HSP70-2 is required for desynapsis of synaptonemal complexes during meiotic prophase in juvenile and adult mouse spermatocytes

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SUMMARY

Spermatogenic cells synthesize a unique 70-kDa heat shock protein (HSP70-2) during prophase of meiosis I, and targeted disruption of the Hsp70-2 gene has shown that this protein is required for spermatogenic cell differentiation in adult mice. HSP70-2 is associated with synaptonemal complexes formed between paired homologous chromosomes during meiotic prophase. The present study focuses on the nearly synchronous first wave of spermatogenesis in 12- to 28-day old juvenile mice to determine more precisely when HSP70-2 is required and what meiotic processes are affected by its absence. Spermatogenesis in homozygous mutant mice (Hsp70-2−/−) proceeded normally until day 15 when increasing numbers of pachytene spermatocytes became apoptotic and differentiation of cells beyond the pachytene stage began to falter. Synaptonemal complexes assembled in Hsp70-2−/− mice and spermatocytes developed through the final pachytene substage. However, synaptonemal complexes failed to desynapse and normal diplotene spermatocytes were not observed. Metaphase spermatocytes were not seen in tissue sections from testes of Hsp70-2−/− mice, and expression of mRNAs and antigens characteristic of late pachytene spermatocytes (e.g., cyclin A1) and development of spermatids did not occur. Thus, HSP70-2 is required for synaptonemal complex desynapsis, and its absence severely impairs the transition of spermatogenic cells through the late meiotic stages and results in apoptosis beginning with the first wave of germ cell development in juvenile mice.

Key words: heat shock protein, HSP70, meiosis, spermatogenesis, synaptonemal complex, mouse

INTRODUCTION

The 70-kDa heat shock proteins (HSP70s) are chaperones which assist the folding, the assembly and the disassembly of complexes of other proteins (Georgopoulos and Welch, 1993). The expression of some HSP70s is inducible by environmental stress, but expression of other members of the HSP70 family can be either constitutive, or regulated developmentally during gametogenesis (Dix, 1997). HSP70-2 is a unique member of the mouse HSP70 family expressed during prophase of meiosis I in male germ cells (Allen et al., 1988; Zakeri et al., 1988). Hsp70-2 gene expression is regulated developmentally in mouse testis, with transcription beginning in leptotene-stage spermatocytes, under the direction of a promoter within 300 bp of the transcription start site (Dix et al., 1996a). HSP70-2 binds ATP (Allen et al., 1988), CDC2 (Zhu et al., 1997) and other proteins (Allen et al., 1996), and shares over 80% amino acid sequence similarity with the other HSP70s. Genes homologous to Hsp70-2 also are expressed in the spermatocytes of rats (Wisniewski et al., 1990) and humans (Bonnycastle et al., 1994). It has been shown in mice (Rosario et al., 1992; Allen et al., 1996) and rats (Raab et al., 1995) that HSP70-2 is relatively abundant in both the cytoplasm and nucleus of spermatogenic cells.

During the zygotene stage of meiotic prophase, axial elements of homologous chromosomes align and synapse to form synaptonemal complexes (SCs; Moses, 1968). In mice, formation of the 19 fully synapsed autosomal SCs and of the partially synapsed X-Y pair appears to be essential for successful completion of DNA repair and recombination processes (Moens, 1994). SCs in vertebrate spermatocytes are composed of two dense, proteinaceous lateral elements connected by transverse filaments, and a ladder-like central element (Moses et al., 1990). SCs remain synapsed for approximately seven days in mouse pachytene spermatocytes, then desynapse during the diplotene stage, as chromosomes condense for the first meiotic division. We have demonstrated that HSP70-2 is a component of the SC lateral elements in pachytene spermatocytes of mouse and hamster, but is not detected in oocyte SCs (Allen et al., 1996). HSP70-2 is present in spermatocyte SCs from zygotene through diplotene, suggesting that it could be significant for SC formation, DNA repair and recombination.
processes, or SC desynapsis required for progression to metaphase 1.

