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Cloning and nucleotide sequence of the murine *hsp84* cDNA and chromosome assignment of related sequences

(Heat shock; multigene family; peptide analysis; recombinant DNA)

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SUMMARY

The nucleotide (nt) sequence of mouse 84-kDa heat shock protein (Hsp) cDNA has been determined using a combination of molecular cloning and oligodeoxynucleotide priming on poly(A)$^+$ RNA. The cDNA was 2.5 kb long, not including the poly(A) tail. It contained a 5′ leader of about 94 nt that was G + C-rich, and a 243-nt 3′-untranslated region that was A + T-rich in the vicinity of the polyadenylation signal. Gene *hsp84* codes for an acidic polypeptide of 724 amino acid (aa) residues. Mouse Hsp84 had 81% and 63% aa homology to *Drosophila melanogaster* Hsp82 and yeast Hsp90, respectively. The nucleotide sequence had 74% and 59% homology to *Drosophila* and yeast *hsp* sequences, respectively, in the coding regions of these genes. This homology did not extend to the 5′- and 3′-untranslated regions. Chromosomal analysis indicated that *hsp84*-related sequences are on at least three different chromosomes.

INTRODUCTION

One of the major Hsp synthesized when cells are subjected to heat or other stress is an acidic cytosolic protein having an apparent molecular mass between 82 and 90 kDa (reviewed by Lindquist, 1986). The function of this protein is not known although it has been found to be complexed with the oncogene product, pp60^{src} (Lanks et al., 1982) and with steroid receptors (Mendel et al., 1986; Riehl et al., 1985). The synthesis of this protein is decreased upon glucose deprivation (Lanks, 1983) and is increased after viral infection (Khandjian and Türler, 1983), during hepatic regeneration or after exposure to hepatocarcinogens (Carr et al., 1986). This Hsp is post-translationally modified by phosphorylation (Welch et al., 1983; Ullrich et al., 1986).

Our laboratory has shown that one of the tumor-specific transplantation antigens purified from an

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Abbreviations: aa, amino acid(s); bp, base pair(s); Hsp, heat-shock protein(s); *hsp*, gene coding for Hsp; kb, 1000 bp; HPLC, high-performance liquid chromatography; MC, 3-methylcholanthrene; nt, nucleotide(s); PAGE, polyacrylamide gel electrophoresis; PTH, phenylthiohydantoin; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl, 0.015 M Na_3 · citrate (pH 7.0); TFA, trifluoroacetic acid; u, unit(s); UT, untranslated region.

MC-induced mouse tumor, Meth A, was related to or identical with this Hsp (Ullrich et al., 1986). The mouse Hsp was shown to consist of two isoforms of 84 and 86 kDa (Ullrich et al., 1986).

To better understand the function and regulation of these Hsp and to elucidate the nature of its associated tumor-specific transplantation activity, we have isolated cDNA clones using synthetic oligodeoxynucleotide probes designed on the basis of amino acid sequences of the Hsp84 isoform. The amino acid sequence, deduced from the nucleotide sequence of the coding region of *hsp84* cDNA, showed considerable homology to that of *Drosophila* Hsp82 (Blackman and Meselson, 1986). Chromosomal analysis indicated that *hsp84*-related sequences are on at least three different chromosomes.

MATERIALS AND METHODS

(a) Screening of cDNA libraries

A cDNA library constructed in the vector pcDV1 (Okayama and Berg, 1983) with poly(A)$^+$RNA from the MC-induced C3H mouse tumor line, MB66MCAad36 (Shilo and Weinberg, 1981) was the gift of Dr. H. Okayama. The library was screened by colony hybridization (Grunstein and Hogness, 1975; Maniatis et al., 1982) using a mixture of radiolabeled 17-nt oligodeoxynucleotides having the following sequence: 5′ – ^{32}P – T T Py T C Pu A A Py T T N G C Py T T – 3′, where Py = C and T, Pu = A and G, and N = A, C, G, and T. Hybridization to filters was carried out in 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, 0.05% sodium pyrophosphate, 100 μg/ml denatured, sheared salmon sperm DNA and 10^6 dpm/ml radiolabeled probe at 37°C overnight. Filters were washed in 6 × SSC + 0.05% sodium pyrophosphate at 47°C before autoradiography. A second cDNA library constructed in λgt10 with RNA from Meth A (Wolf et al., 1985) was the gift of Dr. V. Rotter. The library was screened by plaque hybridization (Benton and Davis, 1977; Maniatis et al., 1982) using a radiolabeled 40-nt oligodeoxynucleotide synthesized on the basis of nucleotide sequence data (see RESULTS, section **b**). Hybridization was carried out as above except that the temperature of hybridization was 55°C. Filters were washed sequentially at 55°C in 6 × and 1 × SSC solutions containing 0.1% SDS.

