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Genomic Structure of the Spermatid-Specific *Hsp70* Homolog Gene Located in the Class III Region of the Major Histocompatibility Complex of Mouse and Man[1]

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The *Hsc70t* gene is a *Hsp70* homolog gene expressed constitutively in spermatids in mice. This gene is linked to two heat-inducible *Hsp70* genes, *Hsp70.1* and *Hsp70.3*, located in the MHC class III region. The syntenic region of human chromosome 6 contains the *HSPA1B*, *HSPA1A*, and *HSPA1L* genes. Here, we have isolated a *HSPA1L* cDNA clone from human testicular cells. The *HSPA1L* gene contained an intron 13 bp upstream of the initiating ATG. A similar genomic structure was found in the *Hsc70t* gene. The transcription initiation site of the *Hsc70t* gene was located at *ca.* 600 bp upstream of the heat-inducible *Hsp70.3* gene, linked head-to-head. Sequence alignment of the mouse and human genes revealed that the human *HSPA1L* and *HSPA1A* genes were orthologous to the mouse *Hsc70t* and *Hsp70.3* genes, respectively. Conserved sequence stretches observed in the 5′ flanking region and the first exon of the spermatid-specific *Hsp70* gene may be involved in regulation of the specific gene expression.

Key words: genomic structure, *Hsp70*, MHC, promoter, spermatogenesis.

A 70 kDa heat shock protein (HSP70) is evolutionarily conserved and phylogenetically ubiquitous in all organisms. Most eukaryotes have a large family of at least 5 *Hsp70*-related genes (*1-3*). These genes exhibit close homology at the amino acid sequence level, but their expression is regulated differently.

A unique expression pattern of *Hsp70*-related genes has been reported in male germ cell differentiation in the mouse and rat (*4, 5*). The *Hsp70.2* gene in the mouse and the orthologous gene, *Hst70*, in the rat have been cloned, and it has been shown that their expression starts in spermatocytes during spermatogenesis (*6, 7*). Recently, a human homolog of these genes, *HSPA2*, was cloned, and it was shown that *HSPA2* is constitutively expressed in most tissues, with a high level in the testis (*8*). We have isolated another mouse testis-specific *Hsp70* gene, *Hsc70t*, transcribed exclusively after meiosis during mouse spermatogenesis (*9, 10*). At present, the *Hsc70t* gene is the only member of the *Hsp70* gene family known to be expressed in a cell-type specific manner.

The *Hsc70t* gene was physically mapped to the central region of the mouse MHC linked with the heat-inducible *Hsp70.3* and *Hsp70.1* genes (*3, 11*). The genomic organization of these *Hsp70* genes in the mouse MHC is consistent with that of *HSPA1B, HSPA1A*, and *HSPA1L* (alias *HSP70-2, HSP70-1*, and *HSP70-Hom*, respectively) in the class III region of the human MHC (*12, 13*). The amino acid sequence of *HSPA1L* is 90% homologous to those of heat-inducible genes *HSPA1B* and *HSPA1A*. Despite their sequence similarity, these genes differ in the regulation of their expression (*13*).

In the present work, *HSPA1L* cDNA was isolated from a human testis cDNA library, with antiserum against the murine *Hsc70t* gene product. Sequence comparison of cDNA and genomic DNA in mouse and man revealed the evolutionarily conserved, novel genomic structure of the spermatid-specific *Hsp70* gene, in which the 5′ untranslated region is interrupted by an intron. In addition, we mapped the transcription start point of the *Hsc70t* gene approximately 600 bp upstream of that of the *Hsp70.3* gene. Our data allowed us to perform comparative analyses of *Hsc70t* and homologous genes expressed in the mammalian testis to understand the regulation of their gene expression.

MATERIALS AND METHODS

cDNA Screening—To prepare an antiserum against the

[1] The sequence data in this article have been deposited in the DDBJ/EMBL/GenBank DNA databases under accession nos. D85730, D85731, D85732, D85733, and D85734.

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Abbreviations: HSP70, 70 kDa heat shock protein; HSC70t, 70 kDa heat shock protein constitutively expressed in spermatids; MHC, major histocompatibility complex; RACE, rapid amplification of cDNA ends.

mouse HSC70t protein, a 1.1 kb *Pst*I fragment of the pHS2 cDNA clone, which encoded a part of the carboxy terminal amino acids (377-641) of mouse HSC70t (*9*), was subcloned in frame into the pPUR292 *lacZ* expression vector (*14*). The electrophoretically purified fusion protein was injected subcutaneously into rabbits. For the isolation of antibodies specific to testicular germ cells, the antiserum was absorbed with bacterial lysates of *E. coli* cells expressing β-gal derived from the pUR292 vector using an Affigel-15 (Bio-Rad, Hercules, CA) affinity column. The character of the antiserum was described by Matsumoto *et al.* (*15*). On two-dimensional Western blotting analysis, the antiserum strongly reacted with a novel 70 kDa protein in the late spermatid fraction. Immunohistochemical analyses of mouse testis sections revealed that the antigen was present in the cytoplasm of late spermatids. The antigens recognized by this antiserum were also detected on immunohistochemistry in the human testis (*16*).

