HSP70-2 is required for CDC2 kinase activity in meiosis I of mouse spermatocytes

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SUMMARY

Cyclin B-dependent CDC2 kinase activity has a key role in triggering the G2/M-phase transition during the mitotic and meiotic cell cycles. The Hsp70-2 gene is expressed only in spermatogenic cells at a significant level. In Hsp70-2 gene knock-out (Hsp70-2−/−) mice, primary spermatocytes fail to complete meiosis I, suggesting a link between HSP70-2 heat-shock protein and CDC2 kinase activity during this phase of spermatogenesis. Members of the HSP70 protein family are molecular chaperones that mediate protein de novo folding, translocation and multimer assembly. This study used immunoprecipitation-coupled western blot and in vitro reconstitution experiments to show that HSP70-2 interacts with CDC2 in the mouse testis, appears to be a molecular chaperone for CDC2, and is required for CDC2/cyclin B1 complex formation. Previous studies reported that most CDC2 kinase activity in the mouse testis is present in pachytene spermatocytes. Although CDC2 kinase activity for histone H1 was present in the testis of wild-type mice, it was nearly absent from the testis of Hsp70-2−/− mice, probably due to defective CDC2/cyclin B1 complex formation. Furthermore, addition of HSP70-2 to freshly prepared extracts of testis from Hsp70-2−/− mice not only restored CDC2/cyclin B1 complex formation but also reconstituted CDC2 kinase activity in vitro. It appears that one cause of failure to complete meiosis I during spermatogenesis in Hsp70-2−/− mice is disruption of CDC2/cyclin B1 assembly in pachytene spermatocytes, thereby preventing development of the CDC2 kinase activity required to trigger G2/M-phase transition. These studies provide novel in vivo evidence for a link between an HSP70 molecular chaperone and CDC2 kinase activity essential for the meiotic cell cycle in spermatogenesis.

Key words: cyclin-dependent kinase, cyclin B1, molecular chaperone, HSP70, spermatogenesis, mouse

INTRODUCTION

Progression through the mitotic cell cycle in eukaryotic cells is controlled by cyclin-dependent kinases (CDKs) at two primary transitions, from G1 to S-phase and from G2 to M-phase. CDK activities are regulated by several mechanisms, including ubiquitination and proteolysis of cyclins, alterations in CDK phosphorylation, specific inhibitors of cyclin and CDK activities, changes in rate of synthesis of CDKs and cyclins (Morgan 1995), and assembly of CDK/cyclin complexes (Yee et al., 1995; Fisher et al., 1995; Tassan et al., 1995). The G2/M-phase transition requires cyclin B-dependent CDC2 protein kinase activity (Draetta and Beach 1988; Draetta et al., 1988; Dunphy et al., 1988). The CDC2 kinase activity is regulated by two major events: the binding of a cyclin B regulatory subunit to the CDC2 catalytic subunit, and phosphorylation and dephosphorylation of different residues on the CDC2 subunit (Morgan 1995; Solomon 1994). Although CDC2 is present constitutively in most cells, CDC2 kinase activity oscillates during the mitotic cell cycle due to the accumulation and periodic destruction of cyclin B (Draetta et al., 1989).

