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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

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A unique member of the mouse *HSP70* gene family has been isolated and characterized with respect to its DNA sequence organization and expression. The gene contains extensive similarity to a heat shock-inducible *HSP70* gene within the coding region but diverges in both 3′ and 5′ nontranslated regions. The gene does not yield transcripts in response to heat shock in mouse L cells. Rather, the gene appears to be activated uniquely in the male germ line. Analysis of RNA from different developmental stages and from enriched populations of spermatogenic cells revealed that this gene is expressed during the prophase stage of meiosis. A transcript different in size from the major heat-inducible mouse transcripts is most abundant in meiotic prophase spermatocytes and decreases in abundance in postmeiotic stages of spermatogenesis. This pattern of expression is distinct from that observed for another member of this gene family, which was previously shown to be expressed abundantly in postmeiotic germ cells. These observations suggest that specific *HSP70* gene family members play distinct roles in the differentiation of the germ cell lineage in mammals.

Heat shock proteins are activated in response to external stimuli such as elevated temperature in organisms as distantly related as bacteria and humans (9, 19, 33, 39). The genes involved in this highly conserved response are grouped on the basis of the relative molecular weights of their protein products. There is frequently more than one gene within each group. The existence of multiple members of the various *HSP* gene families is widespread in evolution. The yeast *HSP70* gene family consists of at least eight different genes which have been identified at the genetic and molecular level (9). Recent estimates of the number of genes in the mouse and human *HSP70* gene families suggest that they contain at least 5 (21) and 8 to 10 (25) genes, respectively.

Studies on the expression of the *HSP* genes suggest that they may also be activated as part of a normal developmental program. Expression of *HSP70* gene family members has been observed during embryonic development of organisms as diverse as frogs (6), sea urchins (36), and mice (4, 5, 13, 16). Meiotic cells have also been shown to be a site of expression of members of the *HSP* gene families. *HSP20* family members are expressed in growing oocytes and in spermatocytes of *Drosophila* (12, 46) and in sporulating yeast cells (17). *HSP70* genes have been shown to be expressed in the germ line of *Drosophila* (7) and mice, rats, and humans (16, 16a, 45).

Hybridization with a cDNA probe corresponding to a heat shock-inducible member of the *HSP70* gene family has been shown to yield in mammalian testes a uniquely sized transcript that is not found in other tissues (45). This developmentally regulated transcript is expressed at the highest levels in enriched populations of haploid spermatids. The transcript appears to be very stable, since it remains at high levels in RNA isolated from elongating spermatids and residual bodies. A low level of hybridization was also observed in RNA isolated from spermatogenic cells in earlier stages of differentiation. This hybridization might have been attributed to the low level of early spermatids which contaminate the meiotic prophase cellular fraction (43). However, we also considered the possibility that the high level of sequence similarity of the *HSP70* genes across species and among family members might result in our detecting the expression of another member of the *HSP70* gene family.

The high level of sequence conservation of the *HSP70* genes has permitted the identification of multiple members of this gene family. In the present study, we report the isolation of a new member of this gene family and its relationship to other *HSP70* gene family members at the level of DNA sequence analysis. Characterization of the expression of this gene reveals that it is expressed with a unique developmental specificity within the male germ line.

MATERIALS AND METHODS

Isolation of genomic clones. Two mouse genomic libraries (kindly provided by R. Near, Massachusetts Institute of Technology) were constructed by partial digestion of either AJ or BALB/c mouse DNA with *Mbo*I and isolation of 15- to 20-kilobase (kb) fragments on a sucrose gradient (22). The size-selected DNAs were ligated into the *Bam*HI site of the lambda phage vector Charon 30 and packaged (22); 5×10^5 phage from the AJ library were screened by plaque hybridization with a ^{32}P-labeled *Drosophila HSP70* gene probe (14) as described previously (44). An *Eco*RI-*Bam*HI restriction fragment from phage 11 (see Results) was subcloned into pBR322 (pM1.8) and used to screen a second (BALB/c) genomic library. A single recombinant phage, λ 621, was obtained. Restriction and subsequent genomic DNA blot analysis indicated that only a 3.8-kb fragment from an *Eco*RI

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digest of λ 621 DNA contained *HSP70*-related sequences. This fragment was subcloned into pUC18 to generate pM3.8.

Genomic Southern blot analysis. High-molecular-weight mouse DNA was isolated from NIH 3T3 cells (22); ~10 μg of the digested DNA was electrophoresed on a 0.8% agarose gel and transferred onto GeneScreen Plus membrane (New England Nuclear Corp.) according to protocols supplied by the manufacturer. The filters were prehybridized overnight at 65°C in 10% dextran sulfate–1.0 M NaCl–1.0% sodium dodecyl sulfate (SDS) with 100 μg of denatured salmon sperm DNA per ml. Probe was added to 4×10^5 cpm/ml, and hybridization was allowed to proceed at 65°C for a minimum of 16 h. Hybridized filters were washed sequentially, 1 h each, in 2× SSC (1× SSC is 0.15 M NaCl–0.015 M sodium citrate)–1% SDS at 65°C (two times) and then with 0.2× SSC–1% SDS at 65°C (two times). For rehybridizations, filters were treated with 0.4 M NaOH for 30 min at 42°C, neutralized in 100 mM Tris hydrochloride (pH 7.5), monitored for complete probe removal by autoradiography, and then rehybridized. Hybridized filters were exposed at −70°C with intensifying screens.