A human testis cDNA library from a healthy 50-year-old, constructed at the *Eco*RI site of λgt11 (Clontech, Palo Alto, CA), was screened for phages containing the human cDNA counterpart of the mouse *Hsc70t* gene by immunoscreening. Immunoscreening was performed with absorbed rabbit antiserum against the mouse HSC70t protein as the first antibody, followed by alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (Cappel, West Chester, PA). Positive clones were detected with nitroblue tetrazolium as the chromogen. Inserts from positive phage clones were isolated by *Eco*RI digestion and subcloned into the *Eco*RI site of the pBluescript II vector.

An adult mouse BALB/c testis cDNA library constructed at the *Eco*RI site of λgt11 (Clontech, Palo Alto, CA) was screened for *Hsc70t* full length cDNA-containing phages by means of the same immunoscreening. This library was also screened by hybridization to a *Bam*HI-*Eco*RI fragment from pHS2 (a BTBRTF strain partial length cDNA; *9*). Inserts from positive bacteriophage clones were isolated by *Eco*RI digestion and subcloned into the *Eco*RI site of the pBluescript II vector. To obtain the maximum possible amount of the 5′ flanking sequence of *Hsc70t* cDNA, a 5′-RACE reaction was performed with cDNA templates premade from BALB/c testes RNA (Marathon-Ready cDNA; Clontech, Palo Alto, CA) using a Marathon cDNA adapter primer and an *Hsc70t* gene-specific reverse primer (5′-CTTCATATCTGACTGCACAACAGG-3′, corresponding to complementary sequences to sequences encoding amino acid sequence 83 to 90), according to the manufacturer's protocol. The amplified product was a highly specific fragment that was gel-purified and cloned into the pGEM-T vector.

Genomic DNA Screening—A human cosmid genomic library was constructed by ligation of a *Sau*3AI partial digest of DNA from an HLA homozygous B cell line, AKIBA (A24, B52, Cw-, DR2, DQ6, Cp63), into the *Bam*HI site of the pWE15 cosmid vector (*17*). Colonies were screened with ^{32}P-labeled inserts, one of the human cDNA clones, HUM70t, isolated from the testis library, and the human heat-inducible *HSP70* genomic DNA clone, pH 2.3 (*1*). Two positive clones, pAH6051 and pAH6036, were characterized by comparing their restriction maps with those reported previously by Milner and Campbell (*13*). A 3 kb *Bam*HI fragment containing upstream *HSPA1A* sequences was subcloned from pAH6036 into the pBluescript II vector.

A mouse genomic clone λg13 containing the *Hsc70t* gene was isolated from the 129/SvJ mouse genomic library as previously described (*9*). This clone contains 7 kb and 6 kb *Eco*RI fragments, labeled with GE7 and GE6, respectively. The GE6 subclone contains the amino acid coding region of *Hsc70t*. Parts of the GE7 clone, NA16, NA32, NA8, and NA6, were subcloned into the pBS vector to determine the upstream region of the *Hsc70t* gene.

Primer Extension Experiment—Total RNA was extracted from adult testes of the 129/SvJ strain mice according to the method of Chirgwin *et al.* (*18*). Poly(A)$^+$ RNA was prepared using Oligotex-dT30 (Japan Synthetic Rubber/Nippon Roche, Tokyo). Primer extension was conducted as described (*19*). Antisense DNA was extended using poly(A)$^+$ RNA as a template, with reverse transcriptase (Takara, Tokyo). An oligonucleotide primer, P-5 (5′-AGCTCCAGGAACATCCAAACTGAGC-3′), starting at the 79th nucleotide upstream from ATG as an antisense sequence was used to extend the 5′ portion of the cDNA (Fig. 1b). The primer was labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Takara). The extended products were analyzed on a denaturing 8% polyacrylamide gel.

Nuclease Protection Assay—For nuclease protection a 349-nt antisense DNA was generated from primer P-5 through thermal cycle extension of a *Stu*I-digested pNA6 plasmid, containing a *Stu*I-*Sac*I genomic DNA fragment as a template, with *Taq* polymerase (Takara). The extended product was gel-purified and ^{32}P-end-labeled. The mapping of the 5′-end of *Hsc70t* RNA was conducted essentially as described by Berk and Sharp (*20*) except that mung bean DNA nuclease (Takara) was used for digestion of the DNA-RNA hybrids instead of S1 nuclease. The protected fragments were separated on a denaturing 8% polyacrylamide gel. The P-5 primer was used for parallel sequencing of the pNA6 plasmid DNA, which allowed comparison with the corresponding genomic nucleotide sequence as the size marker.

DNA Sequencing—The nucleotide sequences of genomic and complementary DNAs, and 5′-RACE products were determined by the dideoxy-chain termination method using fluorescein auto DNA sequencers (Genesis 2000, Dupont; and ABI PRISM377, Perkin Elmer). Sequence data were analyzed with MacMolly Tetra (Soft Gene GmbH) or GeneWorks (IntelliGenetics, Mountain View, CA) software packages on a Macintosh personal computer.