(b) Primer extension on RNA

Poly(A)$^+$RNA was prepared from Meth A ascites cells according to the method of Chirgwin et al. (1979). Dideoxynucleotide sequencing of RNA was performed as described (Geliebter et al., 1986) with the following minor modifications. To denature RNA secondary structure, the RNA was heated for 5 min at 65°C then cooled on an ice-water bath. RNA and oligodeoxynucleotide primer were annealed at 55°C for 1 h in 0.33 M KCl. Other specified reaction components, except reverse transcriptase, were then added. Human placental RNase inhibitor (800 u/ml) was included as a reaction component. Primer extension was initiated by the addition of reverse transcriptase.

(c) Somatic cell hybrids

Hybrids were generated by fusion of Chinese hamster E36 cells with peritoneal or spleen cells of Balb/c, A/HeJ, or NFS.*Akv-2* mice. The production and characterization of these hybrids have been described previously (Kozak et al., 1975; Kozak and Rowe, 1979; 1980). High-M_r DNA was extracted from cultured hybrid cells within a few passages of their characterization for mouse chromosomes.

(d) Other methods

Oligodeoxynucleotides were synthesized by the phosphoramidite method (Adams et al., 1983) using an automated synthesizer (Vega Coder 300) and were purified by HPLC. They were labeled at the 5′ end with ^{32}P using [γ-^{32}P]ATP (6000 Ci/mol) and T4 polynucleotide kinase. Northern blots were performed as described (Maniatis et al., 1982).

RESULTS AND DISCUSSION

(a) Peptide analysis

Nine peptides generated by CNBr cleavage of the Hsp84 isoform were isolated by Superose 12 column

chromatography and HPLC; these were subjected to Edman degradation. The sequence of one of these (residues 115–125) has been previously reported (Ullrich et al., 1986). Another, sequenced for 21 cycles, was the N-terminal peptide (Ullrich et al., 1986). The sequences of the other seven peptides, together with their position in the protein sequence deduced from nucleotide sequence data, are shown in Table I. All of the peptide sequences are in agreement with the nucleotide sequence data.

(b) Cloning and sequence analysis of *hsp84* cDNA

A cDNA library constructed with RNA derived from an MC-induced C3H mouse tumor line was screened for *hsp84* clones using an oligodeoxynucleotide probe designed to cover all codon possibilities for a portion of peptide V in Table I (see MATERIALS AND METHODS, section **b**). Seven positive signals were obtained from approx. 10^5 recombinants. Of these, the one with the longest insert (approx. 1.3 kb) was subjected to sequence analysis (Fig. 1, clone A). The probe sequence aligned with the clone at nt positions 1669–1685 (Fig. 2). This cDNA clone contained about one-half of the protein coding region together with the entire 3′-untranslated region and approx. 80 adenosine residues from the poly(A) tract.

A cDNA library constructed in λgt10 with RNA derived from the MC-induced tumor, Meth A, was also screened. The probe utilized was a synthetic 40-mer derived from the 5′ portion of the clone from the first library (nt positions 1334–1374, Fig. 2). About 80 positive signals were found out of 10^6 recombinant phage. Out of nine isolates, three contained cDNA from the region 5′ to that previously isolated. The representative structure of these clones is shown in Fig. 1 (clone type B). One of the longest clones was sequenced in its entirety. Several of the others were also sequenced and found to consist of the same stretch or portions thereof. Only one base difference was noted between the various cDNA clones from the latter library, a non-conservative substitution of a T for a G within the coding