Two different clones were utilized for the genomic analysis. Clone pM9.5 is a pBR322 subclone of an approximately 9.5-kb *Bam*HI genomic fragment which contains a heat-inducible mouse *HSP70* gene (C. Hunt and S. K. Calderwood, submitted for publication). The 1.5-kb probe from pM9.5 is a *Bal*I-*Xho*I fragment which spans amino acids 1 through 542 of the coding portion of the *HSP70* gene. The gene represented by this clone will be referred to as *HSP70.1* in this manuscript. The second probe was the 1.8-kb *Eco*RI-*Bam*HI insert from pM3.8. DNA sequence analysis (see Results) revealed that this fragment contains ~500 base pairs (bp) of upstream sequences and ends at a *Bam*HI site which encodes amino acid 462. The *HSP70* gene family member represented by this clone will be referred to as *HSP70.2* in this manuscript. Both probes were labeled with [^{32}P]dXTP by random priming on purified DNA inserts (11).

DNA sequence analysis. Sequence analysis of *HSP70.2* was carried out by the dideoxy-chain termination method of Sanger et al. (38), substituting 7-deaza GTP for dGTP in the reaction to eliminate GC compression (23). From pM1.8 and pM3.8, specific subclones were constructed in M13mp18 or M13mp19 by forced directional cloning. Sequence data were compiled on a Vax2060 computer with Intelligenetics programs.

Recombinant DNA clones used in RNA analysis. The following probes were obtained from cloning and sequencing studies for use in analysis of expression of the gene: (i) pM1.8, see above; (ii) pM1.8-200, an ~230-bp *Sma*I-to-*Taq*I fragment of pM1.8 which contains 121 bp of 5′ untranslated sequences, 30 bp of the most 5′ region of the putative coding region of *HSP70.2*, and vector sequences.

The following probes were obtained from other investigators for use in our analysis of RNA from various tissues and cell lines: (i) pMHS213, a *Hin*dIII-*Eco*RI insert containing 1.3 kb of cDNA for a heat-inducible member of the *HSP70* family (21; a gift from L. Moran); (ii) pab1 sub9, a plasmid comprising sequences derived from the Abelson murine leukemia virus (41; a gift from S. Goff).

Source of tissues and cells. Swiss Webster male mice were used as the source of normal mouse tissues. For enrichment of particular testicular cell types by the developmental progression of spermatogenesis in the mouse, testes were collected from animals on days 7 and 17 of life (3, 28, 42). Enriched populations of cells in specific stages of spermatogenesis were separated by sedimentation at unit gravity according to procedures described by Wolgemuth et al. (43).

Mouse L cells were grown in Dulbecco minimal essential medium with 10% fetal calf serum at 37°C with 5% CO_2 and subjected to heat shock as described previously (45). The heat shock treatment was a modification of that described by Lowe and Moran (20): L cells were heat shocked at 43°C for 90 min and allowed to recover for 2 h at 37°C. Cells were lysed directly on the culture plates, and RNA was isolated and analyzed as described below.

Analysis of mRNA. RNA was isolated from the different tissues and separated testicular cell populations by using the LiCl precipitation method of Cathala et al. (8). Poly(A)$^+$ RNA was selected through one cycle of oligo(dT)-cellulose chromatography (2). RNA that is not retained by oligo(dT) is termed the flow-through RNA.

RNA samples were electrophoresed on denaturing 0.8% agarose–2.2 M formaldehyde gels. Gels were blotted onto nitrocellulose or GeneScreen Plus (22) and baked for 3 h at 80°C (nitrocellulose) or exposed to UV light (GeneScreen Plus) to fix RNA to the filters. Probes were labeled by nick translation or random priming (11). Hybridization was essentially as described by Wahl et al. (40) at high stringency in the presence of 10% dextran sulfate, 50% formamide, and 4× SSC. After hybridization, filters were washed sequentially for 20 min each in 2× SSC–0.1% SDS (two times), 1× SSC–0.1% SDS, 0.1× SSC–0.1% SDS, and 0.1× SSC alone, all at 65°C. Filters were exposed to autoradiographic film with intensifying screens.

