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Isolation and Characterization of a cDNA Encoding a Protein Homologous to the Mouse 70 kDa Heat Shock Protein

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Hsp70, a 70 kDa protein, is the major protein expressed when cells are heat-shocked. A cDNA library from mouse ID13 cells was screened with the human hsp70 gene as a probe, and a positive clone was obtained. The positive clone was subcloned into pUC19 and the precise restriction was obtained. The cDNA was sequenced by the Sanger's dideoxy termination method. Single open reading frame that codes for a protein of 70 kDa was found. The DNA sequence of the cloned mouse DNA shows great homology (66-90%) with other mouse hsp70 genes and somewhat less homology (50%) with *E. coli* hsp70 gene (dnaK). With the exception of one amino acid, the protein sequence deduced from the cDNA is identical to the mouse that shock cognate protein 70 (hsc70) that is constitutively expressed at normal temperature. The result suggests that the cloned cDNA encodes a hsc70 family rather than a heat-inducible family.

KEY WORDS: Heat shock protein, cDNA cloning, DNA sequencing

Cells of all organisms, even phylogenetically as distant as *E. coli* and human, respond to an increase in temperature (hyperthermia) or other environmental stresses such as drugs, arsenite poisoning and ethanol by expression of a small number of proteins, while the expression of most other cellular proteins declines (for review, Pelham, 1986). The major member of these stress proteins is a 70 kDa gene product. The hsp70 proteins have been highly conserved, showing 60-78% identity among eukaryotic proteins, and 40-60% identity between *E. coli* and the eukaryotic hsp70 (Linquist, 1986). While the overall region shows homology, the two-thirds of the amino terminal of hsp70 is much more highly conserved than the carboxy-terminal region.

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All hsp70 proteins bind ATP with high affinity and possess a weak ATPase activity (Linquist and Craig, 1988). It is shown that hsp70 can interact with variety of peptides and their release is dependent upon the hydrolysis of ATP, and the typical example is the uncoating enzyme of coated vesicles (Chappel *et al.*, 1986). The ATP binding and ATPase activity are resided in the two-thirds of the amino terminal of hsp70 protein (Chappel *et al.*, 1987). It is thought that the carboxyl terminal region may function as a domain which interacts with substrate proteins.

DnaK is the only known hsp70 protein in *E. coli*, while eukaryotes have multiple copies of hsp70 genes (hsp multigene family). In the yeast *Saccharomyces cerevisiae*, there appears to be at least eight members of the hsp70 gene family. Several of these hsp70 genes are expressed in response to hyperthermia or other stresses, and others are expressed in normal unstressed condition (Ellwood *et al.*, 1984). These constitutively

expressed proteins are called heat shock cognate proteins (hsc 70). Some hsc70 genes are expressed at all developmental stages, others are expressed only in embryos. Hsc70 is found at higher concentrations in dividing cells than in resting cells (Pelham, 1986). Many data support the idea that hsp70 proteins interact with a variety of cellular proteins and are essential for the movement of proteins with cell. There are many evidences that hsp70 proteins are participating in transportation of proteins into mitochondria, lysosome, and relating to secretory pathway.

The evolutionary conservation of the structural and regulatory elements of eukaryotic hsp70 genes among variable species has made this system a good model for the study of eukaryotic gene expression and the regulation. As a first step to understand the function and the regulation of the genes in the eukaryotic hsp70 multigene family, we cloned mouse hsp70 cDNA gene from mouse ID13 cDNA library. We confirmed by Southern analysis that there exists several hsp70 like genes in ID13 cells and screened the mouse ID13 cDNA library by using human hsp70 as a probe (Hunt and Morimoto, 1985). Here we report DNA sequence analysis of a positive hsp70 clone.

Materials and Methods

Cell Strains, Library, Plasmids, and Phages

E. coli cell, JM105 was used as a host organism for all transformations, and *E. coli* cell C600hfl was used as a host cell of bacteriophage lambda. The mouse cell ID13 was used to purify mouse genomic DNA for southern analysis. cDNA library of ID13 previously constructed by Choe *et al.* (1989) was used to clone the hsp70 gene. Plasmid pUC18, 19 was used as a vector to clone and sequence the DNA fragments, and bacteriophage M13mp18, 19 were also used as sequencing vectors. The plasmid pH 2.3 (Hunt and Morimoto, 1985) containing human hsp70 gene was used as a probe to clone mouse hsp70 cDNA gene.

Media and Cultures

E. coli cells were cultured in LB medium (10 g Bacto-tryptone, 5 g Yeast extract, 10 g NaCl, per liter, pH 7.3) at 37°C and LB agar plate (LB with 1.5% Bactoagar). Ampicillin (50 μg/ml) was used to select the cells harboring plasmids. X-gal (50 μg/ml) and IPTG (40 μg/ml) were used as a selection dye for *E. coli* transformants. ID13 mouse cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum.