To determine if HSP70-2 has a critical role in SC function or meiosis we used homologous recombination to disrupt the Hsp70-2 gene in mice (Dix et al., 1996b). The testes of adult Hsp70-2−/− mice were one third the weight of those from wild-type mice and lacked postmeiotic germ cells. Though SCs formed in Hsp70-2−/− spermatocytes, very few of these cells progressed to the first meiotic division. Males were infertile, while females were unaffected. The failure of meiosis in Hsp70-2−/− male mice was associated with an increase in spermatocyte apoptosis (Mori et al., 1997), and a lack of CDC2 kinase activity in Hsp70-2−/− spermatocytes (Zhu et al., 1997). However, these observations were made in adult Hsp70-2−/− mice and it was a concern that some effects may be secondary to a general disruption of spermatogenesis. The current study monitored the progression of the first wave of spermatogenesis in juvenile Hsp70-2−/− mice to identify the substages of meiotic prophase at which features of the abnormal phenotype appear and to determine if the changes seen are primary or secondary effects of the Hsp70-2−/− genotype.

### MATERIALS AND METHODS

**Hsp70-2−/− mice**

Hsp70-2−/− mice were developed as previously described (Dix et al., 1996b) and were cared for and utilized according to protocols approved by NIEHS and US EPA Institutional Animal Care and Use Committees and in accordance with U.S. Public Health Service guidelines. The majority of these studies were performed with juvenile and adult mice of a predominantly C57BL/6N genetic background. Three to four generations were produced by backcrossing from a male chimera created with E14TG2a embryonic stem cells derived from strain 129/SvOla with wild-type female C57BL/6N mice. The C57BL/6N Y chromosome was introduced into the backcross line by mating an N1 generation Hsp70-2−/− female with a C57BL/6N wild-type male. Additional studies utilized adult mice generated by backcrossing the same male chimera with wild-type female mice (Taconic, Germantown, NY).

**Histology and immunohistochemistry**

The day of birth was designated as day 0. Testes were collected from juveniles on days 10 to 28 after birth or from adults at approximately 12 weeks of age, immersed in Bouin’s fixative, paraffin-embedded and sectioned. Sections were either stained with hematoxylin and eosin, or stained with antibodies against various antigens, as described below. In situ hybridization experiments were performed on seminiferous tubules from adult mice using probes generated by PCR with the primers shown in Table 1. The human GCNA1 monoclonal antibody (Enders and May, 1994; Mori et al., 1997), a mouse IgG anti-bovine vimentin monoclonal antibody (Progen Biotech GmbH, Heidelberg, Germany), or rabbit antiserum 2A for HSP70-2 (Rosario et al., 1992).

**Table 1. PCR primers used to generate northern probes**

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<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Probe length (bp)</th>
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<tr>
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<tr>
<td>Histone H1</td>
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<tr>
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<td>194</td>
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**Probe length (bp)**

**Detection and characterization of apoptotic germ cells**

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) was used for in situ visualization of DNA fragmentation indicative of apoptosis (MEBSTAIN Apoptosis Kit, Medical and Biological Laboratories Co., Nagoya, Japan; Mori et al., 1995). Testes were fixed in 4% paraformaldehyde for 16 hours at 4°C, embedded in paraffin and sections placed on silanized slides. After proteinase K treatment, 3′-OH DNA ends were labeled with biotin dUTP and detected with avidin-conjugated FITC (Fig. 5A-F) or rhodamine B-labelled streptavidin (Fig. 5G-H). After processing for TUNEL, sections were immunostained with a rat IgM anti-mouse GCNA1 monoclonal antibody (Enders and May, 1994; Mori et al., 1997), a mouse IgG anti-bovine vimentin monoclonal antibody (Progen Biotech GmbH, Heidelberg, Germany), or rabbit antiserum 2A for HSP70-2 (Rosario et al., 1992).