RESULTS

Isolation and genomic organization of the *HSP70.2* gene. Approximately 5×10^5 recombinant lambda phage from an AJ strain mouse genomic library were screened with a probe for the *Drosophila HSP70* gene. Eight clones were confirmed as bona fide *HSP70* clones. Restriction enzyme mapping of the clones demonstrated that three unique phages, designated λ 4, λ 11, and λ 14, had been isolated. λ 14 was shown to contain a mouse *HSP70* gene which recognizes a heat-inducible mRNA of 3.1 kb in mouse NIH 3T3 cells (Hunt and Calderwood, submitted). The sequence in the coding region of this gene is identical to that of pMHS213, a cDNA isolated from a cDNA library prepared from heat-shocked mouse L cells (20). λ 4 appears to contain an *HSP70*-related pseudogene (data not shown). The results for λ 11 are described below.

Restriction enzyme digestion and Southern blot hybridization analysis yielded the restriction map shown in Fig. 1A. A 4.0-kb *Bam*HI fragment contained sequences which hybridized to the *Drosophila HSP70* gene probe. *HSP70* cross-hybridizing sequences were further localized to a 1.8-kb *Eco*RI-*Bam*HI subfragment which was subcloned into pBR322 to form the clone pM1.8.

DNA sequence analysis (see below) indicated that clone pM1.8 contained only the 5′ 462 amino acids of the putative gene product for this gene, ending at a *Bam*HI restriction site. Because this site formed the junction between the insert DNA and the right arm of the lambda phage cloning vector, it was apparent that only part of the gene had been cloned. A second mouse genomic library was therefore screened with pM1.8 as the probe. Among the phage giving positive hybridization, one clone liberated a 3.8-kb *Eco*RI fragment that hybridized with pM1.8. This 3.8-kb fragment was subcloned into the *Eco*RI site of pUC18 to generate plasmid pM3.8 (Fig. 1A).

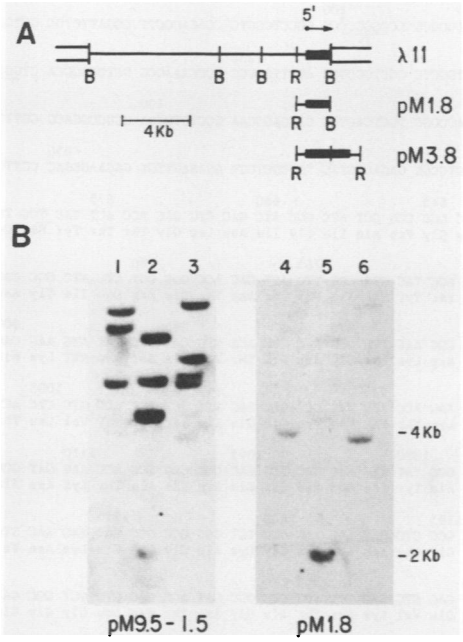

FIG. 1. Restriction and Southern blot analyses of the *HSP70.2* gene. (A) Restriction map of λ 11 showing its relationship to the plasmid subclones. The locations of all *Bam*HI restriction endonuclease sites (B) are indicated for the insert DNA (single line). Phage DNA is represented by double lines, and the single thick line is the *HSP70.2* coding sequence. The arrow indicates the direction of transcription. Clone pM1.8 is a pBR322 subclone containing 1.8 kb of DNA from the indicated *Eco*RI site (R) to the *Bam*HI site at the junction between the insert and phage sequences. Clone pM3.8 was created by subcloning a 3.8-kb *Eco*RI fragment from λ 621 into pUC18. It overlaps pM1.8, extending past the 3' end of the gene. (B) Genomic blot analysis. Genomic DNA from mouse NIH 3T3 cells was restricted with *Bam*HI (lanes 1 and 4), *Bam*HI-*Eco*RI (lanes 2 and 5), or *Eco*RI (lanes 3 and 6), separated by gel electrophoresis, and transferred to GeneScreen Plus membranes. Lanes 1 through 3 were probed with a radiolabeled 1,500-bp fragment isolated from pM9.5 containing sequences spanning amino acids 1 through 542 of this gene. Lanes 4 through 6 are the same filter as in lanes 1 through 3 after the first probe was removed and annealed with the 1.8-kb insert from pM1.8. This fragment spans the complete 5' end of the *HSP70.2* gene, to amino acid 462. Experimental conditions are described in Materials and Methods. Exposure time was 1 day.

Southern blot hybridization analysis was used to determine the number of genes in the mouse genome related to pM1.8. High-molecular-weight DNA was digested with *Eco*RI or *Bam*HI or both restriction endonucleases. The resulting fragments were analyzed by Southern blot hybridization analysis with the mouse genomic probe pM1.8 (see above) or pM9.5, a subclone of λ 14 (Hunt and Calderwood, submitted). Clone pM9.5 detected three or four bands in all digests (Fig. 1B); the bands were not the same sizes as the *Eco*RI and *Bam*HI fragments found in clone λ 11 (Fig. 1A). The filter was then rehybridized with clone pM1.8. A single major band was detected in each digest: a 4.0-kb fragment for *Bam*HI, a 1.8-kb *Bam*HI-*Eco*RI fragment, and a 3.8-kb *Eco*RI fragment. We concluded that pM1.8 represents a single-copy gene which differed enough in its DNA sequence to be distinguished from other *HSP70*-related genes. As noted in Materials and Methods, we have termed this gene *HSP70.2* for the purposes of this discussion.