Chemicals and Enzymes

[α-^{35}S]dATP and [α-^{32}P]dATP were obtained from Amersham. Restriction enzymes, T4 DNA ligase and calf intestine phosphatase were from Boeringer Manheim and New England Biolabs. DNA sequencing kit was purchased from United States Biochemical Corporation. Nick translation kit was from BRL. GENE CLEAN kit was from BIO101.

Southern Analysis

Chromosomal DNAs of mouse cells ID13 were isolated as described (Sambrook *et al.*, 1989). After digestion of chromosomal DNAs with several restriction endonucleases, DNAs were sized on 0.8% agarose gel and transferred to a nitrocellulose filter as described (Southern, 1975). pH 2.3, containing human hsp70 gene (Hunt and Morimoto, 1985) was labeled by nick translation and used as a probe. The hybridization solution was 50% Formamide/0.5% SDS/5X Denhart's solution/6X SSPE/10 μg/ml denatured salmon sperm DNA. The filter was incubated with probe overnight at 37°C after a 2 hour incubation in hybridization buffer in the absence of probe. The filter was then washed in 1X SSC/0.1% SDS at 25-65°C for 1 hour with several changes of the wash solution.

Screening of ID13 cDNA Library

To isolate heat shock protein gene from a cDNA library of mouse ID13 cells, the plasmid containing human hsp70 gene (pH 2.3) was labeled with ^{32}P and used to screen the ID13 cDNA library by the method of Benton and Davis (1977). 4×10^5 plaques were screened, and we obtained 10 positive clones. The positive clones were screened two times more to selects a well isolated plaque, and we obtained a true positive clone.

Restriction Endonuclease Mapping

Phage DNA of positive clone was isolated, subcloned into pUC19, and digested with several enzymes (EcoR I, Pst I, Kpn I, BamH I, Hind III). The cleaved DNAs were separated by agarose gel (0.8%) electrophoresis. From the banding patterns of DNA fragments, restriction endonuclease map was obtained.

Subcloning of Mouse Hsp70 Fragments into pUC18 and M13mp18, 19

General subcloning techniques were done as described by Sambrook *et al.* (1989). The 2.1 Kb DNA fragment of mouse hsp70 gene was divided into 6 fragments and subcloned into pUC18 and M13mp18, 19.

Nucleotide Sequencing

Restriction fragments of mouse hsp70 cDNA clone were cloned into either M13mp18, 19 phage DNA or pUC18, 19 DNA and the nucleotide sequence was determined by the Sanger's dideoxy chain termination procedure using [α-^{35}S]dATP (Sanger *et al.*, 1977).

Results

Southern blot analysis

The chromosomal DNA of ID13 cells was isolated and digested by several restriction enzymes. The digested DNA was fractionated on 0.8% agarose gel and transferred to a nitrocellulose filter. Human hsp70 DNA was used as a probe for hybridization. After hybridization, the filter was first washed at 25°C and gradually washed at higher temperature. At 50°C, the autoradiogram shows a band at 4.3 Kb DNA fragment of BamH I digestion (Data not shown). At 65°C, all DNA bands were dissappeared indicating that human hsp70 DNA has some homology but does not have complete homology. From these results, we proceeded to isolate mouse hsp70 gene from a mouse cDNA library using human hsp70 as a probe.

Isolation of cDNA homologous to the mouse hsp70 gene

ID13 cDNA library was screened with the human hsp70 gene as a probe and a positive clone was isolated. DNA of the positive clone was purified and the insert was subcloned into Hind III site of pUC19. The plasmid containing mouse hsp70 cDNA gene was named pHSP4. We tried to digest the DNA with many restriction enzymes, but only a few enzymes (Pst I, Sma I, and Sac I) could digest the DNA. The restriction map of pHSP4 is shown in Fig. 1. The cloned DNA was sequenced using [α-^{35}S]dATP and Sequenase version 2.0. Sequensing directions and length of DNA sequenced are shown in Figure 1.