**Northern blots**

Northern blots were prepared with total RNA extracts from testes of Hsp70-2−/− and wild-type mice. Blots were loaded with 10 μg total RNA per lane and hybridized with probes generated by PCR with the primers shown in Table 1. The human β-actin cDNA control probe was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). Blots were hybridized in 6x SSC, 0.5% SDS, 100 μg/ml salmon DNA, and 50% formaldehyde at 42°C overnight, washed twice at room temperature in 2× SSC and 0.1% SDS, subjected to a final wash at 50°C in 0.1× SSC and 0.1% SDS, and exposed to X-ray film for 1-3 days.

**Synaptonemal complex analysis**

Surface-spreads were prepared and stained by using previously described methods for light and electron microscopy (Dresser and Moses, 1979). Briefly, testicular cells were spread on the surface of 0.5% NaCl and transferred to slides precoated with either polylysine or poly-L-lysine. Slides were fixed in 4% paraformaldehyde, rinsed in 0.4% PhotofloTM (Kodak), air-dried, and silver stained using 50% silver nitrate in gelatin developer. The polylysine film was floated off the slide onto distilled water and transferred to 50-mesh copper grids for electron microscopy (JEOL, JEM 100C transmission electron microscope; magnification 1300-4000×). The poly-L-lysine coated slides were used for light microscopy (Olympus BH-2, 100× objective). 200 prophase spermatocytes per animal were identified as leptotene, zygotene, pachytene (substages I to V), or diplonema stage according to criteria described by Moses (1980,1981). These staging criteria include the morphology of nucleoli, the prominence of the sex body, and different synaptic states of the SCs.
RESULTS

Disruption of spermatogenesis prior to the G2/M transition of meiosis I in juvenile Hsp70-2−/− mice

HSP70-2 was detected with antibody 2A by immunostaining cross-sections of seminiferous tubules from 12- to 22-day-old juvenile wild-type mice (Fig. 1). At day 12, spermatogenesis had progressed to early prophase of meiosis I and HSP70-2 synthesis was beginning in leptotene and zygotene spermatocytes (Fig. 1A). By day 14, early pachytene spermatocytes were present and contained readily detectable amounts of HSP70-2 in their cytoplasm and nuclei (Fig. 1B). HSP70-2 was present throughout the remainder of the meiotic and post-meiotic phases of spermatogenesis (Fig. 1C, day 22).

The progression of the first wave of spermatogenesis was compared in Hsp70-2+/− and Hsp70-2−/− litter mates to minimize possible developmental and genetic differences. On day 15, pachytene spermatocytes with discernible XY chromosome bodies were visible in sections of testes from Hsp70-2+/− and Hsp70-2−/− mice (Fig. 2A,B). However, in the Hsp70-2−/− mice an increased number of pachytene spermatocytes appeared to be apoptotic, containing dense nuclei devoid of recognizable chromosomal structure (Fig. 2B). By day 18, tubules from Hsp70-2+/− mice showed an organized progression of cell types from spermatogonia at the periphery to late pachytene spermatocytes at the innermost layers (Fig. 2C). In contrast, tubules from Hsp70-2−/− mice lacked this clear organization, and pachytene cells were mixed with pre-meiotic and apoptotic cells (Fig. 2D). Even more substantial differences were apparent between Hsp70-2+/− and Hsp70-2−/− mice at postnatal day 22. Spermatocytes with chromosomes aligned at the metaphase plate and round spermatids were present in wild-type and Hsp70-2+/− mice (Fig. 2E), but not identified in Hsp70-2−/− mice (Fig. 2F). Instead, all spermatocytes in

![Fig. 1.](image1.png) Immunohistochemical detection of HSP70-2 in the testes of juvenile Hsp70-2+/− mice at days 12 (A), 14 (B) and 22 (C) postnatal. Only faint peroxidase staining is visible in leptotene/zygotene spermatocytes in seminiferous tubules from a day-12 testis. By day 14 HSP70-2 is readily detectable in the cytoplasm and nucleus of pachytene cells. At day 22 MI/II spermatocytes with chromosomes aligned on the metaphase plate are present. HSP70-2 is present throughout the spermatocyte complement of these tubules. Bar equal, 25 μm.