RESULTS AND DISCUSSION

cDNA Cloning and Genomic Structure of the Human HSPA1L Gene—Antigens, recognized by an antiserum against the fusion protein from the mouse *Hsc70t* cDNA clone, were detected by immunohistochemistry in the human testis (*16*). On the basis of this evidence, we attempted to isolate a member of the human *HSP70* gene family from a human testis cDNA expression library using the antiserum. Sequence alignment of positive clones showed a continuous 2,371 bp stretch of cDNA containing an open reading frame, coding for a 641 amino acid protein, similar to the heat-inducible HSP70 protein. We have referred to this cDNA clone as HUM70t (see database, M85730). Although there were 11 single-base discrepancies between the published *HSPA1L* genomic sequence

a

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genome -3053 AAATTTGCAGGGAGGGACGGGGTGGGGGGTGGGGGGACCCCGGTTGTGCAGTTTGATATT
  cDNA  -135                  GACGGGGTGGGGGGTGGGGGGACCCCGGTTGTGCAGTTTGATATT

       -2993 GAGGGAGCCCCCACCTACTCGCTGGGGCTGCGTAATCTGGACGTTTCCAAACTGAAGCGA
         -9  GAGGGAGCCCCCACCTACTCGCTGGGGCTGCGTAATCTGTACGCTTCCAAACTGAAGCGA

       -2933 AGGCGTCGGGAGACTAGGTGAGCCAGTAGGGATCTGATTGATTGTGAACCTGAAAGGCTC
         -30 AGGCGTCGGGAGACTAG----------------------------------------

       -2873 AGTAGGTCCTTGTTTGG      (2770bp)     AAGACCCTGTCTCAAAAAAATAAATA
             ----------------------------------------------------------

         -61 ACATTGAAAAGGAACTCTCCCAAAAGTATCTTATTCTTTCTCCATAGGCCTCAGAGAACC
         -13 ----------------------------------------------GCCTCAGAGAACC

          +1 ATGGCTACTGCCAAGGGAATCGCCATAGGAATCGACCTGGGCACCACCTACTCCTGTGTG
          +1 ATGGCTACTGCCAAGGGAATCGCCATAGGCATCGACCTGGGCACCACCTACTCCTGTGTG
             M  A  T  A  K  G  I  A  I  G  I  D  L  G  T  T  Y  S  C  V
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b

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genome -4249 AAAGTTGCCGGGAGGGGCGGGGTGGGGGGTGGGGGGGACCCCGGCTGCTCAGTTTGGATG
  cDNA  -144       TGCCGGGAGGGGCGGGGTGGGGGGTGGGGGGGACCCCGGCTGCTCAGTTTGGATG

       -4189 TTCCTGGAGCTCCCTGCTCGTAGGGGCTCAGCGACTTCAAATTTTCCAAACTGGATCGAA
         -89 TTCCTGGAGCTCCCTGCTCGTAGGGGCTCAGCGACTTCAAATTTTCCAAACTGGATCGAA

       -4129 GGCGTAGAGATCCCAGGTGAGCTGGGAAGGCTCTGATTGGAGTGGGGCAGAAGCCTCTGT
         -29 GGCGTAGAGATCCCAG----------------------------------------

       -4069 GTCCCAGTTGGTCCTC      (3970bp)     ATAGCTTAAGTTTTATTTAAAA
             ----------------------------------------------------------

         -61 AGAAAACACTAGCCTAAATATGATTTTCAAGTCTCCTTTCTCCATAGGCCTCGGAAAACC
         -13 ----------------------------------------------GCCTCGGAAAACC

          +1 ATGGCTGCTAATAAAGGAATGGCGATCGGCATCGACTTGGGCACCACCTACTCGTGCGTG
          +1 ATGGCTGCTAATAAAGGAATGGCGATCGGCATCGACTTGGGCACCACCTACTCGTGCGTG
             M  A  A  N  K  G  M  A  I  G  I  D  L  G  T  T  Y  S  C  V
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Fig. 1. **Sequence comparisons of the genome and cDNA in man (a) and mouse (b).** The upper line shows the sequence of the genome and the lower line the sequence of the cDNA, in which the split region is indicated by a dashed line. The third line shows the deduced amino acid sequences. Nucleotides are numbered on the left with the translation initiation site designated as +1. Alignment of the 5′ untranslated sequences indicates that both genes are divided by intervening sequences. The splice junction dinucleotides (GT/AG) are underlined. In the human *HSPA1L* gene (a), the downstream genomic sequences after the abridged intron were taken from Milner and Campbell (*13*). The 30th base, A, in the sequence deposited by Milner and Campbell (M34269) is replaced by C in the *HSPA1L* cDNA sequence. The complete sequence of the *HSPA1L* cDNA, and total upstream sequence of the *HSPA1L* gene are registered in GenBank, D85730 and D85731, respectively. In the mouse *Hsc70t* gene (b), the complementary sequences (−103 to −79) to the P-5 primer used for determination of the transcription start point are double-underlined. The complete cDNA sequence of *Hsc70t* and the total intron sequence of the genome are registered in GenBank, D85732 and D85733, respectively.