DNA sequence analysis of cloned hsp70 cDNA gene

The DNA sequence of the cloned cDNA is shown in Fig. 2. After computer analysis, single open reading frame that could encode a protein of 70 kDa was identified. The amino acid deduced

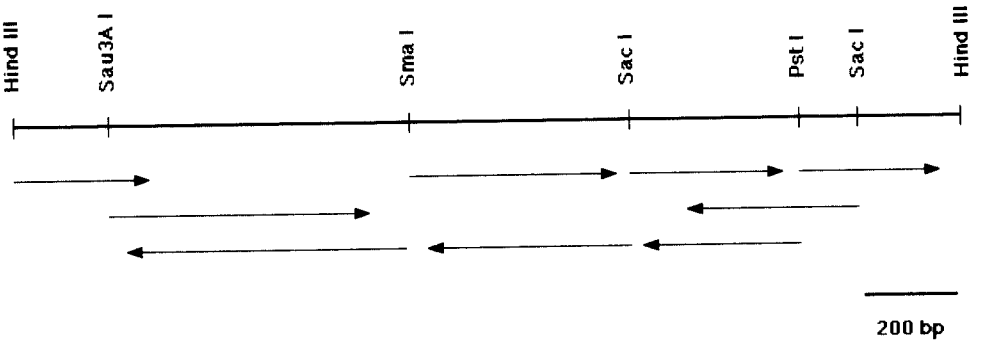

Fig. 1. Restriction enzyme map and sequencing strategy of cloned hsp70 cDNA gene. The restriction sites of several enzymes are noted. The direction and the length of arrows are proportional to the region analyzed from the restriction sites.

```
   1 GGA CCT GCA GTT GGC ATT GAT CTC GGC ACC ACC TAC TCC TGT GTG GGT GTC TTC CAG CAT
   1 Gly Pro Ala Val Gly Ile Asp Leu Gly Thr Thr Tyr Ser Cys Val Gly Val Phe Gln His
     GGA AAG GTG GAA ATT ATT GCC AAT GAC CAG GGT AAC CGC ACC ACA CCA AGC TAT GTT GCT
     Gly Lys Val Glu Ile Ile Ala Asn Asp Gln Gly Asn Arg Thr Thr Pro Ser Tyr Val Ala
     TTC ACG GAC ACA GAG AGA TTA ATT GGG GAT GCG GCC AAG AAT CAG GTT GCA ATG AAC CCC
     Phe Thr Asp Thr Glu Arg Leu Ile Gly Asp Ala Ala Lys Asn Gln Val Ala Met Asn Pro
     ACC AAC ACA GTT TTT GAT GCC AAA CGT CTG ATC GGG CGT AGG TTT GAT GAT GCT GTT GTT
     Thr Asn Thr Val Phe Asp Ala Lys Arg Leu Ile Gly Arg Arg Phe Asp Asp Ala Val Val
     CAG TCT GAT ATG AAG CAC TGG CCC TTC ATG GTG GTG AAT GAT GCA GGC AGG CCC AAG GTC
     Gln Ser Asp Met Lys His Trp Pro Phe Met Val Val Asn Asp Ala Gly Arg Pro Lys Val
 301 CAA GTG GAA TAC AAA GGG GAG ACA AAA AGT TTC TAC CCA GAG GAA GTG TCC TCC ATG GTT
 101 Gln Val Glu Tyr Lys Gly Glu Thr Lys Ser Phe Tyr Pro Glu Glu Val Ser Ser Met Val
     CTG ACA AAG ATG AAG GAA ATT GCA GAA GCG TAC CTC GGA AAG ACT GTT ACC AAC GCT GTG
     Leu Thr Lys Met Lys Glu Ile Ala Glu Ala Tyr Leu Gly Lys Thr Val Thr Asn Ala Val
     GTC ACA GTG CCC GCT TAC TTC AAT GAC TCT CAG CGA CAG GCA ACA AAA GAT GCT GGA ACT
     Val Thr Val Pro Ala Tyr Phe Asn Asp Ser Gln Arg Gln Ala Thr Lys Asp Ala Gly Thr
     ATT GCT GGC CTC AAT GTA CTT CGA ATC ATC AAT GAA CCA ACT GCT GCT GCT ATT GCT TAT
     Ile Ala Gly Leu Asn Val Leu Arg Ile Ile Asn Glu Pro Thr Ala Ala Ala Ile Ala Tyr
     GGC TTA GAT AAG AAG GTC GGA GCT GAA AGG AAT GTG CTC ATT TTT GAC TTG GGA GGT GGC
     Gly Leu Asp Lys Lys Val Gly Ala Glu Arg Asn Val Leu Ile Phe Asp Leu Gly Gly Gly
 601 ACT TTT GAT GTG TCA ATC CTC ACT ATT GAG GAT GGA ATT TTT GAG GTC AAA TCA ACA GCT
 201 Thr Phe Asp Val Ser Ile Leu Thr Ile Glu Asp Gly Ile Phe Glu Val Lys Ser Thr Ala
     GGA GAC ACC CAC TTA GGT GGA GAA GAT TTT GAC AAC CGA ATG GTC AAT CAT TTC ATT GCT
     Gly Asp Thr His Leu Gly Gly Glu Asp Phe Asp Asn Arg Met Val Asn His Phe Ile Ala
     GAG TTC AAG CGA AAG CAC AAG AAA GAC ATC AGT GAG AAC AAG AGA GCT GTC CGC CGT CTC
     Glu Phe Lys Arg Lys His Lys Lys Asp Ile Ser Glu Asn Lys Arg Ala Val Arg Arg Leu
     CGC ACG GCC TGC GAG CGG GCC AAG CGC ACC CTC TCC TCC AGC ACC CAG GCC AGT ATT GAG
     Arg Thr Ala Cys Glu Arg Ala Lys Arg Thr Leu Ser Ser Ser Thr Gln Ala Ser Ile Glu
     ATT GAT TCT CTC TAT GAG GGA ATT GAC TTC TAT ACC TCC ATT ACC CGG GCT CGA TTT GAG
     Ile Asp Ser Leu Tyr Glu Gly Ile Asp Phe Tyr Thr Ser Ile Thr Arg Ala Arg Phe Glu
 901 GAG TTG AAT GCT GAC CTG TTC CGT GGC ACA CTG GAC CCT GTA GAG AAG GCC CTT CGA GAT
 301 Glu Leu Asn Ala Asp Leu Phe Arg Gly Thr Leu Asp Pro Val Glu Lys Ala Leu Arg Asp
     GCC AAG CTG GAC AAG TCA CAG ATC CAT GAT ATT GTC TTG GTG GGT GGT TCT ACC AGA ATC
     Ala Lys Leu Asp Lys Ser Gln Ile His Asp Ile Val Leu Val Gly Gly Ser Thr Arg Ile
     CCC AAG ATT CAG AAA CTT CTG CAA GAC TTC TTC AAT GGA AAA GAG CTG AAC AAG AGC ATT
     Pro Lys Ile Gln Lys Leu Leu Gln Asp Phe Phe Asn Gly Lys Glu Leu Asn Lys Ser Ile
     AAC CCC GAT GAA GCT GTT GCC TAT GGT GCA GCT GTC CAG GCA GCC ATT CTA TCT GGA GAC