![Fig. 2.](image2.png) Morphology of seminiferous tubules from Hsp70-2+/− (A,C,E) and Hsp70-2−/− (B,D,F) mice at days 15 (A,B), 18 (C,D), and 22 (E,F). Pachytene spermatocytes with prominent XY bodies are relatively abundant by day 15 (arrows in A,B), as are apoptotic cells in Hsp70-2−/− mice (arrowheads in B). By day 18 the tubule lumens of Hsp70-2−/− mice are filled with pachytene cells (C), while tubules from homozygous mice contain a disorganized mix of pachytene, leptotene/zygotene, and dying cells (D). Approximately 8 days after the first pachytene cells develop, MI/II spermatocytes with chromosomes aligned at the metaphase plate are present in day 22 tubules from Hsp70-2−/− mice (arrows in E). MI/II spermatocytes are absent from tubules from Hsp70-2−/− mice (F). Bars equal 25 μm.
Hsp70-2−/− mice seemed to arrest development at the end of the pachytene stage and to become apoptotic.

Following meiosis, the haploid genome is remodeled and repackaged into round spermatids at day 24 (A) and elongating spermatids at day 28 (C) in Hsp70-2+/− mice, while tubules from Hsp70-2−/− mice contain degenerating cells with dense nuclei devoid of recognizable chromosomal structure (B). By adulthood (F), most tubules from Hsp70-2−/− mice contained only pre-meiotic and meiotic germ cells. This is in contrast to a stage VI tubule (E) of an adult Hsp70-2+/− mouse, which contains spermatocytes at pachytene (arrow), round spermatids (arrowhead) and elongating spermatids being released into the tubule lumen. Bars equal 25 μm.

**Fig. 3.** Morphology of seminiferous tubules from Hsp70-2+/− (A,C,E) and Hsp70-2−/− (B,D,F) mice at days 24 (A,B) and 28 (C,D), and at adulthood (E,F). Adluminal cells are primarily round spermatids at day 24 (A) and elongating spermatids at day 28 (C) in Hsp70-2+/− mice, while tubules from Hsp70-2−/− mice contain degenerating cells with dense nuclei devoid of recognizable chromosomal structure (B,D). By adulthood (F), most tubules from Hsp70-2−/− mice contained only pre-meiotic and meiotic germ cells (Fig. 3F).

**Apoptosis in pachytene spermatocytes**

TUNEL staining was used in combination with morphological and immunofluorescent analyses to identify and characterize apoptotic germ cells in juvenile mice. The apparent frequencies of apoptotic germ cells in wild-type, Hsp70-2+/−, and Hsp70-2−/− mice were similar on day 12 (Fig. 4A,B). However, by day 15 apoptotic pachytene spermatocytes were more common in Hsp70-2+/− mice (Fig. 4C,D) than in wild-type or Hsp70-2−/− mice. This difference was maintained in 17-day old mice (Fig. 4E,F).

To confirm the identity of apoptotic cells in testes from Hsp70-2−/− mice, an antibody to germ cell nuclear antigen 1 (GCNA1) was used to identify spermatogonia and spermatocytes (green fluorescence in Fig. 4G) and an antibody to vimentin was used to identify Sertoli cells (green fluorescence in Fig. 4H). TUNEL-positive nuclei were present in the central portion of tubules (rhodamine/orange cells in Fig. 4G), coincident with GCNA1-positive spermatocytes. However, vimentin-positive cells lacked GCNA1 staining. These results confirmed that the apoptotic cells in the adluminal region of tubules of Hsp70-2−/− mice were spermatocytes (Fig. 4H).

