 1986. The dynamic state of heat shock proteins in chicken embryo fibroblasts. J. Cell Biol. 103:1495–1507.
- Delisle, A. J., R. A. Graves, W. F. Marzluff, and L. F. Johnson. 1983. Regulation of histone messenger RNA production and stability in serum stimulated mouse 3T3 fibroblasts. Mol. Cell. Biol. 3:1920–1929.
- Dragon, E. A., S. R. Sias, E. A. Kato, and J. D. Gabe. 1987. The genome of *Trypanosoma cruzi* contains a constitutively expressed, tandemly arranged multicopy gene homologous to a major heat shock protein. Mol. Cell Biol. 7:1271–1275.
- Dubrow, R., V. G. H. Riddle, and A. B. Pardee. 1979. Different responses to drugs and serum of cells transformed by various means. Cancer Res. 39:2718–2726.
- 19. Dworniczak, B., and M. E. Mirault. 1987. Structure and expression of a human gene coding for a 71 kd heat shock 'cognate' protein. Nucleic Acids Res. 15:5181–5197.
- 20. Farrelly, F. W., and D. B. Finkelstein. 1984. Complete sequence of the heat shock-inducible HSP90 gene of *Saccharomyces cerevisiae*. J. Biol. Chem. 259:5745-5751.
- Favaloro, J., R. Treisman, and R. Kamen. 1980. Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S1 gel mapping. Methods Enzymol. 65:718–749.
- 22. Geliebter, J. 1987. Dideoxynucleotide sequencing of RNA and uncloned cDNA. Focus 9:5-8.
- Glass, D. J., R. I. Polvere, and L. H. Van Der Ploeg. 1986. Conserved sequences and transcription of the *hsp70* gene family in *Trypanosoma brucei*. Mol. Cell Biol. 6:4657–4666.
- Graham, F. L., and A. J. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52:456–467.
- Haire, R. N., M. S. Peterson, and J. J. O'Leary. 1988. Mitogen activation induces the enhanced synthesis of two heat-shock proteins in human lymphocytes. J. Cell. Biol. 106:883–891.
- Heintz, N., H. L. Sive, and R. G. Roeder. 1983. Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. Mol. Cell. Biol. 3: 539–550.
- 27. Hickey, E., S. E. Brandon, R. Potter, G. Stein, J. Stein, and

L. A. Weber. 1986. Sequence and organization of genes encoding the human 27 kDa heat shock proteins. Nucleic Acids Res. 14:4127–4145.

- Hickey, E., S. E. Brandon, S. Sadis, G. Smale, and L. A. Weber. 1986. Molecular cloning of sequences encoding the human heat-shock proteins and their expression during hyperthermia. Gene 43:147–154.
- 29. Hickey, E., and L. A. Weber. 1982. Modulation of heat-shock polypeptide synthesis in HeLa cells during hyperthermia and recovery. Biochemistry 21:1513–1521.
- Howard, K. J., and C. W. Distelhorst. 1988. Effect of the 90 kDa heat shock protein. HSP90. on glucocorticoid receptor binding to DNA-cellulose. Biochem. Biophys. Res. Commun. 151:1226– 1232.
- Hunt, C., and R. I. Morimoto. 1985. Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc. Natl. Acad. Sci. USA 82: 6455-6459.
- Ingolia, T. D., E. A. Craig, and B. J. McCarthy. 1980. Sequence of three copies of the gene for the major *Drosophila* heat shock induced protein and their flanking regions. Cell 21:669–679.
- Joab, I., C. Radanyi, M. Renoir, T. Buchou, M.-G. Catelli, N. Binart, J. Mester, and E.-E. Baulieu. 1984. Common nonhormone binding component in non-transformed chick oviduct receptors of four steroid hormones. Nature (London) 308:850– 853.
- 34. Kay, R. J., R. H. Russnak, D. Jones, C. Mathias, and E. P. Candido. 1987. Expression of intron-containing *C. elegans* heat shock genes in mouse cells demonstrates divergence of 3' splice site recognition sequences between nematodes and vertebrates, and an inhibitory effect of heat shock on the mammalian splicing apparatus. Nucleic Acids Res. 15:3723–3741.
- Kelley, P. M., and M. J. Schlesinger. 1982. Antibodies to two major chicken heat shock proteins cross-react with similar proteins in widely divergent species. Mol. Cell. Biol. 2:267–274.
- Kleinsek, D. A., W. G. Beattie, M. J. Tsai, and B. W. O'Malley. 1986. Molecular cloning of a steroid-regulated 108K heat shock protein gene from hen oviduct. Nucleic Acids Res. 14:10053– 10069.
- 37. Koyasu, S., E. Nishida, T. Kadowaki, F. Matsuzaki, K. Iida, F. Harada, M. Kasuga, H. Sakai, and I. Yahara. 1986. Two mammalian heat shock proteins, HSP90 and HSP100, are actinbinding proteins. Proc. Natl. Acad. Sci. USA 83:8054–8058.
- Kozak, M. 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell 44:283-292.
- Lagrimini, L. M., S. T. Brentano, and J. E. Donelson. 1984. A DNA sequence analysis package for the IBM personal computer. Nucleic Acids Res. 12:605–614.
- Lai, B.-T., N. W. Chen, A. E. Stanek, W. Keh, and K. W. Lanks. 1984. Quantitation and intracellular localization of the 85K heat shock protein by using monoclonal and polyclonal antibodies. Mol. Cell. Biol. 4:2802–2810.
- 41. Lanks, K. W. 1983. Metabolite regulation of heat shock protein levels. Proc. Natl. Acad. Sci. USA 80:5325-5329.
- Lanks, K. W., and E. J. Kasambalides. 1979. Purification and characterization of a major component from the cytoplasmic matrix of cultured murine L cells. Biochim. Biophys. Acta 578: 1-12.
- 42a.Lees-Miller, S. P., and C. W. Anderson. 1989. Two human 90-kDa heat shock proteins (hsp90) are phosphorylated in vivo at conserved serines that are phosphorylated in vitro by casein kinase II. J. Biol. Chem. 264:2431-2437.
- Lawn, R. M., A. Efstratiadis, C. O'Connell, and T. Maniatis. 1980. The nucleotide sequence of the human β-globin gene. Cell 21:647–651.
- 44. Lawn, R. M., E. F. Fritsch, R. C. Parker, G. Blake, and T. Maniatis. 1978. The isolation and characterization of a linked δ and β -globin gene from a cloned library of human DNA. Cell 15: 1157–1174.
- Lindquist, S. 1986. The heat-shock response. Annu. Rev. Biochem. 55:1151–1191.
- 46. Lipsich, L. A., J. R. Cutt, and J. S. Brugge. 1982. Association of