     Asn Pro Asp Glu Ala Val Ala Tyr Gly Ala Ala Val Gln Ala Ala Ile Leu Ser Gly Asp
     AAG TCT GAG AAC GTT CAG GAT TTG CTG CTC TTG GAT GTC ACT CCT CTT TCC CTT GGT ATT
     Lys Ser Glu Asn Val Gln Asp Leu Leu Leu Leu Asp Val Thr Pro Leu Ser Leu Gly Ile
1201 GAA ACT GCT GGC GGA GTC ATG ACT GTC CTC ATC AAG CGC AAT ACC ACC ATC CCC ACC AAG
 401 Glu Thr Ala Gly Gly Val Met Thr Val Leu Ile Lys Arg Asn Thr Thr Ile Pro Thr Lys
     CAG ACA CAG ACT TTC ACC ACC TAC TCT GAC AAC CAG CCT GGT GTA CTC ATT CAG GTA TAT
     Gln Thr Gln Thr Phe Thr Thr Tyr Ser Asp Asn Gln Pro Gly Val Leu Ile Gln Val Tyr
     GAA GGT GAA AGG GCC ATG ACC AAG GAC AAC AAC CTG CTT GGA AAG TTC GAG CTC ACA GGC
     Glu Gly Glu Arg Ala Met Thr Lys Asp Asn Asn Leu Leu Gly Lys Phe Glu Leu Thr Gly
     ATC CCT CCA GCA CCC CGT GGG GTC CCT CAG ATT GAG GTT ACT TTT GAC ATC GAT GCC AAT
     Ile Pro Pro Ala Pro Arg Gly Val Pro Gln Ile Glu Val Thr Phe Asp Ile Asp Ala Asn
     GGC ATC CTC AAT GTT TCT GCT GTA GAT AAG AGC ACA GGA AAG GAG AAC AAG ATC ACC ATC
     Gly Ile Leu Asn Val Ser Ala Val Asp Lys Ser Thr Gly Lys Glu Asn Lys Ile Thr Ile
1501 ACC AAT GAC AAG GGC CGC TTG AGT AAG GAA GAT ATT GAG CGC ATG GTC CAA GAA GCT GAG
 501 Thr Asn Asp Lys Gly Arg Leu Ser Lys Glu Asp Ile Glu Arg Met Val Gln Glu Ala Glu
     AAG TAC AAG GCT GAG GAT GAG AAG CAG AGA GAT AAG GTT TCC TCC AAG AAC TCA CTG GAG
     Lys Tyr Lys Ala Glu Asp Glu Lys Gln Arg Asp Lys Val Ser Ser Lys Asn Ser Leu Glu
     TCC TAT GCC TTC AAC ATG AAA GCA ACT GTG GAA GAT GAG AAA CTT CAA GGC AAG ATC AAT
     Ser Tyr Ala Phe Asn Met Lys Ala Thr Val Glu Asp Glu Lys Leu Gln Gly Lys Ile Asn
     GAT GAG GAC AAA CAG AAG ATT CTT GAC AAG TGC AAT GAA ATC ATC AGC TGG CTG GAT AAG
     Asp Glu Asp Lys Gln Lys Ile Leu Asp Lys Cys Asn Glu Ile Ile Ser Trp Leu Asp Lys
     AAC CAG ACT GCA GAG AAG GAA GAA TTT GAG CAT CAG CAG AAA GAA CTG GAG AAA GTC TGC
     Asn Gln Thr Ala Glu Lys Glu Glu Phe Glu His Gln Gln Lys Glu Leu Glu Lys Val Cys
1801 AAC CCT ATT ATT ACC AAG CTG TAC CAG AGT GCA GGT GGC ATG CCT GGG GGA ATG CCT GGT
 601 Asn Pro Ile Ile Thr Lys Leu Tyr Gln Ser Ala Gly Gly Met Pro Gly Gly Met Pro Gly
     GGC TTC CCA GGT GGA GGA GCT CCC CCA TCT GGT GGT GCT TCT TCA GGC CCC ACC ATT GAA
     Gly Phe Pro Gly Gly Gly Ala Pro Pro Ser Gly Gly Ala Ser Ser Gly Pro Thr Ile Glu
     GAG GTG GAT TAA GTC AGT CCA AGA AGA AGG TGT AGC TTT GTT CCA CAG GGA CCC AAA ACA
     Glu Val Asp ***
     AGT AAC ATG GAA TAA TAA AAC TAT TTA AAT TGG CAC CAA AAA AAA AAA AAA AAA AAA AAA
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Fig. 2. Nucleotide and amino acid sequences of cloned mouse hsp70 cDNA gene. One open reading frame that can code 643 amino acids was found. This open reading frame does not have ATG initiation code at it's 5'end. By amino acids sequence comparison with other closely related mouse hsp70 gene (Giebel *et al.*, 1988), it is possible to predict that 3 amino acids (Met, Ser, and Lys) were deleted from the 5'end of the open reading frame. *** indicates the translation termination codon and polyadenylation site are underlined.

from the DNA sequences is also shown in Fig. 2. Unfortunately, our sequenced cDNA does not contain ATG initiation codon. When we compare nucleotide sequence of our cDNA and already cloned mouse hsp70 cDNA (Giebel *et al.*, 1988), we find that 9 nucleotides at 5' end are missing in our hsp70 cDNA. Therefore, we could predict that our hsp70 cDNA consists of the open reading frame of 1944 nucleotides (including TAA termination codon) and codes for 647 amino acids.