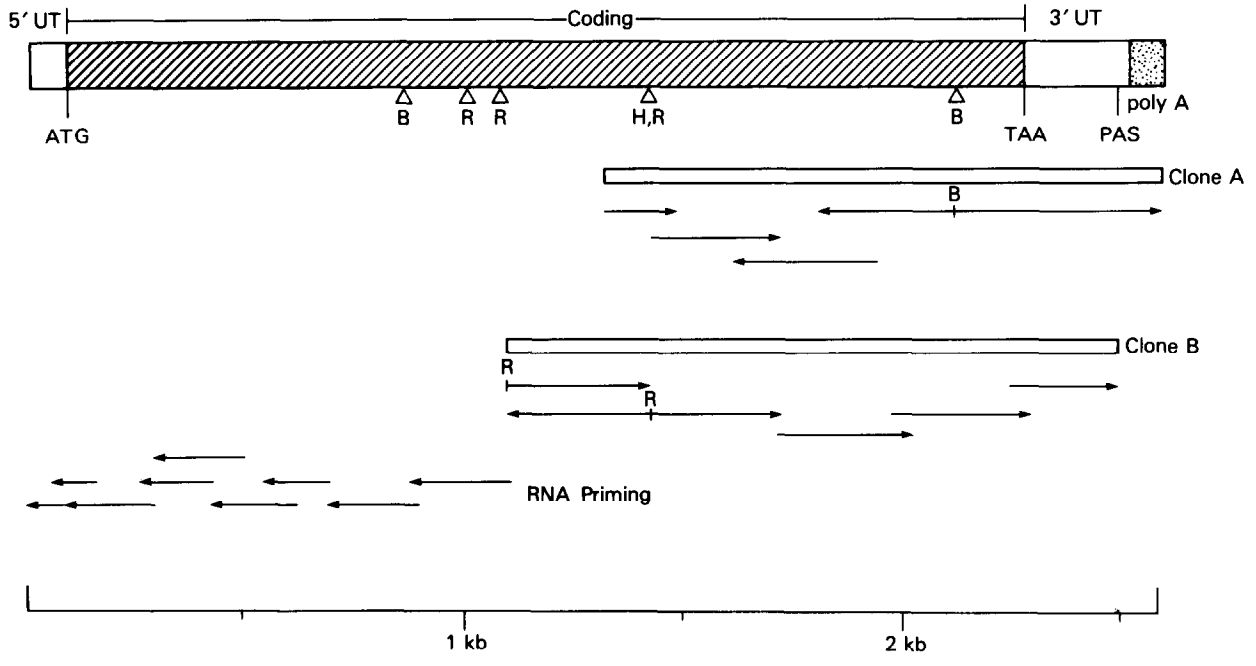

Fig. 1. Structural organization of mouse *hsp84* cDNA. Clone A was the longest insert obtained from a cDNA library constructed with RNA from the C3H tumor line, MB66MCAad36. Clone B represents the longest variety of insert obtained from a cDNA library constructed from RNA derived from a cell line of the Balb/c tumor, Meth A. For nucleotide sequence analysis, inserts, or portions thereof, were subcloned into either M13mp18 or M13mp19 (Yanisch-Perron et al., 1985). Sequence analysis was performed by the chain-termination method (Sanger et al., 1977; Sanger and Coulson, 1978) on single-stranded templates prepared from M13 recombinants using [α-^{35}S]dATP as label. Arrows denote the direction and extent of determined sequences. RNA priming was performed on Meth A poly(A)$^+$ RNA with synthetic oligodeoxynucleotides in the presence of chain terminators (see MATERIALS AND METHODS, section **b**). B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; PAS, polyadenylation signal; UT, untranslated region.

TABLE I

Amino acid sequence of Hsp84 peptides[a] I through VI

	Peptide number						
	I(pmol)[b]	II(pmol)	III(pmol)	IV(pmol)	V(pmol)	VI(pmol)	VII(pmol)
	Residue number						
Cycle	93–113	126–162	467–476	477–513	554–566	595–613	684–720
1	T (188)	I (59)	T (82)	K (144)	E (287)	A (98)	I (155)
2	K (305)	G (65)	S (95)	E (108)	E (320)	K (41)	K (142)
3	A (277)	Q (36)	L (97)	T (54)	S (62)	K (61)	L (137)
4	D (133)	F (41)	S (44)	Q (88)	K (106)	H (56)	G (92)
5	L (222)	G (30)	E (45)	K (106)	A (132)	L (56)	L (121)
6	I (167)	V (35)	Y (57)	S (65)	K (79)	E (33)	G (93)
7	N (113)	G (26)	V (48)	I (90)	F (94)	I (51)	I (92)
8	N (137)	F (28)	S (38)	Y (73)	E (85)	N (31)	D (71)
9	L (137)	Y (35)	R (22)	Y (79)	N (64)	P (36)	E (67)
10	G (80)	S (31)	M[c](7)	I (74)	L (61)	D (31)	D (49)
11	T (42)	A (25)		T (39)	C[d](37)	H (31)	E (50)
12	I (72)	Y (24)		G (45)	K (25)	P (27)	V (44)
13	A (81)	L (22)		E (32)	L (12)	I (17)	T (33)
14	K (57)	V (24)		S (47)		V (16)	A (45)
15	S (48)	A (20)		K (39)		E (8)	E (30)
16	G (31)	E (15)		E (19)		T (3)	E (32)
17	T (18)	K (24)		Q (28)		L (15)	P (29)
18	K (24)	V (25)		V (29)		R (8)	S (19)
19	A (27)	V (18)		A (27)		Q (7)	A (25)
20	F (13)	V (17)		N (18)			A (29)
21	M[c](5)	I (13)		S (22)			V (18)
22		T (5)		A (20)			P (15)
23		K (11)		F (17)			D (12)
24		H (8)		V (16)			E (19)
25		N (7)		E (8)			I (13)
26		D (6)		R (11)			P (19)
27		D (8)		V (13)			P (11)
28		E (4)		R (10)			L (9)
29		Q (5)		K (6)			E (4)
30		Y (4)		R (6)			G (4)
31		A (5)		G (12)			D (4)
32		X[e]		F (9)			E (2)
33		E (2)		E (2)			D (2)
34		S (0.4)		V (6)			A (4)
35		S (0.7)		V (7)			S (2)
36		A (1)		Y (6)			R (3)
37				M[c](4)			M[c](1)

[a] Hsp84/86 mixture was purified from Meth A ascites cells and the Hsp84 isoform separated by Mono Q HR5/5 chromatography (Ullrich et al., 1986). Carboxy-methylated or untreated Hsp84 was cleaved with CNBr, and fragments size-fractionated on Superose 12 HR10/30 in 0.15 M NH_4HCO_3. The lower M_r fractions were subjected to reverse-phase HPLC (Synchropak RP-8) in 0.1% TFA-H_2O with either acetonitrile or acetonitrile : 1-propanol (3 : 1) as mobile phase on a Hewlett Packard 1090A. Peptides were sequenced on an Applied Biosystems 470A Sequencer equipped with an on-line 120A PTH Analyser.