Primary sequence analysis of *HSP70.2*. Clones pM1.8 and pM3.8 were used to determine the complete nucleotide sequence of *HSP70.2* (Fig. 2). A single unspliced open reading frame was observed, capable of encoding a 634-amino-acid protein with a predicted molecular weight of 69,734. A TATA box was found at nucleotide 631, upstream of the translation start site. Assuming that transcription starts 32 nucleotides 3' of the box, an untranslated 121-nucleotide leader is expected. Further upstream, several additional sequence motifs characteristic of eucaryotic promoters were found. At nucleotide 442, an inverted CCAAT box was found 36 bp 5' of the TATA sequence. The core Sp1 sequence CCGCCC is present at nucleotide 419, and much further 5' several more Sp1 and CCAAT sequences are present. *HSP70.2* lacks an exact match to the heat shock element (HSE) consensus sequence CNNGAANNTTC NNG (31, 32); 12 of 14 nucleotides were the same beginning at nucleotide 445: CTGAGAGTTTCCAG.

The nucleotide and predicted amino acid sequences of *HSP70.2* were compared to several mammalian *HSP70* gene family members (Table 1). *HSP70.2* is closely related to the heat-inducible members of the *HSP70* gene family, with 79% nucleotide similarity to either a human (15) or mouse (Hunt and Calderwood, submitted) gene, and 72% similarity to the published partial sequence of the cDNA pMHS213 which was isolated from a heat-shocked mouse L-cell library (21). The percent similarity rises to 83% when both species are compared at the amino acid level. Compared with the clathrin-uncoating enzyme, the nucleotide relationship is 73% similarity for the human gene (10) and 74% for the rat gene (29). Unexpectedly, the corresponding amino acid comparison between *HSP70.2* and the clathrin-uncoating enzyme genes yielded the highest similarity found, 86% for the human gene and 87% for the rat gene. The least similarity was observed when *HSP70.2* was aligned with the rat glucose-regulated protein, with only 63% similarity at the amino acid level (26).

Expression of the *HSP70.2* gene. To identify the transcripts produced by *HSP70.2*, we isolated RNA from mouse L cells, from heat-shocked L cells, and from selected adult mouse tissues and assayed for the presence of transcripts recognized by *HSP70.2* by Northern (RNA) blot hybridization analysis. Probe pM1.8 detected heat-inducible transcripts of ~2.4 and 3.5 kb in total RNA from heat-shocked L cells (Fig. 3) and from somatic tissues such as liver and brain (data not shown) but not in non-heat-shocked L cells (Fig. 3). This probe also recognized an abundant transcript of ~2.7-kb in length in RNA from adult mouse testes (Fig. 3). A similar pattern was obtained when RNAs from the same sources were hybridized with pMHS213, a cDNA isolated from a heat-shocked L-cell cDNA library (Fig. 3).

Because of the high level of sequence similarity among various members of the *HSP70* gene family, we then examined these RNA samples with probe pM1.8-200, which contains sequences unique for the gene *HSP70.2* (Fig. 3). In contrast to the pattern observed using the longer genomic probe pM1.8, which contains regions of extensive sequence identity to pMHS213, the 200-bp subclone did not recognize transcripts in the RNA from heat-shocked L cells. However, probe pM1.8-200 readily detected the ~2.7-kb transcripts in RNA isolated from adult mouse testis (Fig. 3).

Expression of *HSP70.2* in the male germ line. As noted above, the tissue specificity of expression and the size of the resulting testicular transcripts were strikingly similar to our previous observations on another member of the *HSP70* gene family, recognized by the cDNA clone pMHS213 (45). We therefore compared the cellular and developmental specificity of expression of *HSP70.2* with that observed for