So far, 5 members of hsp70 gene family are isolated in mouse and 3 of them are partially cloned and sequenced (Lowe and Moran, 1986) and 2 members are completely sequenced (Zakeri *et al.*, 1988; Giebel *et al.*, 1988). Comparison among their protein coding sequences and our cDNA shows 99.3% (1930 out of 1944 nucleotides; Giebel *et al.*, 1988) and 56% (Zakeri *et al.*, 1988) homology, respectively. However, there is only one amino acid change (Leuo Phe) at amino acid position 425 compared to the amino acid sequence of Giebel *et al.* (1988). The genetic code of that amino acid is changed from CUC to UUC. Changes of 13 nucleotides result in silent change.

A comparison of the protein coding region between the plant 'petunia' hsp70 gene and the cDNA cloned in this experiment reveals 53.0% identity at the nucleotide level and 53.6% at the amino acid level. The sequence of cloned hsp70 cDNA gene also shows homology with many hsp70 genes of other species. Fig. 3 shows amino acid sequence comparison among mouse, human, yeast and *E. coli*. The degree of sequence identity among many species shows the phylogenetic distance between species.

The cloned gene in this experiment uses the codon TAA (Ochre) as a termination codon. The distance measured by the number of nucleotides between the termination codon TAA and the canonical poly (A) signal, 'AATAAA' is 61 nucleotides. Poly (A) signal was followed by the poly (A) sequence by 19 base pairs.

The eight carboxyl terminal amino acids in eukaryotic hsp70, including the cloned mouse hsp70 in this study, are highly conserved (Fig. 3). The carboxyl terminal sequence is 'Gly-Pro-Thr-Ile (Val for yeast)-Glu-Glu-Val-Asp'. Although *E. coli* hsp70 (dnaK) has somewhat different sequence, Ala-Glu-Phe-Glu-Glu-Val-Cys-Asp-Lys-Asp-Lys-Lys, the underlined three amino acids sequence, Glu-Glu-Val is invariantly conserved. The location of the nucleotide sequences conserved among mouse, human, yeast and *E. coli* hsp70 does not appear to be random. When we divide the hsp70 into eleven parts, the parts 3, 4, 7-9 are more conserved than the other parts of protein. Perhaps these conserved regions form functional domains.

Discussion

We cloned a mouse cDNA that is a member of mouse hsp70 gene family. This conclusion is supported by the fact the cDNA shows great homology to many hsp70 genes of other species, and has all conserved sequences of other mouse hsp70 gens. Although many hsp70 genes have been cloned and sequenced (Linquist and Craig, 1988), several members of hsp70 family are not heat inducible and known as heat shock cognate proteins (hsc). We speculate that the cloned gene in this study is a hsc gene according to the following criteria. First, the cDNA library used in this study was made from mouse, ID13 cells growing at normal temperature (in condition of no heat shock). Second, the cloned gene in this study shows much more homology to the coding sequence of the 70 kDa mouse heat shock cognate protein (99.3%) than to heat inducible mouse hsp70 genes (56%). Giebel *et al.* (1988) reported that mouse 70 kDa heat shock cognate protein (hsc70) genes are higly expressed in early stage of mouse development, and is then down-regulated towards the end of embryogenesis. In adult tissues, only the brain retains high level of hsc70 gene expression.

The rate of evolution of hsp70 can be approximated from the percent change in the amino acids sequence of hsp70. The mouse and *E. coli* hsp70 differ in 47% of amino acid sequence. It is well known that cytochrome C and β-subunit of ATP synthetase are highly conserved protein among different species (Dayhoff *et al.*, 1979). Cytochrome C and β-subunit of ATP synthetase show 10-48% and 65% similarity between eukaryotic and prokaryotic species, respectively. The homology between the mouse hsp70 and *E. coli* dnaK is