Although cell cycle control during meiosis has not been as well studied as during mitosis in higher organisms, the maturation promoting factor (MPF) required for meiosis in frog oocytes is composed of CDC2 and cyclin B (Dunphy et al., 1988; Draetta et al., 1989). In addition, cDNAs for B- and A-type cyclins expressed in the mouse testis have been cloned and the expression patterns of various CDK and cyclin mRNAs have been examined during mouse spermatogenesis (Chapman and Wolgemuth 1992, 1993, 1994; Ravnik et al., 1995; Sweeney et al., 1996). Cdc2 and CdK2 transcripts were found in pachytene spermatocytes (meiotic I prophase), and Cdc2 transcripts were most abundant in late pachytene and diplotene spermatocytes preparing to undergo the first meiotic division (Rhee and Wolgemuth 1995). In addition, cyclin B1 and CDC2 proteins were present at highest levels in pachytene spermatocytes of wild-type mice, but little CDC2 protein and no cyclin B1 protein were detected in the testis of germ cell-deficient mice (Chapman and Wolgemuth 1994). Furthermore, CDC2 kinase activity was present mainly in pachytene spermatocytes and at low or undetectable levels in somatic cells and early germ cells of the testis of wild-type mice (Chapman and...
The generation of Hsp70-2−/− mice was described previously (Dix et al., 1996b) and the knock-out mice were maintained by back-crossing with C57BL/6N mice. The animals were housed in the National Institute of Environmental Health Science (NIEHS) animal facilities.

All procedures involving animals were performed in accordance with USPHS guidelines and were approved previously by the NIEHS Animal Care and Use Committee.

Preparation of protein extracts from mouse tissues
Testis and thymus were collected from the same wild-type and Hsp70-2−/− adult male mice. Total lysates were prepared with a dounce homogenizer in fresh ice-cold protein lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1% NP-40, 50 mM NaF, 1 mM NaVO₃, 1 mM DTT, 1 mM PMSF, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, 10 μg/ml aprotinin). The reagents were obtained from Sigma Chemical Co. (St. Louis, MO). The extracts were cleared by centrifugation at 440 g for 10 minutes at 4°C. The supernatants were collected for SDS-PAGE, western blotting, in vitro CDC2 kinase assays and immunoprecipitation studies. The protein concentrations of the testis and thymus extracts were determined by Bradford dye assay and samples were used immediately or stored at −70°C.

Immunoprecipitation and western blot analysis
For immunoprecipitations, 50 μg of total proteins were clarified by incubation with 15 μl (1:1 slurry in 1× PBS) of protein A/G-Sepharose (Santa Cruz Technology Inc.) in the immunoprecipitation buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1% NP-40, 1 mM EDTA pH 8.0, 1 mM PMSF, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, 10 μg/ml aprotinin) for 1 hour at 4°C with shaking. Immunoprecipitations were performed by incubating aliquots of the precleared lysates with either preimmune serum (control), specific antibodies or specific antibodies preincubated with the immunogen peptide for overnight at 4°C with shaking. Protein A/G-Sepharose (15 μl) was added and incubated for 1 hour at 4°C with shaking. The resulting immunoprecipitates were collected by centrifugation at 440 g for 5 minutes at 4°C and the pellets were washed 4 times with 750 μl of ice-cold immunoprecipitation buffer. The pellets were resuspended in 20 μl of 1× SDS protein sample buffer (62.5 mM Tris pH 6.8, 40 mM DTT, 2% SDS, 0.025% bromophenol blue, 10% glycerol) and were subjected to western blot analysis.

Total protein (50 μg) extracts from testis and thymus for western blot analysis were boiled for 3 minutes and then fractionated by SDS-PAGE and transferred to nitrocellulose. The blots were blocked with 10% dried milk in Tris-buffered saline with 0.1% Tween-20 (TBS-T) for 30 minutes at room temperature, probed with antibodies to CDC2, cyclin B1 (Santa Cruz Technology Inc., Santa Cruz, CA) or Hsp70-2 (Rosario et al., 1992) for 1 hour at room temperature and then incubated with horseradish peroxidase-conjugated anti-IgG secondary antibody (Santa Cruz Technology Inc.) for 1 hour at room temperature. The proteins were detected using the ECL system (Amersham Corp., Arlington, IL).

For western blot analysis of phosphorylation status of CDC2 protein, total protein extracts (80 μg) from the testis of wild-type and Hsp70-2−/− mice were separated by electrophoresis using 10% SDS-PAGE (25 cm) for 6 hours at 150 V. The proteins were transferred at 0.1 Amp onto a nitrocellulose membrane overnight at 4°C. The blot was then probed with the antisera to CDC2. The antisera to CDC2 and the CDC2 peptide immunogen were gifts of Dr Y. Xiong, University of North Carolina at Chapel Hill.