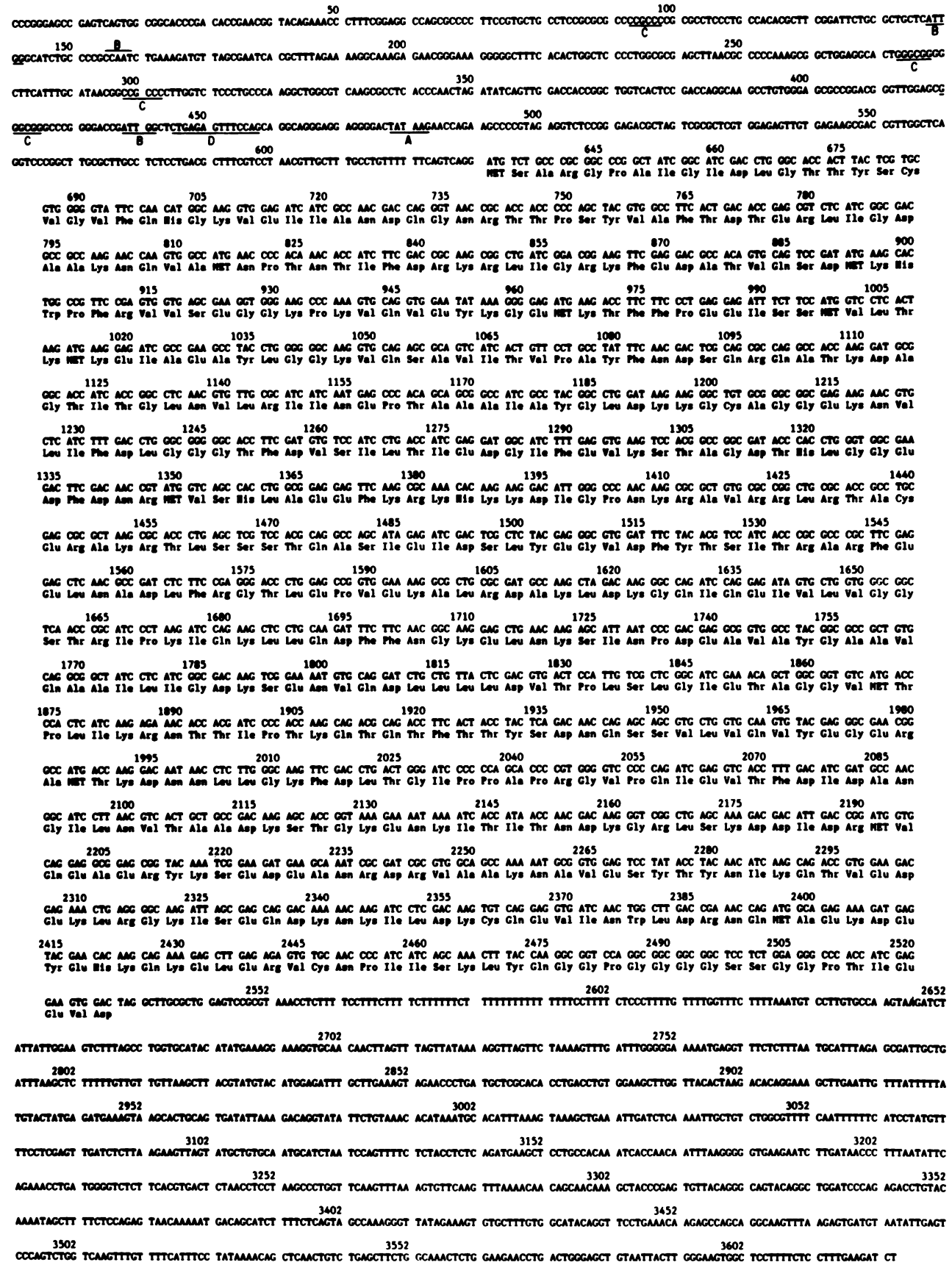

FIG. 2. Nucleotide sequence of the *HSP70.2* gene. The complete nucleotide sequence of *HSP70.2* is presented, as determined from the genomic DNA in clones pM1.8 and pM3.8. Nucleotide sequences similar to elements known to function in transcription, either by functional or protein binding assays, are indicated as follows: A, TATA box; B, inverted CCAAT box; C, SP1 elements CCGCCC; D, HSEs, CNNGANNTTCNNG.

TABLE 1. Nucleotide and amino acid sequence similarities among *HSP70* genes

HSP70 gene or product (reference)	% Similarity of *HSP70.2*	
	Nucleotides	Amino acids
Human uncoating enzyme (10)	73	86
Rat uncoating enzyme (29)	74	87
Human *HSP70* (15)	79	83
Mouse *HSP70.1*[a]	79	83
Rat *GRP* (26)		63
pMHS213 (21)	72	76

[a] Hunt and Calderwood, submitted.

the gene detected by pMHS213 in a series of Northern blot hybridization analyses.

RNA was isolated from testes at three stages of postnatal development to determine whether *HSP70.2* is expressed in testes which contain premeiotic or postmeiotic germ cells. Testes recovered from mice on postnatal day 7 or 8 contain germ cells in various stages of the stem cell differentiation cycle but do not contain germ cells which have entered meiotic prophase (3, 28). Testes from animals on day 17 of postnatal development contain germ cells which have entered meiotic prophase and progressed as far as the pachytene stage, in addition to the mitotic stem cells. Testes from adult animals contain virtually the complete spermatogenic cell lineage, from mitotic stem cells to fully differentiated spermatozoa. Testes at each of these stages contain the full complement of somatic cells, including Leydig cells and Sertoli cells.