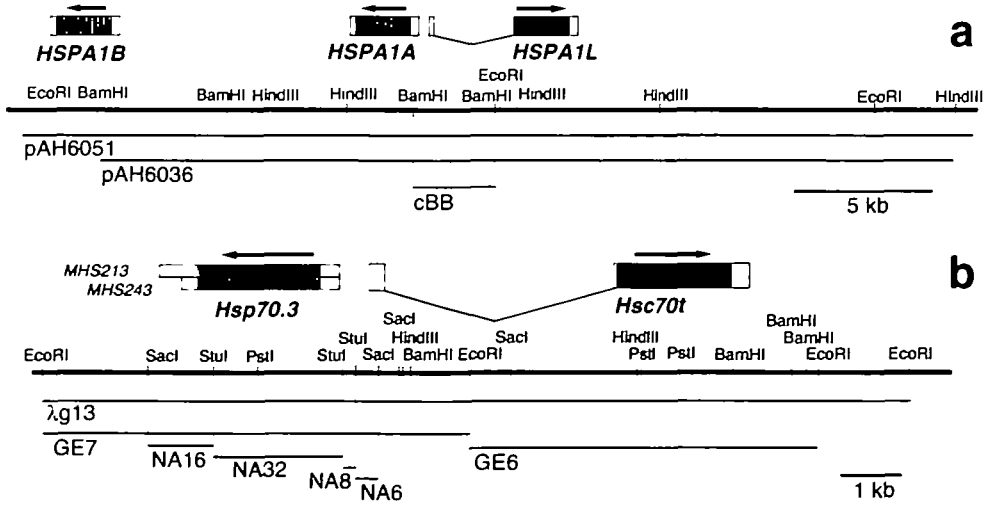

Fig. 2. **Restriction map and genomic organization of a part of the MHC class III region containing a cluster of *HSP70* homologs in man (a) and mouse (b).** The exons are indicated by boxes and the shaded areas represent the coding regions. An arrow indicates the direction of transcription of each gene. The human genome (a) was isolated from two cosmid clones, pAH6051 and pAH6036. The restriction mapping sites mostly correspond with data of Milner and Campbell (*13*). Genomic subclone cBB, containing a *Bam*HI fragment, was used for detection of the first exon of *HSPA1L*, which is shown in the top scheme. The mouse genome (b) was isolated from a λg13 clone. The mapping sites are compared with sites found in the cosmid clones (*11*). *Hsp70.3* encodes two transcripts, *hsp68* MHS243 and MHS213 (*22*). Genomic subclones GE6 and GE7, containing *Eco*RI fragments, were used to determine the genomic structures of these genes. Clone NA6 was used for determination of the transcription initiation site of the *Hsc70t* gene.

(*13*) and the sequence of the cDNA clone, the sequences matched each other in the coding and 3′ untranslated regions. As shown in Fig. 1a, however, the 119 bp of the 5′ untranslated sequence found in the cDNA clone was not present in the published sequences of *HSPA1A* and *HSPA1L* (*13*). This suggests that the *HSPA1L* gene may contain at least one intron in its 5′ untranslated region. The missing sequences could be located in the *Bam*HI 3.0 kb fragment between the *HSPA1A* and *HSPA1L* loci (Fig. 2a). Nucleotide sequencing of the *Bam*HI 3.0 kb fragment confirmed that a sequence completely matching the 5′ untranslated region of the cDNA was present in this region (Fig. 1a).

Genomic Structure of the Mouse Hsc70t Gene—In order to compare the genomic structure of the human *HSPA1L* with that of murine *Hsc70t*, cDNA clones carrying 5′ untranslated sequences of *Hsc70t* were isolated. The sequence of one of the cDNA clones, FB-1, isolated from a BALB/c mouse testes cDNA expression library with the antibody and with a DNA probe for the 3′ untranslated region of the *Hsc70t* cDNA almost completely matched the previously reported *Hsc70t* sequence (see database, D85732, *9*; the published sequences have been corrected in GenBank, M32218, *21*). The newly isolated murine *Hsc70t* cDNA has mismatched sequences in the 5′ untranslated region compared to the GE6 genomic *Eco*RI 6 kb fragment previously isolated, as does the human *HSPA1L* cDNA (Fig. 1b).

To detect further sequences upstream in the genomic clone, the GE7 genomic *Eco*RI 7 kb fragment was subcloned from the same λg13 phage clone containing the GE6 clone. The resultant *Eco*RI fragments corresponded with the recently reported restriction map sites of the region containing the *Hsp70.3* and *Hsc70t* genes, defined by overlapping cosmid clones (Fig. 2b; *11*). Actually, the GE7 genomic clone contains the sequences of *Hsp70.3* encoding two mRNAs carrying different poly(A) signal sites, MHS213 and MHS243, the *hsp68* cDNA clones described by Lowe and Moran (*22*) (data not shown; the sequences are registered in GenBank, D85734). Nucleotide sequencing of the GE6 and GE7 genomic clones made it clear that sequences corresponding to the 5′ untranslated sequences of the *Hsc70t* cDNA are located in the upstream region of the *Hsp70.3* gene in the GE7 clone (Fig. 1b). Thus, we detected the presence of only one intervening sequence of *ca.* 3 kb and *ca.* 4 kb in the human and mouse genomes, respectively (Fig. 2). The sequence at the exon-intron junction border in both genes agrees with the splicing consensus sequence (*23*; Fig. 1).

