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 (Wnisiewski 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 I.

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 129/SvEv 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 immunostained with an Elite ABC-peroxidase kit (Vector Laboratories, Burlingame, CA) as previously described (Dix et al., 1996a). Antibodies used include rabbit polyclonal antibody 2A, which is specific for a peptide sequence in HSP70-2 (Rosario et al., 1992), mouse IgM monoclonal antibody J1, which recognizes a stage-specific for a peptide sequence in HSP70-2 (Rosario et al., 1992), and mouse IgM monoclonal antibody J1, which recognizes a stage-

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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<td>Hsp70-2</td>
<td>upper 5′-CAGACGCCACCTTTCCATAC-3′ lower 5′-TTTTGTCTGCCTGCAATCT-3′</td>
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<tr>
<td>Histone H1t</td>
<td>upper 5′-GCCCCCATACTCCCACT-3′ lower 5′-TTTCTCTGCTGGCTTGT-3′</td>
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<td>Proacrosin</td>
<td>upper 5′-GTGTGTGAGGTATGGTCAAGGA-3′ lower 5′-GCCGAAGAGAGAGGTAAAGAG-ACCAT-3′</td>
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<tr>
<td>CyclinA1</td>
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<tr>
<td>Sprm-1</td>
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<td>219</td>
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<tr>
<td>Hsc70t</td>
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<td>222</td>
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<tr>
<td>Gapd-s</td>
<td>upper 5′-AACATCATCCCCATGGCCAC-3′ lower 5′-GATCCGGGGATAGTGTCGAGCA- CGAGATAGGAAG-3′</td>
<td>194</td>
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Detection and characterization of apoptotic germ cells

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labelling (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% formamide 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 polystyrene 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 diplotene 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 synaptonemal 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+/ mice 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

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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 which are prevalent at day 24 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 were nearly normal except for a few apoptotic cells (arrowhead). This is in contrast to a stage VI tubule 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.

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 Hsp70-2−/− 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 synthesize 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 of Sprm1 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.

Failure of synaptonemal complex desynapsis in Hsp70−/− mice

Silver-stained SCs were analyzed by light and electron microscopy to correlate the stage of spermatocyte developmental arrest with the stage of SC assembly or disassembly in Hsp70-2−/− mice. Leptotene, zygotene, pachytene (substages I to V) and diplotene stage spermatocytes were identified in juvenile and adult wild-type, Hsp70-2+/− and Hsp70-2−/− mice. In adult C57BL/6N Hsp70-2−/− mice, mid to late pachytene cells with fully formed SCs accounted for over half the cells scored (Figs 7A, 8). Most SCs of pachytene spermatocytes of Hsp70-2−/− mice appeared normal, forming fully synapsed autosomal bivalents with no evident increased asynapsis or mispairing. However, desynapsing SCs typical of diplotene cells from a Hsp70-2+/− mouse are shown for comparison (Fig. 7C). These results were consistent with what was observed previously in pachytene cells from adult Hsp70-2−/− mice (Dix et al., 1996b). Similar results were obtained from electron microscopic analysis of SC spreads of pachytene cells from 129Sv/Ev 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 indirectly 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 constitute 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 observations). 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 triggering 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 spermatocytes 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 desynapsis in Hsp70-2′ mice suggests that HSP70-2 chaperone...
function is required for SC desynthesis. 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
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 SCPI/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 (Mieuwissen et al., 1992; Dobson et al., 1994). Since phosphorylation is a common mechanism for protein complex disassembly (Draetta and Beach, 1988), this suggests that failure of SC desynthesis in Hsp70-2-/- spermatocytes might be due to loss of the ability of CDC2 to phosphorylate SCPI/SYN1. The presence of HSP70-2 in SCs could facilitate the activation of CDC2 for this process in wild-type mice.

The function of other components of the cell cycle machinery may also depend on HSP70-2, and these in turn may affect SC desynthesis and disassembly. In the mouse, a unique transcript for CDC25C, a phosphatase which activates CDC2, is present in late pachytene-diplotene spermatocytes (Wu and Wolgemuth, 1995). Mouse pachytene spermatocytes can be induced to undergo the G2/M transition in vitro with okadaic acid, indicating that regulation of protein phosphorylation status is important in this process (Wiltshire et al., 1995). In addition, the gene for cyclin A1 is expressed only in late spermatocytes and oocytes in the mouse (Sweeney et al., 1996). Although the role of cyclin A1 is unknown, cyclin A1 mRNA was not found in the testes of Hsp70-2-/- mice in this study, suggesting that the protein does not function in the processes disrupted in Hsp70-2-/- spermatocytes. Furthermore, several genes have been identified in *Drosophila* that are required for G2/M progression in spermatocytes. Mutations in *Dmcdc2* (a *Cdc2* homologue; Sigrist et al., 1995) or *twine* (a *Cdc25* homologue; Lin et al., 1996) cause spermatocytes to skip the meiotic division and initiate limited spermatid differentiation. Several other *Drosophila* meiotic-arrest mutant genes cause chromosome condensation to stop at a similar point as occurs in *twine* mutants, suggesting that they may be required for activation of either the *Cdc2* or the *Cdc25* homologue (Lin et al., 1996). However, since *Drosophila* spermatocytes do not assemble SCs nor undergo genetic recombination during meiotic prophase (Orr-Weaver, 1995), they apparently use different strategies than the mouse to coordinate SC function, cyclin-dependent kinase activities, and the G2/M transition.

The mutant phenotype seen in Hsp70-2-/- mice has several distinct features, each of which may relate to a specific aspect of HSP70-2 function during meiotic prophase. First, there is increased apoptosis in early to mid-pachytene spermatocyte development, suggesting that a process activating apoptosis is potentiated, or a process inhibiting apoptosis is compromised in the absence of HSP70-2. Second, the SCs fail to desynapse and pachytene spermatocyte development is blocked at the end of meiotic prophase. Whether lack of SC desynthesis is a direct effect of the absence of HSP70-2 protein, or an indirect effect of disrupting other essential cellular functions remains to be determined. Third, spermatocytes fail to undergo the G2/M transition, which probably occurs largely due to disruption of cell cycle machinery regulating the G2/M checkpoint. Recent studies have shown that HSP70-2 is required to chaperone 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.

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**REFERENCES**