[b] Numbers in parentheses are pmol of PTH amino acid detected.

[c] Actually homoserine.

[d] Actually carboxymethylcysteine.

[e] Undetermined amino acid residue.

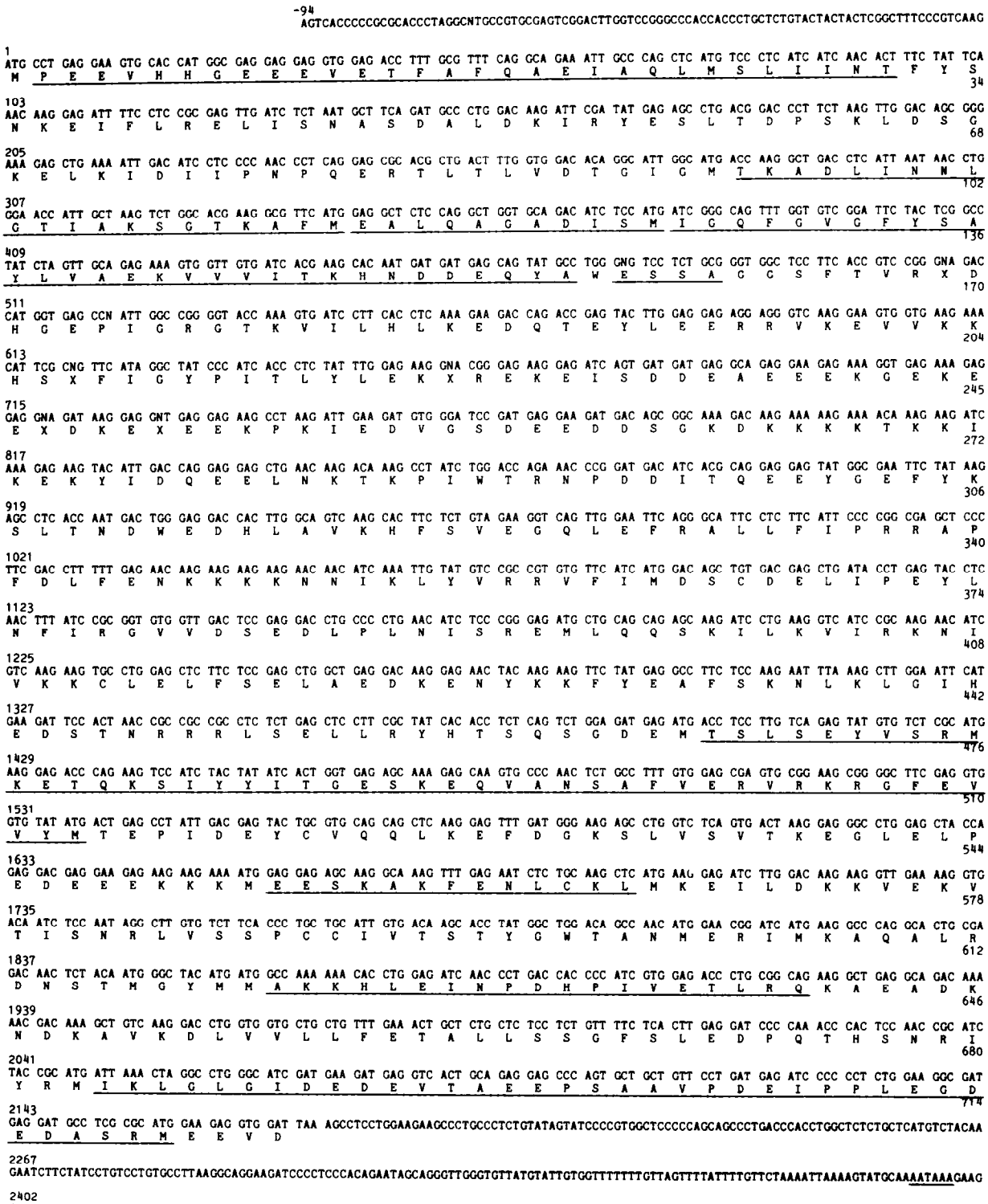

Fig. 2. Combined nucleotide sequence of mouse *hsp84* cDNA. Shown is the anti-sense strand. Sequences from the 5′ end to nt position 2388 were derived from a cell line of the Balb/c tumor, Meth A. Sequences from nt 2389 to the 3′ end were from the C3H tumor line, MB66MCAad36. The polyadenylation signal, AATAAA (Proudfoot and Brownlee, 1978), is underlined. N's indicate positions in the nucleotide sequence that were ambiguous on nucleotide sequencing gels. Predicted aa residues are given in single letter code beneath the nucleotide sequence. X's indicate unassigned amino acid residues. Those amino acid residues confirmed by peptide analysis are underlined.

region at nt position 1511 (Fig. 2). Also, one nt difference was noted between clones from the two libraries, a substitution of a T for an A within the 3′-untranslated region of the clone from the first library. That these permutations are the result of passage of mammalian DNA in *E. coli* cannot be ruled out.