In vitro CDC2 kinase assays
The CDC2/cyclin B1 complexes were immunoprecipitated with antisera to CDC2 as described above. The resulting immune complexes were collected and washed 3 times with ice-cold immunoprecipitation buffer and 2 times with ice-cold kinase assay buffer (50 mM Heps pH 7.5, 10 mM MgCl₂, 80 mM β-glycerophosphate pH 7.3, 20 mM EGTA, 1 mM DTT, 10 mM ATP). Histone H1 kinase activity was assayed in the kinase assay buffer (50 μl) with addition of 5 μM Ca²⁺-dependent kinase inhibitor (Sigma Chemical Co.), 50 μg of calf thymus histone H1 (Boehringer Mannheim Corp., Indianapolis, IN) and 0.1 mCi/ml [γ-³²P]ATP. The reactions were
Expression and purification of His6-tagged HSP70-2

The pDMEX1.9 plasmid containing a 1.9 kb EcoRI/HindIII HSP70-2 cDNA fragment in His6 expression vector pTrcHisB (Invitrogen, San Diego, CA) was kindly provided by Dr Daniel Mamelak (Department of Microbiology, Hospital for Sick Children, Toronto, Ontario, Canada). The plasmid DNA was introduced into the Escherichia coli strain Top10 (Invitrogen). The His6-tagged HSP70-2 was purified from the bacterial cells expressing His6-HSP70-2 as described by Zhu et al. (1995).

In vitro reconstitution of the CDC2/cyclin B1 complexes and CDC2 kinase assay

Fifty µg of total protein extracts from the testis of Hsp70-2−/− mice were mixed with different amounts of affinity purified recombinant His6-HSP70-2 (0, 100 ng, 200 ng, 300 ng, 400 ng and 500 ng) and the mixtures incubated for 30 minutes at 30°C in 150 µl protein lysis buffer. The reaction mixtures were immunoprecipitated with antiserum to CDC2 and the immunoprecipitated materials either subjected to western blot analysis with antiserum to cyclin B1 or were used for CDC2 kinase assay as described above.

RESULTS

HSP70-2 is associated with CDC2 in mouse testis

Spermatocytes do not progress through the G2/M checkpoint of meiosis I in Hsp70-2−/− mice (Dix et al., 1996b). To test the hypothesis that HSP70-2 functions as a chaperone for CDC2, we examined their interactions by immunoprecipitation-coupled western blot analysis. Protein extracts of testis were immunoprecipitated with polyclonal antiserum 2A that recognizes a peptide sequence specific to HSP70-2 (Rosario et al., 1992) and the immunoprecipitated proteins were subjected to western blot analysis with antibodies to CDC2 or cyclin B1. CDC2 coimmunoprecipitated with HSP70-2 from extracts of testis from wild-type mice (Fig. 1A, lane 1). However, cyclin B1 did not coimmunoprecipitate with HSP70-2 from extracts of testis from wild-type mice (Fig. 1B). HSP70-2 was also found to associate with CDC2 when extracts of testis from wild-type mice were immunoprecipitated with antiserum to CDC2 and western blots were probed with antiserum 2A (Fig. 1C). CDC2 was not coimmunoprecipitated with antiserum 2A from extracts of testis from Hsp70-2−/− mice (Fig. 1, lane 3) or when pre-immune serum was used (Fig. 1A, lane 5), and neither CDC2 nor cyclin B1 was precipitated from control thymus extracts by antiserum 2A (Fig. 1). Since cyclin B1 did not coimmunoprecipitate with CDC2 and HSP70-2, these studies suggest that HSP70-2 associates with CDC2 only when it is not in the CDC2/cyclin B1 complex.