Linkage between the MHC and *Hsp70* genes, and physical mapping of three *Hsp70* homologs, *Hsp70-1*, *Hsp70-2*, and *Hsp70-3*, have been shown in the rat (*24*). The homologous relationship between mouse *Hsc70t* and rat *Hsp70-3* is supported by the results obtained on interspecies sequence comparisons, in particular of the 3′ untranslated regions. Recently, it was shown that the rat *Hsp70-3* gene contains an intron in the same genomic region (*25*).

Characterization of the Transcription Initiation Site of the Mouse Hsc70t Gene—The 5′ end of the murine *Hsc70t* gene was determined by means of the primer extension and single-stranded DNA nuclease mapping methods. As a result, a major initiation site was found, with a corresponding band, with both methods at A, the position being assigned as +1 (Fig. 3). Five nucleotides were removed from this initiation site at the 5′-end of the cDNA generated by the 5′-RACE method using the P-5 primer, as shown in Fig. 1b. This may have been caused by the action of T4 DNA polymerase, which was used to create blunt ends on the double stranded cDNA for adapter ligation. As shown in Fig. 3, the 71 nt protected band observed on nuclease protection assaying in lane 1 is consistent with the major band observed on the primer extension assaying in lane 2. Although the major site on nuclease protection assaying appeared to be 2 bp beyond this band, this may be due to steric interference by the cap structure of RNA (*26*). As a result, a major initiation site was found at A, at the position denoted by an asterisk (Fig. 3b).

There was no canonical TATA element located at the characteristic position near the transcription initiation site (*27*). The transcription start site was, however, overlapped by the loose consensus sequence of an initiator (Inr), PyPyA+1NT/APyPy, which is sufficient for determining the start site location in TATA-less promoters (*28*). These sequences are almost completely conserved in the mouse

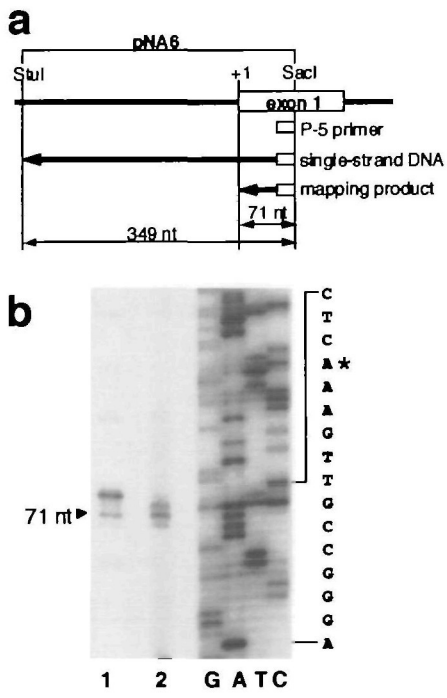

Fig. 3. **Determination of the transcription initiation site (+1) of the mouse *Hsc70t* gene.** (a) The strategy used to determine the location of +1. For mung bean nuclease protection mapping, oligonucleotide primer P-5 was used to generate an antisense 349-nt single-strand DNA probe from a *Stu*I-digested pNA6 plasmid containing a *Stu*I-*Sac*I genomic fragment by thermal cycle extension. (b) Lane 1, mung bean nuclease protection assay results with mouse testis RNA. Lane 2, primer extension analysis with primer P-5 was performed on testis poly(A)$^+$ RNA. Lanes G, A, T, and C are DNA sequence ladders of genomic clone NA6 with the same primer, P-5. The sequence of 16 bases containing the transcription initiation site (*) is shown on the right. The 71-nt bands corresponding to the protected fragment observed on mung bean nuclease protection assaying and the extended product observed on primer extension analysis are indicated by an arrowhead. The major band in lane 1 may have been caused by steric interference by the cap structure of RNA.