Since full-length cDNA clones were not found, and Northern blot analysis indicated a rather large mRNA (approx. 3 kb) (not shown), the sequence was completed using oligodeoxynucleotide priming on Meth A poly(A)$^+$ RNA. The combined sequence is shown in Fig. 2. The first nucleotide of the sequence was the 5′-most base that was discernible as a band on the autoradiograms of sequencing gels. Ten consecutive bands, however, appeared in all lanes in these sequencing gels above this 5′-most discernible nt (not shown). It is not known whether this indicates that the mRNA is actually 10 nt longer than the determined sequence or whether there are multiple start points.

(c) The *hsp84* gene product

The gene product, deduced from the coding region of *hsp84* cDNA, is a polypeptide chain of 724 aa (Fig. 2). Five aa could not be assigned due to seven ambiguities in the nucleotide sequence. The amino acid sequence is in agreement with the sequence analysis of N-terminal and internal peptides and with amino acid composition data (Ullrich et al., 1986). Hsp84 shows 81% and 63% aa homology to the *Drosophila* 82-kDa Hsp (Blackman and Meselson, 1986) and yeast 90-kDa Hsp (Farrelly and Finkelstein, 1984), respectively (Fig. 3; unassigned amino acids were omitted from the calculations). There are several long blocks of amino acid sequence homology shared among all three species. Such regions are presumably important to the function of this Hsp.

It is interesting to note that the final 4 aa residues at the C terminus of Hsp84 are homologous to residues at the C termini of the Hsp70 proteins as well as 4 aa residues near the terminus of the *E. coli* DnaK product (compiled in Lindquist, 1986). The evolutionary and functional significance of this isolated homology is unknown.

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M    1  MPEEVHHGEEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNASDALDKIRYESLTDPSKLDSGKELKIDIIPNPQERTLTLVDTGIGMTKADLIN
D    1  ..--------..A.....................................................Y.KL...KTAG...II.........S..V.
Y    1  .---------AS...E.....T.........V.....................K..S..KQ.ETEPD.F.R.T.K.EQKV.EIR.S.......E...

M  101  NLGTIAKSGTKAFMEALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRXDHG-EPIGRGTKVILHLKEDQTEYLEERRVK
D   94  ..........................................D..T.TS.N......V.............A.NS-..L.....IV.YI.....D....SKI.
Y   92  ..................S....V.............LF...DR.Q..S.S......I...N........TL.EVN.R.....ILR.F..D..L.....K.I.
C           ..................................T...ST

M  200  EVVKKHSXFIGYPITLYLEKXREKEISDDEAEEEKGEKEEXDKEXEEKPKIEDVGSDEEDDSGKDKKKKTKKIKEKYIDQEELNKTKPIWTRNPDDITQE
D  193  .I.N...Q......K.LV..E....V.....DD..K.GD.KKEMETDE.......ED.DA.KKDKDA..K.T.....TED.................S..
Y  192  ..I.R..E.VA...Q.VVT.EV...--VPIP....KDE.KK.E.K--.DEDDKKPKL..V.EEEE..P....V..EVQEI........L......S.....

M  300  EYGEFYKSLTNDWEDHLAVKHFSVEGQLEFRALLFIPRRAPFDLFENKKKKNNIKLYVRRVFIMDSCDELIPEYLNFIRGVVDSEDLPLNISREMLQQSK
D  293  ..........................................T.......Q..R..............N.ED........MK.....................N.
Y  288  ..NA....IS.....P.Y..............I....K........S................T.EAED....W.S.VK...........L.......N.

M  400  ILKVIRKNIVKKCLELFSELAEDKENYKKFYEAFSKNLKLGIHEDSTNRRRLSELLRYHTSQSGDEMTSLSEYVSRMKETQKSIYYITGESKEQVANSAF
D- 393  V.......L...TM..IE..T..........DQ.........V....N..AK.ADF..F...A...DFC..AD......DN..HV.F......D..S....
Y  388  .M..........LI.A.N.I...S.QFE...S.....I...V...TQ..AA.AK....NSTK.V..L...TD..T..P.H..N.........LKA.EK.P.