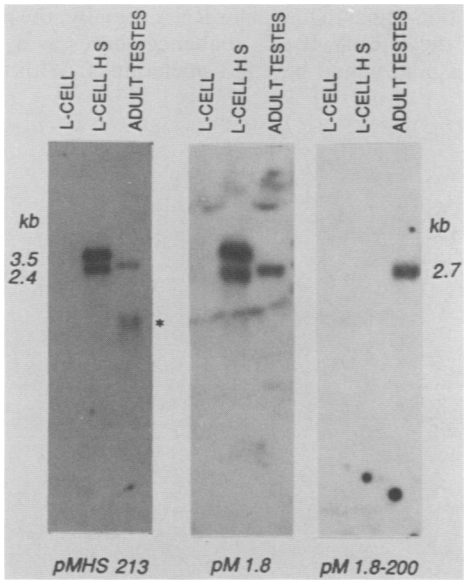

FIG. 3. *HSP70* gene transcripts in mouse L cells and normal mouse tissues. RNAs were electrophoresed in denaturing 0.8% agarose–2.2 M formaldehyde gels and processed for Northern blot hybridization analysis with ^{32}P-labeled pMHS213, the genomic clone pM1.8, or the genomic subclone pM1.8-200. Hybridization conditions were at high stringency (22, 40). L cells were heat shocked as previously described (45). Lanes marked L-cell and L-cell HS (heat shocked) contained 15 μg of total RNA; lanes marked adult testes contained 20 μg of total RNA. The asterisk indicates a residual *Hox-1.4* (42) transcript from a previous hybridization. Exposure times: pMHS213, 1 day; pM1.8, 3 days; pM1.8-200, 7 days.

The results are depicted in Fig. 4. Both pM1.8 and the *HSP70.2*-specific subclone pM1.8-200 readily detected transcripts in RNA from testes from day 17 as well as in RNA from adult animals. In contrast, pMHS213 only detected 2.7-kb transcripts in RNA from the adult animal (Fig. 4), consistent with our earlier observations (45). These data demonstrate that *HSP70.2* is expressed earlier in the differentiation pathway of the germ cell lineage, most abundantly at or about the time the cells enter meiotic prophase, than is the gene whose transcripts are recognized by the probe pMHS213. This observation further suggests that there are two distinct *HSP70* genes, which exhibit discrete developmental specificity of expression in the mouse male germ line but which yield similarly sized transcripts.

Further support for the expression of two genes and additional data defining the cellular specificity of expression of *HSP70.2* were obtained by examining RNA isolated from enriched populations of spermatogenic cells. The rationale and methods for this experimental approach have been discussed in detail in previous studies from our laboratory (35, 42). In brief, RNA was isolated from enriched populations of spermatogenic cells in meiotic prophase (predominantly pachytene), of early (round) spermatids, and of cytoplasmic fragments of elongating spermatids and residual bodies. Analysis for the presence of the 2.7-kb transcripts was performed by Northern blot hybridization (Fig. 5). Transcripts detected by pM1.8-200 (and by pM1.8) were most abundant in the meiotic prophase cells. In certain blots, it appeared that *HSP70.2* transcripts decreased in abundance in early spermatids and in residual bodies and cytoplasmic fragments (Fig. 5 and data not shown). In contrast, the 2.7-kb transcripts detected by pMHS213 were most abundant in early spermatids and residual body fractions (Fig. 5). c-*abl*, which has been shown to produce a novel transcript uniquely in early spermatids and residual bodies (35), served as a positive control for RNA integrity.

DISCUSSION

DNA sequence and structure of *HSP70.2*. Examination of the DNA sequence data for *HSP70.2* suggests that *HSP70.2* may occupy a unique position in the *HSP70* gene family. Structurally, it is closely related to the heat-inducible members of the family. Like all of the known heat-inducible members, it lacks introns in the coding sequence. It is also most similar at the nucleotide level to sequences of the inducible members (79%). Additionally, *HSP70.2* contains a sequence with 12 of 14 bases matching the HSE consensus sequence CNNGAANNTTCNNG, located in the same relative position of the HSEs in the chicken *HSP70* gene (24). This partial HSE sequence, however, appears to be nonfunctional with respect to heat inducibility. All known heat-inducible eucaryotic *HSP70* promoters contain either exact HSEs or multiple overlapping HSEs of higher homology than that found in *HSP70.2* (19, 31, 32). Other members of the *HSP70* gene family which contain inexact HSEs, such as the genes encoding glucose-regulated protein or clathrin-uncoating enzyme, are also not induced by heat or are only marginally induced (1, 10, 18). The predicted amino acid sequence of *HSP70.2* is most highly similar to the sequence of the gene encoding clathrin-uncoating enzyme, an *HSP70* family member involved in removing the clathrin network surrounding coated pits (37). The *HSP70.2* gene, therefore, may have been derived from the heat-inducible *HSP70* genes but may have evolved functional properties more similar to those of genes encoding the uncoating enzyme.