**The developmental program of spermatogenesis is disrupted by late-pachytene stage in Hsp70−/− mice**

Monoclonal antibodies to a spermatogenic cell surface carbohydrate antigen (J1) and a protein in the fibrous sheath of the sperm flagellum (ATC) were used to determine if spermatocytes from Hsp70-2−/− mice synthesized components that appear in late pachytene spermatocytes and spermatids of wild-type mice. Antibody J1 bound to the cell surface of late pachytene spermatocytes at day 22 (Fig. 5A), spermatids at days 24 and 28 (Fig. 5C,E), and sperm of adult Hsp70-2+/− mice (Fig. 5G). However, the antibody did not bind to spermatocytes from juvenile Hsp70-2−/− mice (Fig. 5B,D,F), indicating that biosynthesis of this antigen was disrupted in the first wave of spermatogenesis. The antigen was present in low amounts in adult Hsp70-2−/− mice, indicating that it can be synthesized in these animals (Fig. 5H). Monoclonal antibody ATC reacted with the fibrous sheath in elongating spermatids of day
28 and adult Hsp70-2+/− mice. However, no reaction was seen in Hsp70-2−/− mice (data not shown).

We also examined the transcription of genes known to be developmentally expressed in spermatogenic cells to determine when the genetic program of male germ cell development was disrupted in Hsp70-2−/− mice. Expression of the histone H1t and proacrosin genes is reported to occur in mid-pachytene spermatocytes in wild-type mice (Drabent et al., 1996; Kashiwabara et al., 1990; Kremling et al., 1991). Northern analysis demonstrated that transcripts from both genes were present in testes of Hsp70-2−/− mice (Fig. 6). These transcripts were more abundant in wild-type than in Hsp70-2−/− mice, probably because they are also present in spermatids in wild-type mice. Expression ofSprml and cyclin A1 is reported to occur at the end of prophase of meiosis I (Anderson et al., 1993; Sweeney et al., 1996). Northern analysis determined that neither gene was expressed in testes of Hsp70-2−/− mice (Fig. 6). These results suggest that there is neither premature down-regulation of genes expressed during prophase of meiosis nor premature initiation of expression of genes transcribed in postmeiotic spermatids in Hsp70-2−/− mice.

**DISCUSSION**

Juvenile Hsp70-2−/− mice were studied to determine when different features of the mutant phenotype appear during the first wave of spermatogenesis. These findings were correlated with the progression of SC disruption during prophase of meiosis I in pachytene spermatocytes of adult Hsp70-2−/− mice.
It was found that late pachytene spermatocytes in juvenile
Hsp70-2−/− mice failed to complete meiotic prophase and
became apoptotic (Fig. 9). However, SC disruption occurred
prior to the diakinesis phase of meiosis I, before the G2/M-
phase transition. These results strongly suggest that HSP70-2
is required for SC disassembly and the completion of meiosis
I. Since the abnormal phenotype occurs during the first wave
of spermatogenesis in Hsp70-2−/− mice, the results also suggest
that HSP70-2 is involved in SC disassembly and G2/M-phase
transition and that the mutant phenotype is not caused indi-
rectly by the disruption of spermatogenesis.

Apoptosis begins in some Hsp70-2−/−
spermatocytes prior to meiotic arrest
All late pachytene spermatocytes underwent
apoptosis by day 19 in juvenile C57BL/6N
Hsp70-2−/− mice. However, apoptosis was
more commonly seen at day 15 by TUNEL in
Hsp70-2−/− than in wild-type mice. This
suggests either that a process activating
apoptosis is potentiated or that a process
inhibiting apoptosis is compromised in
pachytene spermatocytes of Hsp70-2−/− mice
prior to meiotic arrest. Because it is well
known that p53-dependent mechanisms con-
stitute a major pathway for activating
apoptosis (Clarke et al., 1994), Hsp70-2−/−
female mice were mated with p53−/− male
mice to produce double-homozygous mutant
males (Hsp70-2−/−, p53−/−) to determine if the
absence of p53 would prevent apoptosis in
spermatocytes and rescue fertility in Hsp70-
2−/− mice. However, all spermatocytes of
double-homozygous mutant males were seen
to undergo apoptosis (unpublished observa-
tions). This strongly suggests that apoptosis in
Hsp70-2−/− spermatocytes is activated through a p53-independent mechanism. It
remains to be determined if this occurs
directly through another pathway for trigger-
ing apoptosis, or indirectly due to disruption of essential cellular processes caused by the
absence of HSP70-2.