CDC2 kinase activity is not detected in the testis of Hsp70-2−/− mice

The interaction demonstrated between HSP70-2 and CDC2 in the testis of wild-type mice supported the hypothesis that HSP70-2 is a molecular chaperone of CDC2 and suggested that CDC2 kinase activity might be altered in the testis of Hsp70-2−/− mice. We assayed CDC2 kinase activity in immunoprecipitates from testis and control thymus extracts of wild-type or Hsp70-2−/− mice, as determined by the ability to phosphorylate exogenous histone H1. Significant kinase activity was present in CDC2 or cyclin B1 immunoprecipitates from extracts of testis or thymus from wild-type mice (Fig. 2 lanes 3, 4, 7, 8) and from extracts of thymus from Hsp70-2−/− mice (Fig. 2 lanes 6, 10). However, little or no kinase activity was detected in immunoprecipitates from extracts of testis from Hsp70-2−/− mice (Fig. 2, lanes 5, 9). These findings suggest that CDC2 kinase activity depends upon the presence of HSP70-2 in spermatogenic cells.

HSP70-2 is required for the formation of CDC2/cyclin B1 complexes in mouse spermatocytes

It was possible that the absence of CDC2 kinase activity in the testis of Hsp70-2−/− mice was due to disruption of expression or stability of either cyclin B1 or CDC2. However, western blot analysis showed that both proteins were present...
in the testis of Hsp70-2/− mice (Fig. 3). Along with the above results, this suggested that HSP70-2 might be involved in the formation of CDC2/cyclin B1 complexes in mouse pachytene spermatocytes. To examine this possibility further, CDC2 protein was immunoprecipitated with an antibody to CDC2 and the immunoprecipitates were analyzed by western blot with an antibody to cyclin B1. CDC2 and cyclin B1 coimmunoprecipitated from extracts of testis and thymus from wild-type mice (Fig. 4A, lanes 1, 2) and from extracts of thymus from Hsp70-2/− mice (Fig. 4A, lane 6), but not from extracts of testis from Hsp70-2/− mice (Fig. 4A, lane 5). Cyclin B1 was not detected in any of the immunocomplexes when the antibody to CDC2 was preincubated with the peptide immunogen (Fig. 4A, lanes 3, 4, 7, 8), confirming the specificity of the assay. However, only CDC2 and not cyclin B1 was present when the CDC2 antibody was used to immunoprecipitate with extracts of testis from Hsp70-2/− mice (Fig. 4B, lane 5). Similar results were obtained with the reciprocal experiments, in which the extracts were immunoprecipitated with an antibody to cyclin B1 and then the immunoprecipitates were analyzed by western blot analysis with an antibody to CDC2 (Fig. 4C,D). These results indicate that CDC2 and cyclin B1 proteins are present in the testis of Hsp70-2/− mice, but that CDC2/cyclin B1 complexes do not form.

The phosphorylation status of CDC2 is altered in the testis of Hsp70-2/− mice

Binding of cyclin B to CDC2 is required to modify the phosphorylation of CDC2 and for CDC2 to acquire kinase activity (Solomon et al., 1990, 1991). Therefore, if cyclin B1/CDC2 complex formation does not occur, the phosphorylation status of CDC2 should be altered in the testis of Hsp70-2/− mice. This was examined by western blot, which has been used previously to determine the phosphorylation status of CDC2 (Draetta and Beach 1988; Solomon et al., 1990). Three CDC2 protein variants were present in extracts from testis of wild-type mice (Fig. 5, lane 1). However, two of these CDC2 variants were substantially reduced in extracts from testis of Hsp70-2/− mice (Fig. 5, lane 2). Most of the CDC2 protein in the testis of Hsp70-2/− mice migrated in the lowest band, the
Hsp70-2 gene These studies examined why disruption of the Hsp70-2 gene caused primary spermatocytes to arrest prior to the G2/M-phase transition (Dix et al., 1996b). The results support the hypothesis that HSP70-2 is a chaperone for CDC2 in pachytene spermatocytes, with their direct or indirect interaction required for completion of meiosis I. We show that HSP70-2 associates with CDC2, but not with cyclin B1 in the mouse testis (Fig. 1) and that loss of this interaction is likely to be a primary cause of the meiotic arrest in Hsp70-2−/− male mice, suggesting the model for formation of the CDC2/cyclin B1 complex shown in Fig. 7. The present study appears to be the first to demonstrate that interaction is required between an HSP70 protein and a CDK to enable an essential step in cell cycle progression.