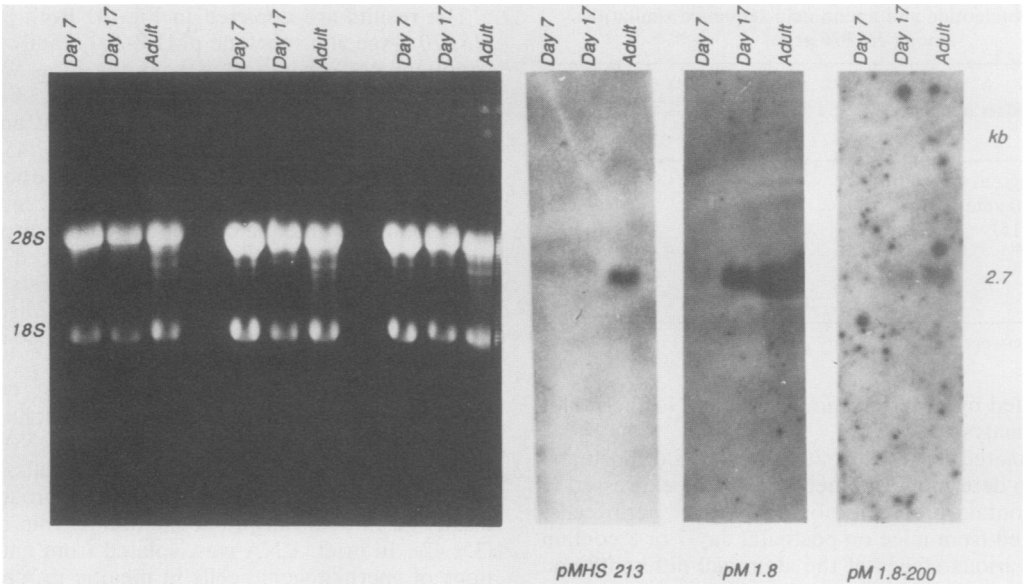

FIG. 4. Developmental stage-specific expression of *HSP70* mRNAs. RNA was isolated from mouse testes recovered from animals on day 7 or 8 and on day 17 of life and from adult animals. Northern blot hybridization analysis was performed as described in the legend to Fig. 3. The left panel shows the ethidium bromide-stained gel; the right panels are the autoradiographs. Each lane contained 25 μg of total RNA. Exposure time for each panel was 1 day.

No consensus polyadenylation signal (AATAAA) was observed within 1 kb of the translation stop codon. We suggest that the sequence TACAAA, at position 3350, may represent the polyadenylation signal, based on the following evidence. Oppi et al. (30) recently suggested that this sequence serves as the polyadenylation signal of the developmentally regulated c-*abl* testicular transcript (35). Northern blot hybridization analysis with various genomic fragments in this region demonstrated a positive hybridization signal to testicular RNA when fragments which are 5′ to this sequence were used (Zakeri, Hunt, and Wolgemuth, unpublished observations). Conversely, fragments which originate from regions 3′ to this sequence do not yield positive hybridization signal to testicular RNA. Finally, the predicted length of the mRNA, if this sequence serves as a polyadenylation signal, would be 2,861 nucleotides. With the addi-