Disruption of synaptonemal complex
desynapsis and meiosis
Developmental progression of spermatocytes
in juvenile and adult testes was analyzed in
surface-spread germ cells by light and
electron microscopy, and by morphological
analysis of paraffin sections. Analysis of
surface-spread spermatocytes from Hsp70-
2−/− mice indicated that SCs failed to
desynapse, and that some SCs fragmented.
Spermatocytes were not differentiated beyond
pachytene spermatocyte stage V (prior to
diplotene, diakinesis, and G2/M transition),
suggesting that HSP70-2 is required for SC
disassembly (discussed below). Normal
diplotene spermatocytes were not seen in
surface-spreads, and metaphase stage sperma-
tocytes were not seen in sections. Furthermore, the failure to
detect the J1 antigen or mRNA from genes normally expressed
in late prophase I spermatocytes (Sprm-1, cyclin A1) and post-
meiotic spermatids (Gapd-s, Fsc1, Hsc70t) indicated that the
genetic program of spermatogenesis arrests prior to the late
pachytene spermatocyte stage in Hsp70-2−/− mice.

The HSP70 proteins are chaperones which assist the
folding and the assembly and disassembly of other proteins
(Georgopoulos and Welch, 1993). The presence of HSP70-2
in the SC (Allen et al., 1996) and the failure of SC desynap-
sis in Hsp70-2−/− mice suggests that HSP70-2 chaperone

Fig. 5. Immunohistochemical detection of spermatogenic cell surface antigens with J1
antibody in Hsp70-2+/− (A,C,E,G) and Hsp70-2−/− (B,D,F,H) mice. In Hsp70-2+/−
mice, the antigen is first detectable at day 22 in late prophase spermatocytes (arrows in
A). By day 24 immunostaining of round spermatids in Hsp70-2+/− mice is evident (C),
and round and elongating spermatid surface staining continues in day 28 (E) and adult
(G) mice. Differentiation-arrested spermatocytes in Hsp70-2−/− mice are not
immunoreactive at day 22 (B), day 24 (D) or day 28 (F). Only in testes from adult
Hsp70-2−/− mice are spermatocytes immunoreactive (H). Bars equal to 25 μm.
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4601HSP70-2 and meiosis is required for SC desynapsis. Structural proteins of the SC that have been identified include SYN1/SCP1 (Moens and Spyropoulos, 1995), COR1 (Dobson et al., 1994), and SC65 (Chen et al., 1992). Other proteins known to be associated with the SCs that have roles in DNA recombination or repair include RAD51 (Haaf et al., 1995), PMS2 (Baker et al., 1995), MLH1 (Baker et al., 1996; Edelmann et al., 1996), DNA topoisomerase II (Moens and Earnshaw, 1989; Cobb et al., 1997), BRCA1 (Scully et al., 1997), ATM, ATR (Keegan et al., 1996), and UBC9 (Kovalenko et al., 1996). If HSP70-2 is required to chaperone such proteins during SC disassembly, it would not be surprising if defects in this process

Fig. 6. Gene expression in the testes of wild-type (lane 1) and Hsp70-2−/− mice (lanes 2-7). Northern blot analysis was used to detect transcripts from genes expressed only in spermatogenic cells. Each lane contains 10 µg of total RNA from adult testes. The blots were probed for (A) histone H1t, (B) proacrosin, (C) Sprm1, (D) cyclin A1, (E) Hsc70t, (F) Fsc1, (G) Gapd-s, and (H) actin transcripts. Three separate Northern blots were used, one was probed for H1t, proacrosin, and Hsc70t; one for Fsc1, Gapd-s, and actin; and another for Sprm1 and cyclin A1. RNA from the same animals was used on all three blots. The approximate sizes of the transcripts detected were (A) 0.7 kb, (B) 1.6 kb, (C) 1.2 kb, (D) 1.8 kb, (E) 2.7 kb, (F) 3 kb, (G) 1.5 kb, (H) 2.2 kb.