M  500  VERVRKRGFEVVYMTEPIDEYCVQQLKEFDGKSLVSVTKEGLELPEDEEEEKKKMEESKAKFENLCKLMKEILDKKVEKVTISNRLVSSPCCIVTSTYGWT
D  493  ....KA..............VI.H...YK..Q..............S....R..D.....S.....S...N.....VV.....D........QF..S
Y  488  LDALKAKN...LFL.D.....AFT.....E..T..DI..D-F..E.TD...AER.KEIKEY.P.T.AL....GDQ....VV.YK.LDA.AA.R.GQF..S

M  600  ANMERIMKAQALRDNSTMGYMMAKKHLEINPDHPIVETLRQKAEADKN-DKAVKDLVVLLFETALLSSGFSLEDPQTHSNRIYRMIKLGLGIDEDEVTAE
D  593  ..............TA.....AG..Q.................D....-........I.....S........DS..V.AS..............PMTT
Y  587  ..............S.MSS..SS..TF..S.KS..IKE.KKRVDEGGAQ..T....TK..Y.....T.....DE.TSFAS..N.L.S...N.....E.ET

M  699  EPSAAVPDEIPPLEGDEDASRMEEVD
D  692  DDAQSAG.APSLV.DT....H.....
Y  687  A.EASTAA---.V.EVPADTE.....
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Fig. 3. Comparison of mouse Hsp84 with *Drosophila* Hsp82, yeast Hsp90 and chicken Hsp90 amino acid sequences. The amino acid sequences were derived from cloned sequences of each gene and have been aligned to maximize areas of homology. Identical aa residues are noted by a dot. Regions of high charge density (see RESULTS, section **c**) are overlined. X's in the sequence indicate undetermined amino acids. Dashes indicate gaps introduced to maintain alignment. Only a small portion of the chicken sequence has been published. M, mouse Hsp84; D, *Drosophila* Hsp82 (Blackman and Meselson, 1986); Y, yeast Hsp90 (Farrelly and Finkelstein, 1984) and C, chicken Hsp90 (Catelli et al., 1985).

The largest region of amino acid sequence diversity among the three species occurs near the C termini. Presumably, this region is not critical to Hsp84 function. A similar diversity between species occurs in this same region in the Hsp70 proteins.

The most striking difference between amino acid sequence of the mouse and the other two sequences is the insertion of a 7-aa stretch at the N terminus of the mouse sequence. Nucleotide sequence data indicate that the insertion is apparently not the result of a removal of sequence by a splicing event in *Drosophila* and yeast which does not occur in the mouse.

The hydrophobic/hydrophilic character of the *Drosophila* Hsp82 and yeast Hsp90 proteins have been examined (Blackman and Meselson, 1986). A large charged region covering 98 aa residues and a smaller region covering 17 aa residues were noted (overlined in Fig. 3). Although both of these regions occur in sections of low homology between species, the density of charged residues has been maintained in *Drosophila*, yeast and mouse.

(d) Structural features of *hsp84* cDNA

The nucleotide sequence shows 74% and 59% homology over the coding region to *Drosophila* and yeast sequences, respectively (unassigned nucleotides were omitted from the calculations). Outside the coding region, however, there is little or no discernible homology.

The *hsp84* cDNA has a 5′-untranslated region of approx. 94 nt. Sequences immediately preceding the ATG start signal have been proposed to interact with sequences at the base of a loop in the 18S rRNA component of the eukaryotic ribosome (Kozak, 1986). The rat consensus sequence is 5′–CACC–3′ (Kozak, 1984). The *hsp84* cDNA contains the sequence 5′–CAAG–3′ at this position. Only the first 2 nt are homologous to sequences at the nucleotide of the rat rRNA loop. However, the sequence does contain an A 3 nt upstream from the ATG, a condition thought to be especially important for translation initiation (Kozak, 1984).

A computer search for G + C-rich regions in the *hsp84* cDNA revealed that the longest G + C-rich region was contained within the 5′-untranslated region (72% of the residues are either G or C from nt positions −29 to −93). G + C-rich regions in the 5′ leader have been observed in other eukaryotic genes; however, it is neither a property of genes in general nor a property of *hsp* genes. A computer search for regions of dyad symmetry indicated that no stable hairpin loops should form in the 5′-untranslated portion. Such a condition interferes with efficient translation of mRNA (Pelletier and Sonenberg, 1985), and is in keeping with the notion