the transforming proteins of Rous, Fujinami, and Y73 avian sarcoma viruses with the same two cellular proteins. Mol. Cell. Biol. **2:**875–880.

- Lowe, D. G., W. D. Fulford, and L. A. Moran. 1983. Mouse and Drosophila genes encoding the major heat shock protein (hsp70) are highly conserved. Mol. Cell. Biol. 3:1540–1543.
- 48. Lowe, D. G., and L. A. Moran. 1986. Molecular cloning and analysis of DNA complementary to three mouse $M_r = 68,000$ heat shock protein mRNAs. J. Biol. Chem. 261:2102–2112.
- 49. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 50. Messing, J. 1983. New M13 vectors for cloning. Methods Enzymol. 101:20-78.
- Moore, S. K., C. Kozak, E. A. Robinson, S. J. Ullrich, and E. Appella. 1987. Cloning and nucleotide sequence of the murine hsp84 cDNA and chromosome assignment of related sequences. Gene 56:29–40.
- Morange, M., A. Diu, O. Bensaude, and C. Babinet. 1984. Altered expression of heat shock proteins in embryonal carcinoma and mouse early embryonic cells. Mol. Cell. Biol. 4:730–735.
- Nishida, E., S. Koyasu, H. Sakai, and I. Yahara. 1986. Calmodulin-regulated binding of the 90-kDa heat shock protein to actin filaments. J. Biol. Chem. 261:16033–16036.
- Ogden, R. C., and D. A. Adams. 1987. Electrophoresis in agarose and acrylamide gels. Methods Enzymol. 152:61–87.
- 55. Opperman, H., W. Levinson, and J. M. Bishop. 1981. A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat shock protein. Proc. Natl. Acad. Sci. USA 78:1067–1071.
- 56. Pelham, H. R. B. 1982. A regulatory upstream promoter element in the *Drosophila* hsp-70 heat shock gene. Cell **30**:517–528.
- Plumb, M., J. Stein, and G. Stein. 1983. Coordinate regulation of multiple histone mRNAs during the cell cycle in HeLa cells. Nucleic Acids Res. 11:2391-2410.
- Pratt, W. B. 1987. Transformation of glucocorticoid and progesterone receptors to the DNA-binding state. J. Cell Biochem. 35: 51-68.
- Ramachandran, C. M., M. G. Catelli, W. Schneider, and G. Shymala. 1988. Estrogenic regulation of uterine 90 kilodalton heat shock protein. Endocrinology 123:956–961.
- Ratajczak, T., M. J. Brockway, R. Hahnel, R. L. Moritz, and R. J. Simpson. 1988. Sequence analysis of the nonsteroid binding component of the calf uterine estrogen receptor. Biochem. Biophys. Res. Commun. 151:1156–1163.
- Rebbe, N. F., J. Ware, R. M. Bertina, P. Modrich, and D. W. Stafford. 1987. Nucleotide sequence of a cDNA for a member of the human 90-kDa heat-shock protein family. Gene 53:235-245.
- 62. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**: 237–251.
- Rose, D. W., R. E. Wettenhall, W. Kudlicki, G. Kramer, and B. Hardesty. 1987. The 90-kilodalton peptide of the heme-regulated eIF-2 alpha kinase has sequence similarity with the 90-kilodalton heat shock protein. Biochemistry 26:6583–6587.
- 64. Russnak, R., and E. P. M. Candido. 1985. Locus encoding a family of small heat shock genes in *Caenorhabditis elegans*: two genes duplicated to form a 3.8-kilobase inverted repeat. Mol. Cell. Biol. 5:1268–1278.
- Sadis, S., E. Hickey, and L. A. Weber. 1988. Effect of heat shock on RNA metabolism in HeLa cells. J. Cell Physiol. 135:377–386.
- 66. Sanchez, E. R., S. Meshinchi, W. Tienrungroj, M. J. Schlesinger, D. O. Toft, and W. B. Pratt. 1987. Relationship of the 90-kDa murine heat shock protein to the untransformed and transformed states of the L cell glucocorticoid receptor. J. Biol. Chem. 262:6986–6991.
- 67. Sanchez, E. R., T. Redmond, L. C. Scherrer, E. H. Bresnick,