Fig. 7. Electron micrograph of a portion of a late pachytene nucleus from an Hsp70-2−/− mouse (A) containing fully synapsed autosomal bivalents (arrows) and sex bivalents synapsed only at the XY telomere ends (arrowhead), typical of pachytene substage 5. Normal diplotene nuclei were not observed in Hsp70-2−/− mice (B), only degenerate cells with decondensing sex vesicles (arrow) and degraded autosomal synaptonemal complexes (arrowheads). A portion of a normal diplotene cell from an Hsp70-2+/− mouse, with fully desynapsed XY bivalents (arrow) and desynapsing autosomal bivalents (arrowheads), is shown for comparison (C). Bars equal to 5 µm.

Fig. 8. Frequency distribution of meiotic prophase stages in spermatocytes of wild-type and Hsp70-2−/− mice. Prophase subpopulations of cells (leptotene to diplotene) were staged by light microscopic analysis of silver-stained cell preparations. Hsp70-2−/− mice had no normal diplotene cells, though no reduction of late pachytene cells through to substage V (PV) was observed. Percentages were determined from analyses of 200 cells from each of two control and two Hsp70-2−/− mice (C57BL/6). Knockout of the Hsp70-2 gene results in a failure of the synaptonemal complexes (SCs) of late pachytene cells to desynapse and progress to diplotene stage. Bars represent means, lines represent standard deviations.

Fig. 9. A timeline of juvenile spermatogenesis including the preleptotene (PL), leptotene (L), zygotene (Z), pachytene and diplotene stages of germ cell differentiation during meiotic prophase. In Hsp70-2−/− mice apoptotic spermatocytes increase by day 15, pachytene differentiation falters at substage V, and spermatocytes do not make the transition to meiotic divisions (MI and MII). For an additional timetable of meiotic prophase, see Moses et al. (1990); for a description of the temporal appearance of spermatogenic cell types in prepuberal testis, see Bellvé et al. (1977).
lead to the fragmentation of SCs and disruption of desynapsis seen in Hsp70-2−/− mice.

**Relationship between failure of SC desynthesis and G2/M arrest**

Hsp70-2 was found recently to be a chaperone for CDC2 in pachytene spermatocytes. In Hsp70-2−/− mice the CDC2/cyclin B1 complex failed to assemble and CDC2 kinase activity was not present in the testis. However, addition of recombinant Hsp70-2 protein to homogenates of testes from Hsp70-2−/− mice restored CDC2/cyclin B1 complex formation and CDC2 kinase activity (Zhu et al., 1997). Since the G2/M-phase transition requires cyclin B-dependent CDC2 protein kinase activity (Draetta et al., 1988; Dunphy et al., 1988), it is likely that disruption of CDC2/cyclin B1 complex assembly is one cause of failure for Hsp70-2−/− spermatocytes to complete meiosis I (Zhu et al., 1997). It is unknown if there is a relationship between these findings and the requirement for Hsp70-2 in SC desynthesis. However, the SCP1/SYN1 protein that is a major component of the transverse filaments of the SC contains a carboxy-terminus basic domain that is a potential target site for CDC2 protein kinase (Meuwissen et al., 1992; Dobson et al., 1994). Since phosphorylation is a common target site for CDC2, leading to assembly of the CDC2/cyclin B1 complex that enables CDC2 kinase activity (Zhu et al., 1997). Since Hsp70-2 serves a role in apoptotic mechanisms, SC disassembly, and completion of meiotic prophase in pachytene spermatocytes, we hypothesize that it is a chaperone of proteins that are integral to these processes.

REFERENCES