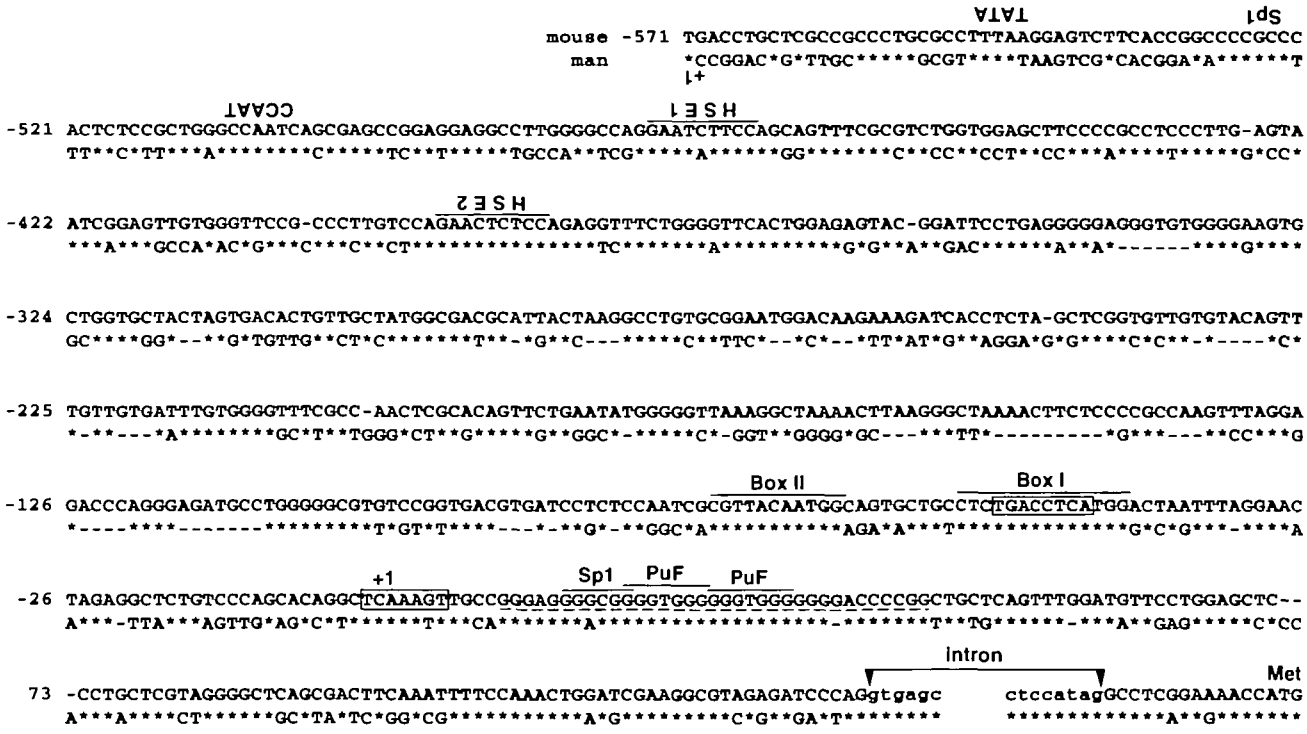

Fig. 4. **Comparison of the nucleotide sequences of the 5′ flanking region, exon 1 and a part of exon 2 of the murine *Hsc70t* and human *HSPA1L* genes.** The *Hsc70t* gene in the upper line is aligned with the *HSPA1L* gene in the lower line. Sequences that differ from *Hsc70t* are shown, identical sequences being indicated by asterisks. Dashes are inserted to optimize alignment of the sequences. The sequence is numbered relative to the transcription start point of the *Hsc70t* gene determined in this study, which is designated as +1. The transcription start point of human *HSPA1A* (*44*), which is transcribed in the opposite direction, is denoted by +1 upside-down. Intron sequences are shown in small letters. The start methionine (ATG) is indicated by Met. Typical elements, TATA box, CCAAT box, GC box (Sp1), and heat shock consensus elements (GAA/TTC) HSE1 and HSE2, in the promoters of the heat inducible *Hsp70* genes are indicated. Highly conserved, long sequence stretches upstream of the transcription start point of the *Hsc70t* gene are shown as Box I and Box II. CRE-like sequences in Box I are boxed. Weak consensus initiator sequences containing the transcription initiation site are boxed. Conserved GC-rich sequences downstream of the transcription start point are underlined with a dashed line. In this region, the Sp1 and GGGTGGG PuF elements are overlined.

and human genomes (Fig. 4). Thus, the transcription initiation site of the mouse spermatid-specific *Hsp70* gene is very close to the 5′ end of the heat-inducible *Hsp70.3* gene, which is transcribed in the opposite direction. The proximal *ca.* 300 bp of the 5′ flanking region of the heat-inducible *Hsp70* gene is highly conserved in man and mouse (*1, 29*). In this region, there are two heat shock elements (HSE), which are the binding sites for the transcription factor (HSF), necessary for inducible transcription of *Hsp70* family genes (see Ref. *30*, for a review).

A comparison of the mouse and human sequences suggested the locations of possible functional elements required for regulation of the spermatid-specific *Hsp70* genes. The sequence, GGGTGGG, conserved in the first exon of the spermatid-specific *Hsp70*, is an element which interacts with PuF, a nuclease hypersensitive element of the human *c-myc* promoter (*31*). This element occurs within the germ-cell promoter of the human *PGK2* gene, as functionally defined in transgenic mice (*32*). Both the mouse protamine-1 and protamine-2 5′ regions contain the sequence, GGGTGGG (*33*). This sequence has also been identified in the germ cell cap site region of the proenkephalin gene, for which spermatogenic-cell transcription is initiated downstream from the proenkephalin somatic promoter (*34*).

Upstream of the transcription initiation site, there are two conserved, long-stretch sequences named Box I and Box II. Box I contains a putative cAMP responsive element (CRE), although the sequence diverges slightly from that of a typical CRE (*35*). The presence of a CRE-like element (TGACCTCA) in Box I is significant in light of the evidence that some haploid-specific genes are regulated transcriptionally with CRE-like sequences (*36-39*). A testis-specific cyclic AMP-responsive element modulator (CREMτ) is expressed in spermatids, and *in vitro* transcription of the haploid-specific RT7 gene is directly regulated by CREMτ (*40, 41*). In fact, it has been shown that, CREM deficiency, generated by homologous recombination, results in a lack of post-meiotic cell-specific gene expression and the complete absence of late spermatids (*42, 43*).