```
    MOUSE  GPAVGIDLGTT YSCVGVFQHG KVEIIANDQG NRTTPSYVAF T-DTERLIGD
    HUMAN  AA========= ========== ========== ========== =-========
    YEAST  ========== ====AH=A=D R=D======= ======F=== =-========
    E.COLI --II======= K===AIMDGT TPRVLE=AE= D=====II=Y =Q=G=T=V=Q

AAKNQVAMNP TNTVFDAKRL IGRRFDDAVV QSDMKHWPFM VVNDAGRPKV QVEYKGETKS
=======L== Q========= ===K=G=P== ========Q =I==GDK=== ==S======A
=====A==== S========= ===N=N==E= =A====F==K L=DVD===QI ===F=====N
P==R=RVT== ===L=AI=== =====Q=EE= =R=VSIM==K I=AAD-NGDA W==V==--QK

FYPEEVSSMV LTKMKEIAEA YLGKTVTNAV VTVPAYFNDS QRQATKDAGT IAGLNVLRII
=====I==== ========== ===YP===== I========= ==========V ==========
=T==Q===== =G====T==S ===A==ND== ========== ========== ==========
MA=PQ==AE= =K===KT==D ===E===E== ==========A =========R ====E=K===

NEPTAAAIAY GLDKKVGAER NVLIFDLGGG TFDVSILTIE DG----IFEV KSTAGDTHLG
========== ===RTGKG== ========== =========D ==------=== =A=======
========== =====GKE=H T========== =====L=F=E ==------=== ==========
=======L== ====-=T=M= TIAVY===== ===I==IE== EVDGEKT=== L==M======

GEDFDNRMVN HFIAEFKRKH KKDISENKRA VRRLRTACER AKRTLSSSTQ ASIEIDSLYE
=======L== ==VE====== =====Q==== ========== ========== ==L=====F=
========== ===Q=====N ===L=T=Q== L========= =========A= T=V=======
=====S==I= YL=====KDQ GI=LRHDPL= MQ==KE===K ==IE===AQ= TDVNLPYITA

GIDFYTSI-- --TRARFEEL NADLFRGTLD PVEKALRDAK LDKSQIHDIV LVGGSTRIPK
========-- ========== CS======E ========== ===A====L= ==========
========-- ========== C========E ====V===== =====VDE== ==========
DATGPKNMNI KV===KL=S= VE==VN=SI= =LKV==Q==G =SV=D=D=VI ====Q==M=M

IQKLLQDFFN GKELNKSINP DEAVAYGAAV QAAILSGDKS ENVQDLLLLD VAPLSLGIET
V====------ =AD====== ====G===== =====M==== ========== =======L==
====VT=Y== ===P=R==== ========== =====T==E= SKT======= ==========
===KVAE==- ===PA=DV== ==E===AI== ===GGV=T== -----=K=V== =T========