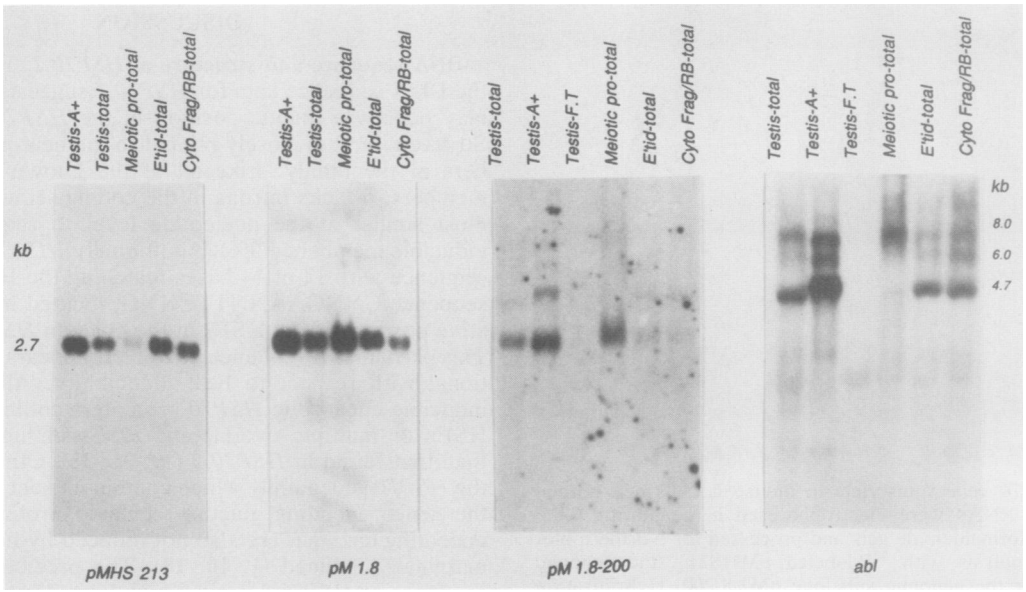

FIG. 5. Northern blot hybridization of RNAs isolated from enriched populations of spermatogenic cells. The cells were purified according to our standard procedures, RNA was isolated, and Northern blot hybridization analysis was performed as described in the legend to Fig. 3. Lanes: Meiotic pro, cells in the prophase stage of meiosis, predominantly pachytene; E'tid, early spermatid cells; cyto frag/RB, cytoplasmic fragments of elongating spermatids and residual bodies fraction of cells; Testis-A$^+$, 5 μg of poly(A)$^+$ RNA from total adult testis; Testis-FT, 25 μg of poly(A)$^-$ RNA from total testis. Total RNAs from the various sources contained 15 μg per lane. The panel labeled *abl* served as a positive control (blot provided by G. Mutter [27]); transcripts detected were identical to those described in our previous studies (35). Exposure times: pMHSP213, 3 days; pM1.8, 2 days; pM1.8-200, 7 days; *abl*, 2 days.

tion of a short poly(A) tail, the final length would be very close to the size predicted from relative migration on formaldehyde gels.

Expression of *HSP70* gene family members in the male germ line. The pattern of expression of *HSP70.2* exhibits certain similarities to that observed previously for another member of the *HSP70* gene family, defined as being recognized by the cDNA clone pMHS213. Both genes are expressed at high levels in the male mammalian germ line and yield transcripts ~2.7 kb in length. Although this size similarity complicated our analysis, it was not totally surprising given the high level of conservation of the overall structure and sequence identity among the *HSP70* genes. All mouse *HSP70* transcripts reported to date fall with the size range of 2.0 to 3.5 kb (16, 21, 45). Lowe and Moran (21) noted two distinct but comigrating transcripts in heat-shocked L cells. The testicular transcripts recognized by clone pMHS213 are indistinguishable in length among mouse, rat, and human samples (45). Delineation of the precise size of the ~2.7-kb transcripts will await the availability of cDNA clones.

The developmental regulation of expression of *HSP70.2* was distinct from that observed for the *HSP70* gene identified by clone pMHS213. High levels of *HSP70.2*-specific transcripts were detected in early stages of spermatogenic development, including the pachytene stage of meiotic prophase. The gene recognized by pMHS213 was expressed later, most abundantly in postmeiotic (haploid) cells in terminal stages of spermiogenesis. We are not aware of any other developmental system in which sequential activation of members of the *HSP70* gene family has been observed, notably in the absence of exogenous stress.

Speculation on the function of *HSP70* gene family members in the male germ line. It is premature to propose specific functions during spermatogenesis for *HSP70* gene family members. However, certain aspects of the precise and evolutionarily conserved structure and pattern of expression of various *HSP* genes allow us to note several themes.

The conservation of expression of the low-molecular-weight heat shock proteins within meiotic cells has been documented in organisms as diverse as *Drosophila* and yeasts (17, 46). *HSP70* gene family members are expressed in the germ cell lineage in evolutionarily divergent organisms, including *Drosophila* (7) and mammals (45). Our previous observations (45) and the studies presented here suggest that expression of more than one member of the *HSP70* gene family is important in the mammalian germ cell lineage. Expression of the *HSP70* family may also be important in early mammalian embryogenesis (4, 13, 16). In each of these examples, specific developmental induction of various *HSP* gene family members is occurring in response to as yet unidentified developmental cues.

It is interesting to note that the expression of *HSP* genes coincides temporally with major differentiative events in these cells. It has been suggested that heat shock proteins or related proteins may function in the assembly and/or disassembly of a variety or cellular macromolecular structures (33). These proteins could thus be involved with the formation and disruption of morphogenetic structures during germ cell differentiation. A remarkable and transient structure such as the synaptonemal complex would be an obvious candidate for a structure that needs to be assembled and broken down during meiotic prophase. Similarly, the round spermatid undergoes striking morphological changes during spermiogenesis, forming a flagellum and an acrosome. Early embryonic morphological changes are less dramatic, although the formation of the somatic-type pronuclei from the sperm and egg nuclei serves as an example of a major cellular structural change.

The association of *HSP* gene expression with the appearance of such precise morphological events should facilitate analysis of the effects of disrupting the normal pattern. That is, overexpression or interference with expression could result in a specific morphological phenotype. Although the presence of multiple *HSP* family members may complicate such analysis (4), mammalian gametogenesis may provide a good test system for ascertaining the effects of disrupting gene function, since it appears from our results that two different members of a specific family have evolved to be expressed at unique times and may thus have evolved unique and critical functions as well.

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