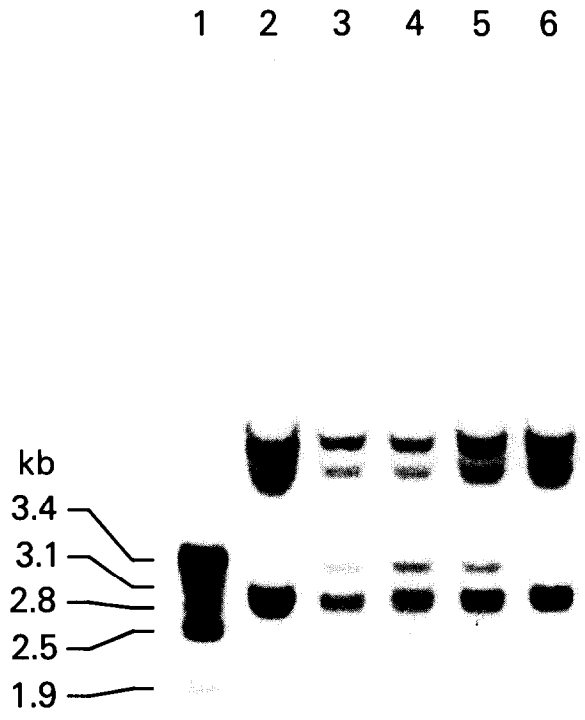

Fig. 4. Southern blot analysis of *Hin*dIII digests of mouse, hamster and selected hamster-mouse hybrid genomic DNAs. Lanes: 1, Balb/c mouse; 2, Chinese hamster; 3–6, selected hybrids. Electrophoretic separation was performed in a 0.7% agarose gel. Southern (1975) blotting (Maniatis et al., 1982) was performed using the hybridization conditions detailed in MATERIALS AND METHODS, section **a**, except that the hybridization temperature was 65°C. The filter was washed sequentially at 65°C with 6× and 1× SCC solutions containing 0.1% SDS. The final wash was in 0.1× SSC + 0.1% SDS at 55°C. The probe was a ^{32}P-nick-translated 0.7-kb *Eco*RI-*Bam*HI segment from the coding region of *hsp84* cloned cDNA (Fig. 1, clone A). The 3.4-, 2.5- and 1.9-kb mouse DNA bands were visible in hybrid DNAs. The 3.1- and 2.8-kb bands were masked by bands from hamster DNA.

that Hsp84 mRNA is efficiently translated upon heat shock. The 5′ leader region of *Drosophila* Hsp70 mRNA has been proposed to contain a distinguishing feature responsible for preferential translation at high temperature. However, the exact nature of this feature still remains unclear (McGarry and Lindquist, 1985; Klemenz et al., 1985).

The 3′-untranslated region is 243 nt long with a consensus polyadenylation signal, 5′–AATAAA–3′ (Proudfoot and Brownlee, 1976), located 18 nt upstream from the site of poly(A) addition. A computer search for A + T-rich regions in the *hsp84* cDNA revealed that the longest A + T-rich region is located at the 3′ end of the cDNA; 82% of the nt residues from positions 2348–2414 are either A or T. A + T-rich sequences in the 3′-untranslated region have been proposed to be involved in the selective degradation of transiently expressed messengers (Shaw and Kamen, 1986). However, human Hsp89 mRNA is thought to be quite stable (Hickey et al., 1986).

(e) Chromosome assignment

High-M_r mouse and Chinese hamster DNA were digested separately with several restriction endonucleases and subjected to Southern blot analysis using a restriction fragment derived from the *hsp84* coding region as probe. *Bam*HI and *Hin*dIII were chosen for use in chromosome assignment experiments.

TABLE II

Assignment of *hsp-1*[a] to mouse chromosome 17

Mouse chromosomes	Number of hybrid clones[b]				
	hsp-1/chromosome retention[c]				% discordant[d]
	+/+	−/−	+/−	−/+	
1	11	5	5	1	27
2	12	3	6	3	35
3	7	5	3	0	20
4	7	5	11	1	50
5	3	5	14	1	64
6	12	4	6	2	33
7	14	2	4	4	33
8	8	6	8	0	36
9	7	4	10	1	50
10	2	6	16	0	67
11	0	5	13	0	72
12	8	2	2	3	33
13	5	3	5	2	47
14	5	5	12	1	56
15	10	1	0	4	27
16	6	4	5	1	38
17	12	5	0	0	0
18	6	4	5	1	38
19	10	3	8	3	46
X	13	5	4	1	22

[a] *hsp-1* designates the locus corresponding to a 3.4-kb *Hin*dIII fragment of mouse DNA.

[b] Fifteen hamster-mouse hybrids were karyotyped; nine hybrids were tested for marker loci.

[c] The number of hybrids which contained both the fragment described in footnote[a] and the designated mouse chromosome, or which lacked both, is indicated in the +/+ or −/− column, respectively. The number of hybrids which contained the fragment but lacked the designated chromosome is indicated in the +/− column, whereas the number which lacked the fragment but contained the chromosome is indicated in the −/+ column.

[d] The % discordance for each mouse chromosome is calculated by dividing the sum of the +/− and −/+ columns by the total number of hybrids examined.

*Bam*HI digestion of Balb/c mouse DNA produced two major bands of 2.3 and 1.5 kb and a minor band of 0.8 kb crossreactive with the probe. An additional band of 1.3 kb was detected in DNA from A/HeJ and NFS strains (not shown). *Hin*dIII produced two major bands of 3.4 and 2.5 kb and three minor bands of 3.1, 2.8 and 1.9 kb (Fig. 4). Chinese hamster DNA also contained sequences crossreactive with the probe and produced 3.3-, 1.5- and 1.4-kb *Bam*HI fragments, and 6.2-, 5.3- and 2.8-kb *Hin*dIII fragments. Thus, the presence or absence of all mouse bands could not be scored in the hybrids since the hamster 1.5-kb *Bam*HI and 2.8-kb *Hin*dIII fragments co-migrated with mouse bands.