AGGVMTVLIK RNTTIPTKQT QTFTTYSDNQ PGVLIQVYEG ERAMTKDNNL LGKFELTGIP
======A=== ==S======= =I======== ========== ========== ==R===SC==
======K==P =====S==KF E==S==R=== ========F== ===K====== ==K=======
M=====T==A K=T=====HS =V=S=RE=== SA=T=H=LQ= ==KAAA==KS ==Q=M=D==M

PAPRGVPQIE VTFDIDANGI LNVSAVDKST GKENKITITN DKGRLSKEDI ERMVQEAEKY
===-======= ========== ===T=TKD== ==A======= ========E= ==========
===R====== ====V=S=== =======E=G= ==S======= ========== =K==A====F
=====M==== ========D== ===S=K==NS ===Q====KA SS=-=NED== QK==RD==AN

KAEDEKQRDK VSSKNSLESY AFNMKATVED EKLQGKINDE DKQKILDKCN EIISWLDKNQ
=====V==ER ==A==A==== =====SA=== =G=K===SEA ==K======Q =V=====A=T
=E====SKQ= IA===Q===I =YSL=N=ISE A=D=LEQA=K =TVTKKAEET --======S==
AEA=RKFE=L =QTR=QGDNL LNSTRKQ==E A=D-==LPAD ==TA=ESALR ALETA=KGED

TAEKEEFEHQ QKELEKVCNP IITKLYQSAG GMPGGMPGGF PGGGAPPSGG ASS--GPTIE
L===D====K A====Q==== ==SG===G== ----------- ---==QGPK= GSGS-======
==S====DD= L===QDIA== =M========= =A=G=GA==A ===FPGGAPP APEAE===V=
K=RI=A---= NQ==A==SQK LMEIAQ=QHA QQQTAGRDAS ANNAKDDDVV DAEF------=