Coordinate activation of a set of spermatid-specific genes, including *Hsc70t* and human orthologous genes, might be regulated through some common *cis*-acting elements. Functional analysis of transcription of these genes will help to reveal the mechanisms of gene expression in haploid germ cells during mammalian spermatogenesis.

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REFERENCES

1. Hunt, C. and Morimoto, R.I. (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
2. Mues, G.I., Munn, T.Z., and Raese, J.D. (1986) A human gene family with sequence homology to *Drosophila melanogaster Hsp70* heat shock genes. *J. Biol. Chem.* **261**, 874-877
3. Hunt, C.R., Gasser, D.L., Chaplin, D.D., Pierce, J.C., and Kozak, C.A. (1993) Chromosomal localization of five murine *HSP70* gene family members: *Hsp70-1*, *Hsp70-2*, *Hsp70-3*, *Hsc70t*, and *Grp78*. *Genomics* **16**, 243-255
4. Zakeri, Z.F., Wolgemuth, D.J., and Hunt, C.R. (1988) Identification 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
5. Krawczyk, Z., Wisniewski, J., and Biesiada, E. (1987) A *hsp70* related gene is constitutively highly expressed in testis of rat and mouse. *Mol. Biol. Rep.* **12**, 27-34
6. Zakeri, Z.F. and Wolgemuth, D.J. (1987) Developmental-stage-specific expression of the *hsp70* gene family during differentiation of the mammalian male germ line. *Mol. Cell. Biol.* **7**, 1791-1796
7. Wisniewski, J., Kordula, T., and Krawczyk, Z. (1990) Isolation and nucleotide sequence analysis of the rat testis-specific major heat-shock protein (*HSP70*)-related gene. *Biochim. Biophys. Acta* **1048**, 93-99
8. Bonnycasle, L.L., Yu, C., Hunt, C.R., Trask, B.J., Clancy, K.P., Weber, J.L., Patterson, D., and Schellenberg, G.D. (1994) Cloning, sequencing, and mapping of the human chromosome 14 heat shock protein gene (*HSPA2*). *Genomics* **23**, 85-93
9. Matsumoto, M. and Fujimoto, H. (1990) Cloning of a *hsp70*-related gene expressed in mouse spermatids. *Biochem. Biophys. Res. Commun.* **166**, 43-49
10. Matsumoto, M., Kurata, S., Fujimoto, H., and Hoshi, M. (1993) Haploid specific activations of protamine 1 and *hsc70t* genes in mouse spermatogenesis. *Biochim. Biophys. Acta* **1174**, 274-278
11. Snoek, M., Jansen, M., Olavesen, M.G., Campbell, R.D., Teuscher, C., and van Vugt, H. (1993) Three *Hsp70* genes are located in the C4-H-2D region: Possible candidates for the *Orch-1* locus. *Genomics* **15**, 350-356
12. Sargent, C.A., Dunham, I., Trowsdale, J., and Campbell, R.D. (1989) Human major histocompatibility complex contains genes for the major heat shock protein HSP70. *Proc. Natl. Acad. Sci. USA* **86**, 1968-1972
13. Milner, C.M. and Campbell, R.D. (1990) Structure and expression of the three MHC-linked *HSP70* genes. *Immunogenetics* **32**, 242-251
14. Ruther, U. and Muller-Hill, B. (1983) Easy identification of cDNA clones. *EMBO J.* **2**, 1791-1794
15. Matsumoto, M., Jimbo, M., and Fujimoto, H. (1991) Expression of a *hsp70*-related gene in mouse spermatid in *Comparative Spermatology 20 Years After* (Serono Symposia Publications from Raven Press, Vol. 75) (Baccetti, B., ed.) pp. 743-746, Raven Press, New York
16. Fujimoto, H., Ito, Y., Ando, A., Matsumoto, M., Fujio, K., Miura, K., Shirai, M., and Inoko, H. (1992) A human *HSP70* homologue gene located in the HLA class III region is expressed in the testicular germ cells in *HLA 1991*, Vol. 2 (Tsuji, K., Aizawa, M., and Sasazuki, T., eds.) pp. 154-157, Oxford Univ. Press, New York
17. Kawai, J., Ando, A., Sato, T., Nakatsuji, T., Tsuji, K., and Inoko, H. (1989) Analysis of gene structure and antigen determinants of DR2 antigens using DR gene transfer into mouse L cells. *J. Immunol.* **142**, 312-317
18. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **24**, 5294-5299
19. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, 2nd ed., Cold Spring Harbor Lab. Press, New York
20. Berk, A.J. and Sharp, P.A. (1977) Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. *Cell* **12**, 721-732
21. Snoek, M., Olavesen, M.G., van Vugt, H., Milner, C.M., Teuscher, C., and Campbell, R.D. (1994) Coding sequences and levels of expression of *Hsc70t* are identical in mice with different *Orch-1* alleles. *Immunogenetics* **40**, 159-162
22. Lowe, D.G. and Moran, L.A. (1986) Molecular cloning and analysis of DNA complementary to three mouse $M_r = 68{,}000$ heat shock protein mRNAs. *J. Biol. Chem.* **261**, 2101-2112