M. J. Welsh, and W. B. Pratt. 1988. Evidence that the 90kilodalton heat shock protein is associated with tubulin containing complexes in L cell cytosol and in intact PtK cells. Mol. Endocrinol. 2:756-760.

- 68. Schwindiger, W. F., and J. R. Warner. 1984. DNA sequence analysis on the IBM-PC. Nucleic Acids Res. 12:601-604.
- Shapiro, M. B., and P. Senapathy. 1987. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res. 15: 7155–7174.
- Simon, M. C., K. Kitchener, H. T. Kao, E. Hickey, L. Weber, R. Voellmy, N. Heintz, and J. R. Nevins. 1987. Selective induction of human heat shock gene transcription by the adenovirus E1A gene products, including the 12S E1A product. Mol. Cell. Biol. 7:2884–2890.
- Sorger, P. K., and H. R. B. Pelham. 1987. Cloning and expression of a gene encoding hsc73, the major hsp70-like protein in unstressed rat cells. EMBO J. 6:993–998.
- Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.
- Theodorakis, N. G., and R. I. Morimoto. 1987. Posttranscriptional regulation of hsp70 expression in human cells: effects of heat shock. inhibition of protein synthesis, and adenovirus infection on translation and mRNA stability. Mol. Cell. Biol. 7: 4357–4368.
- 74. Ullrich, S. J., E. A. Robinson, L. W. Law, M. Willingham, and E. Appella. 1986. A mouse tumor-specific transplantation antigen is a heat shock-related protein. Proc. Natl. Acad. Sci. USA 83:3121–3125.
- 75. van Bergen, E. N., P. M. Henegouwen, G. Berbers, W. A. Linnemans, and R. van Wijk. 1987. Subcellular localization of the 84,000 dalton heat-shock protein in mouse neuroblastoma cells: evidence for a cytoplasmic and nuclear location. Eur. J. Cell Biol. 43:469–478.
- Walker, A. I., T. Hunt, R. J. Jackson, and C. W. Anderson. 1985. Double-stranded DNA induces the phosphorylation of several proteins including the 90,000 mol. wt. heat-shock protein in animal cell extracts. EMBO J. 4:139–145.
- 77. Weber, L. A., T. Nilsen, and C. Baglioni. 1978. Isolation of histone messenger RNA and its translation in vitro. Methods Cell Biol. 19:215-236.
- Welch, W. J., and J. R. Feramisco. 1982. Purification of the major mammalian heat shock proteins. J. Biol. Chem. 257: 14949–14959.
- 79. Welch, W. J., J. I. Garrels, G. P. Thomas, J. J. C. Lin, and J. R. Feramisco. 1983. Biochemical characterization of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca²⁺-ionophore-regulated proteins. J. Biol. Chem. 258:7102-7111.
- Wu, B. J., R. E. Kingston, and R. I. Morimoto. 1986. Human HSP70 promoter contains at least two distinct regulatory domains. Proc. Natl. Acad. Sci. USA 83:629–633.
- Wu, B. J., and R. Morimoto. 1985. Transcription of the human hsp70 gene is induced by serum stimulation. Proc. Natl. Acad. Sci. USA 82:6070–6074.
- 82. Wu, B. J., G. T. Williams, and R. I. Morimoto. 1987. Detection of three protein binding sites in the serum-regulated promoter of the human gene encoding the 70-kDa heat shock protein. Proc. Natl. Acad. Sci. USA 84:2203–2207.
- Xiao, H., and J. T. Lis. 1988. Germline transformation used to define key features of heat-shock response elements. Science 239:1139–1142.
- Yost, H. J., and S. Lindquist. 1986. RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell 45:185-193.
- Ziemiecki, A., M. G. Catelli, I. Joab, and B. Moncharmont. 1986. Association of the heat shock protein hsp90 with steroid hormone receptors and tyrosine kinase oncogene products. Biochem. Biophys. Res. Commun. 138:1298–1307.