EV-D            MOUSE   647 aa*
==-=            HUMAN   640 aa
==-=            YEAST   643 aa
==K=KK          E.COLI  638 aa
```

Fig. 3. Amino acid sequence comparison between several organisms. Double line (=) represents amino acid residues that are the same relative to the mouse hsp70 protein, and dashes indicate gaps to maintain an optimal sequence alignment. The amino acid sequence of hsp70 protein is derived from DNA sequence of hsp70 cDNA from this study, human hsp70 from Hunt and Morimoto (1985), yeast from Ingolia *et al.* (1982), *E. coli* dnaK from Bardwell and Craig (1984).

thus comparable with the homology seen in some proteins known as the most highly conserved.

All hsp70 proteins studied so far have binding affinity to ATP, and ATPase activity. ATP is hydrolyzed when the hsp70 is released from the substrate protein (Flynn *et al.*, 1989). Hsp70 also has binding affinity to many peptides and protein. The injection of denatured protein into *Xenopus* oocytes induces the heat shock responses (Ananthan *et al.*, 1986). The chemicals such as ethanol, puromycin which could make denatured proteins, also induce the heat shock response (Beckman *et al.*, 1990). These data show that the important factor to induce heat shock, is not only heat shock but all stresses which make the denatured proteins. It is possible to make a model of heat shock response as follows. Many stresses which make denatured proteins, increase the activity or the amount of heat shock factor (HSF). The increased activity of HSF induces the expression of hsp70 by binding to heat shock element (HSE). Then the synthesized hsp70 makes the denatured proteins renatured, or brings them into the degradation pathway.

It is an important question that by what mechanism the stresses could increase the activity or the amount of HSF. Craig and Gross (1991) hypothesized a model that HSF are bound to hsp70 in normal state. When cell is heat-shocked, the denatured proteins take the hsp70 protein away from HSF, which makes the HSF trimer and bound to HSE, thus inducing the synthesis of hsp70. This hypothesis sounds plausible because hsp70 has binding affinity to many proteins, which shows possibility of binding to HSF. Another clue to the question is the phosphorylation of HSF. In human or yeast, HSF becomes highly phosphorylated following heat shock, and the transcriptional activity of HSF is closely correlated with the extent of its phosphorylation (Larson *et al.*, 1988). It is not clear, however, how close the phosphorylation is related to the regulation of heat shock responses. The most important but yet unresolved issues of heat shock responses are the mechanisms by which heat shock is sensed, autoregulation is achieved and heat shock factor activates tranacription. These are the puzzles we should really resolve.

References

Ananthan, J., A. R. Goldberg, and R. Voellmy, 1986. Abnormal proteins as eukaryotic stress signals and trigger the activation of heat shock genes. *Science* **232:** 522-524.

Beckmann, R. D., L. Mizzen, and W. Welch, 1990. Interaction of hsp70 with newly synthesized poteins: implication for protein folding and assembly. *Science* **249:** 850-856.

Benton, W. D. and R. W. Davis, 1977. Screening lambda-gt 11 recombinant clones by hybridization to single plaques *in situ*. *Science* **196:** 180-182.

Chappell, T. G., W. J. Welch, D. M. Schlossman, K. B. Palter, M. J. Schlesinger, and J. E. Rothman, 1986. Uncoating ATPase is a member of the 70 kilodalton family of stress proteins. *Cell* **45:** 3-13.

Chappell, T. G., B. B. Konforti, S. L. Schmid, and J. E. Rothman, 1987. The ATPase core of a clathrin uncoating protein. *J. Biol. Chem.* **262:** 746-751.

Choe, J., P. Valliancourt, A. Stenlund, and M. Botchan, 1989. Bovine papillomavirus type 1 encodes two forms of a transcriptional repressor: structural and functional analysis of new viral cDNAs. *J. Virol.* **63:** 1743-1755.

Craig, E. A. and C. A. Gross, 1991. Is hsp70 the cellular thermometer? *Trends Biol. Sci.* **16:** 135-140.

Dayhoff, M. O., 1979. Atlas of protein sequence and structure. National Biomedical Research Foudation, Silver Spring, MD.

Ellwood, M. S., and E. A. Craig, 1984. Differential regulation of the 70 kDa heat shock and related genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **4:** 1454-1459.

Flynn, G. C., T. G. Chapell, and J. E. Rothman, 1989. Peptide binding and release by proteins implicated as catalysts of protein assembly. *Science* **245:** 385-390.

Giebel, L. B., B. P. Dworniczak, and E. K. F. Bautz, 1988. Developmental regulation of a constitutively expressed mouse mRNA encoding a 72 kDa heat shock-like protein. *Devel. Biol.* **125:** 200-207.

Hunt, C. and R. I. Morimoto, 1985. Conserved features of eukaryotic hsp70 genes revealed by comparison with nucleotide sequence of human hsp70. *Proc. Natl. Acad. Sci. USA* **82:** 6455-6459.

Ingolia, T. D., M. R. Slater, and E. A. Craig, 1982. *Saccharomyces cerevisiae* contains a complex multigene family related to the major heat shock-inducible gene of *Drosophila*. *Mol. Cell. Biol.* **2:** 1388-1398.

Larson, J. S., T. J. Shuets, and R. E. Kingston, 1988. Activation in vitro of sequence specific DNA binding

by a human regulatory factor. *Nature* **335:** 372-375.

Linquist, S. 1986. The heat shock response. *Ann. Rev. Biochem.* **55:** 1151-1191.

Linquist, S. and E. A. Craig, 1988. The heat shock proteins. *Ann. Rev. Biochem.* **22:** 631-677.

Lowe, D. G. and L. M. 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.

Pelham, H. R. B., 1986. Speculation on the function of the major heat shock and glucose-regulated protein. *Cell* **46:** 959-961.

Sambrook, J., E. F. Fritsch, and T. Maniatis, 1989. *Molecular Cloning, A Laboratory Manual, Second Edition,* Cold Spring Harbor Laboratory Press. New York.

Southern, E. M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98:** 503-517.

Zakeri, Z. F., D. J. Wolgemuth, and C. R. Hunt, 1988. Indentification and sequence analysis of a new member of the mouse hsp70 gene family and characterization of its unique cellular and developmental pattern of expression in the male germ line. *Mol. Cell. Biol.* **8**: 2925-2932.

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생쥐 섬유아세포에서 70 kDa 고온충격 단백질의 cDNA 클로닝과 염기서열 분석

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고온 충격 단백질 70은 분자량이 70 kD으로 세포를 42°C에서 배양했을 때 생성되는 단백질 중 대표적인 것이다. 우리는 생쥐의 섬유아세포인 ID13세포에서 hsp70 유전자를 분리하기 위하여 사람의 hsp70 유전자를 탐침으로 사용하여 ID13 세포의 cDNA library를 스크리닝하여 양성 클론을 찾아냈다. 얻은 cDNA 유전자를 pUC18, 19 또는 M13mp18, 19에 옮겨 DNA 염기서열을 분석하였다. 그 결과 이 cDNA는 643개 아미노산으로 이루어진 단백질의 정보를 담을 수 있는 것이었다. 이 생쥐의 섬유아세포 hsp70 유전자의 염기서열은 이미 발표된 생쥐의 hsp70과 큰 유사도(60-99%)를 보였고, 대장균의 hsp70(dnaK)과는 50% 정도의 유사도를 보였다. 또한 고온 충격을 주지 않아도 정상 상태에서 발현되는 heat shock cognate protein(hsc70)과 커다란 유사도(99%)를 보이는 것을 볼 때 우리가 클로닝한 유전자는 정상 온도에서도 발현되는 hsc70의 일종으로 추정된다.