23. Shapiro, M.B. and Senapathy, P. (1987) RNA splicing junctions of different classes of eukaryotes: Sequence statics and functional implications in gene expression. *Nucleic Acids Res.* **15**, 7155-7174
24. Walter, L., Rauth, F., and Gunther, E. (1994) Comparative analysis of the three major histocompatibility complex-linked heat shock protein 70 (*Hsp70*) genes of the rat. *Immunogenetics* **40**, 325-330
25. Friedrich, H., Walter, L., and Gunther, E. (1998) Analysis of the 5′-flanking regions of the MHC-linked *Hsp70-2* and *Hsp70-3* genes of the rat. *Biochim. Biophys. Acta* **1395**, 57-61
26. Weaver, R.F. and Weissmann, C. (1979) Mapping of RNA by a modification of the Berk-Sharp procedure: the 5′ termini of 15 S β-globin mRNA precursor and mature 10 S β-globin mRNA have identical map coordinates. *Nucleic Acids Res.* **7**, 1175-1193
27. Breathnach, R. and Chambon, P. (1981) Organization and expression of eukaryotic split genes coding for proteins. *Annu. Rev. Biochem.* **50**, 349-383
28. Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B., and Smale, S.T. (1994) DNA sequence requirements for transcriptional initiator activity in mammalian cells. *Mol. Cell. Biol.* **14**, 116-127
29. Perry, M.D., Aujame, L., Shtang, S., and Moran, L.A. (1994) Structure and expression of an inducible HSP70-encoding gene from *Mus musculus*. *Gene* **146**, 273-278
30. Morimoto, R.I., Sarge, K.D., and Abravaya, K. (1992) Transcriptional regulation of heat shock genes: a paradigm for inducible genomic responses. *J. Biol. Chem.* **267**, 21987-21990
31. Postel, E.H., Mango, S.E., and Flint, S.J. (1989) A nuclease-hypersensitive element of the human *c-myc* promoter interacts with a transcription initiation factor. *Mol. Cell. Biol.* **9**, 5123-5133
32. Robinson, M.O., McCarrey, J.R., and Simon, M.I. (1989) Transcriptional regulatory regions of testis-specific *PGK2* defined in transgenic mice. *Proc. Natl. Acad. Sci. USA* **86**, 8437-8441
33. McCarrey, J.R. (1987) Nucleotide sequence of the promoter region of a tissue-specific human retroposon: comparison with its housekeeping progenitor. *Gene* **61**, 291-298
34. Kilpatrick, D.L., Zinn, S.A., Fitzgerald, M., Higuchi, H., Sabol, S.L., and Meyerhardt, J. (1990) Transcription of the rat and mouse proenkephalin genes is initiated at distinct sites in spermatogenic and somatic cells. *Mol. Cell. Biol.* **10**, 3717-3726
35. Montminy, M.R. and Bilezikjian, L.M. (1987) Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature* **328**, 175-178
36. Means, A.R., Cruzalegui, F., LeMagueresse, B., Needleman, D.S., Slaughter, G.R., and Ono, T. (1991) A novel Ca^{2+}/calmodulin-dependent protein kinase and a male germ cell-specific calmodulin-binding protein are derived from the same gene. *Mol. Cell. Biol.* **11**, 3960-3971
37. Howard, T., Balogh, R., Overbeek, P., and Bernstein, K.E. (1993) Sperm-specific expression of angiotensin-converting enzyme (ACE) is mediated by a 91-base-pair promoter containing a CRE-like element. *Mol. Cell. Biol.* **13**, 18-27
38. Zambrowicz, B.P. and Palmiter, R.D. (1994) Testis-specific and ubiquitous proteins bind to functionally important regions of the mouse protamine-1 promoter. *Biol. Reprod.* **50**, 65-72
39. Sun, Z., Sassone-Corsi, P., and Means, A.R. (1995) Calspermin gene transcription is regulated by two cyclic AMP response elements contained in an alternative promoter in the calmodulin kinase IV gene. *Mol. Cell. Biol.* **15**, 561-571

40. Foulkes, N.S., Mellstrom, B., Benusiglio, E., and Sassone-Corsi, P. (1992) Developmental switch of CREM function during spermatogenesis: from antagonist to activator. *Nature* **355**, 80-84
41. Delmas, V., van der Hoorn, F., Mellstrom, B., Jegou, B., and Sassone-Corsi, P. (1993) Induction of CREM activator proteins in spermatids: Down-stream targets and implications for haploid germ cell differentiation. *Mol. Endocrinol.* **7**, 1502-1514
42. Nantel, F., Monaco, L., Foulkes, N.S., Masquilier, D., LeMeur, M., Henriksen, K., Dierich, A., Parvinen, M., and Sassone-Corsi, P. (1996) Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. *Nature* **380**, 159-162
43. Blendy, J.A., Kaestner, K.H., Weinbauer, G.F., Nieschlag, E., and Shutz, G. (1996) Severe impairment of spermatogenesis in mice lacking the CREM gene. *Nature* **380**, 162-165
44. Wu, B.J., Kingston, R.E., and Morimoto, R.I. (1985) Structure and expression of the human gene encoding major heat shock protein HSP70. *Mol. Cell. Biol.* **5**, 330-341