Analysis of DNA from somatic cell hybrids showed that each of the discernible mouse bands was produced by single genes. The 2.3-kb *Bam*HI and 3.4-kb *Hin*dIII fragment were both present, or both absent, from the different hybrids indicating that they represent the same genetic locus. Three of the genetic loci detected by the *hsp84* probe could be chromosomally assigned. The two major *Hin*dIII bands (3.4 and 2.5 kb) were assigned to chromosomes 17 and 2, respectively (Tables II and III). No discrepancies were noted for the chromosome 17 assignment and two were noted for the chromosome 2 assignment. However, neither of these lines was karyotyped, but they were scored for a chromosome 2 marker. Therefore, it is possible that these lines contained fragments of chromosome 2, or that the chromosome was present in a low percentage of these cells. This analysis indicated that no other chromosome could be implicated for the 2.5-kb fragment. One of the minor *Hin*dIII bands (1.9 kb) was shown to be on chromosome 12 (Table IV). The minor band from the *Bam*HI digest could not be assigned. In keeping with the designation of the genes for Hsp in other organisms, the loci corresponding to the 3.4, 2.5 and 1.9-kb *Hin*dIII bands have been given the designation *hsp*-1, *hsp*-2 and *hsp*-3, respectively.

TABLE III

Assignment of *hsp-2*[a] to mouse chromosome 2

Mouse chromosomes	Number of hybrid clones[b]				
	hsp-2/chromosome retention[c]				% discordant[d]
	+/+	−/−	+/−	−/+	
1	9	7	6	1	30
2	15	8	2	0	8
3	4	6	5	2	41
4	8	7	9	1	44
5	3	7	13	1	56
6	10	6	7	2	36
7	13	4	4	4	36
8	7	7	8	1	39
9	6	6	9	2	48
10	1	7	16	1	68
11	0	8	11	0	58
12	6	3	2	5	44
13	5	7	3	1	25
14	2	4	14	4	75
15	8	3	0	5	31
16	7	5	3	3	33
17	8	4	2	4	33
18	5	6	4	2	35
19	10	6	7	2	36
X	10	4	6	4	42

[a] *hsp-2* designates the locus corresponding to a 2.5-kb *Hin*dIII fragment of mouse DNA.

[b] Twenty-five hybrids were tested; 16 of these were karyotyped, the rest were typed for the presence or absence of specific marker loci.

[c,d] See Table II.

TABLE IV

Assignment of *hsp-3*[a] to mouse chromosome 12

Mouse chromosomes	Number of hybrid clones[b]				
	hsp-3/chromosome retention[c]				% discordant[d]
	+/+	−/−	+/−	−/+	
1	9	3	7	3	45
2	8	2	9	5	58
3	4	2	6	3	60
4	5	4	13	3	64
5	2	5	13	2	68
6	11	4	6	3	38
7	13	2	4	5	38
8	7	5	9	1	45
9	7	5	9	1	45
10	2	7	15	0	63
11	0	5	11	0	69
12	10	6	0	0	0
13	5	3	5	2	47
14	5	7	11	0	48
15	10	2	0	3	20
16	7	5	3	1	25
17	8	3	3	3	35
18	4	3	7	2	56
19	9	1	8	6	58
X	10	4	6	3	39

[a] *hsp-3* designates the locus corresponding to a 1.9-kb *Hin*dIII fragment of mouse DNA.
[b] Fifteen hybrids were karyotyped; ten hybrids were tested for marker loci.
[c,d] See Table II.

(f) Conclusions

(1) We have determined the nucleotide sequence of the mouse *hsp84* cDNA using a combination of molecular cloning and primer extension on RNA. The cDNA codes for a protein of 724-aa residues which bears homology to the yeast Hsp90 and *Drosophila* Hsp82. Regions of high-charge density of amino acids are also conserved between species. The mouse sequence contains a 7-aa stretch near the N terminus that is not present in yeast or *Drosophila*. Homology in nucleotide sequence between species did not extend outside of the coding regions of these genes.

(2) Southern blot experiments of DNA from hamster-mouse hybrids indicated that *hsp84* is a member of a multigene family. Sequences related to *hsp84* were located on at least three different chromosomes.

In screening cDNA libraries with *hsp84* probes, only clones coding for the Hsp84 isoform were found. The nucleic acids coding for the 84- and 86-kDa isoforms are apparently sufficiently different not to cross-hybridize. This notion is supported by a comparison of partial amino acid sequence data available for the two isoforms. Out of 200 aa residues compared between Hsp84 and Hsp86, there was only 85% homology (E.A.R., S.J.U. and E.A., unpublished observations). Also, Hickey et al. (1986) have reported the isolation of two cDNA plasmids for the human 89-kDa Hsp which hybridize to separate RNA species and do not hybridize with each other.

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