Although this is a novel finding, previous studies have implied that HSPs have a role in mitotic cell cycle progression. For example, mutation in the heat-shock factor gene (mas3) caused a temperature-sensitive defect in progression through the G2 phase of the cell cycle in yeast (Smith and Yaffe 1991), while association of HSP70 with unknown proteins p34 and p27 was cell cycle-dependent and enriched in the G2 phase of human HeLa cells (Milarski et al., 1989). In addition, stress-

In vitro reconstitution of CDC2/cyclin B1 complexes and CDC2 kinase activity

To evaluate the possibility that HSP70-2 is required for CDC2/cyclin B1 assembly in spermatocytes, in vitro reconstitution experiments were performed by adding recombinant His6-HSP70-2 to freshly prepared extracts of testis from Hsp70-2−/− mice. Increasing amounts of His6-HSP70-2 were added to aliquots of the extracts, CDC2 was immunoprecipitated and the immunocomplexes were analyzed by western blot for the presence of cyclin B1. Although CDC2 and cyclin B1 did not coimmunoprecipitate from extracts of testis from Hsp70-2−/− mice (Fig. 6A, lane 2), they did coimmunoprecipitate when His6-HSP70-2 was added to the extracts. The coimmunoprecipitation of CDC2 and cyclin B1 was enhanced by adding increasing amounts of His6-HSP70-2 (Fig. 6A, lanes 3-6).

Furthermore, CDC2 kinase activity was present in the immunoprecipitates prepared after addition of His6-HSP70-2. Although little CDC2 kinase activity was detected in the absence of His6-HSP70-2 (Fig. 6B, lane 1) and in the presence of 100 ng or 200 ng His6-HSP70-2 (Fig. 6B, lanes 2 and 3), CDC2 kinase activity increased when additional amounts of His6-HSP70-2 (300 ng-500 ng) were added to the extracts of testis from Hsp70-2−/− mice (Fig. 6B, lane 4, 5 and 6). However, the restored CDC2/cyclin B1 complexes had somewhat lower levels of H1 kinase activity than extracts of wild-type testis. Since CDC2 is largely dephosphorylated in extracts of testis from Hsp70-2−/− mice (Fig 5), the complexes formed in vitro may not achieve full phosphorylation status. However, these findings provide compelling evidence that HSP70-2 has a specific chaperone role necessary for CDC2/cyclin B1 complex formation and for the development of CDC2 kinase activity in mouse spermatocytes.

DISCUSSION

HSP70-2 interacts with CDC2 in mouse spermatocytes

These studies examined why disruption of the Hsp70-2 gene caused primary spermatocytes to arrest prior to the G2/M-phase transition (Dix et al., 1996b). The results support the hypothesis that HSP70-2 is a chaperone for CDC2 in pachytene spermatocytes, with their direct or indirect interaction required for completion of meiosis I. We show that HSP70-2 associates with CDC2, but not with cyclin B1 in the mouse testis (Fig. 1) and that loss of this interaction is likely to be a primary cause of the meiotic arrest in Hsp70-2−/− male mice, suggesting the model for formation of the CDC2/cyclin B1 complex shown in Fig. 7. The present study appears to be the first to demonstrate that interaction is required between an HSP70 protein and a CDK to enable an essential step in cell cycle progression.

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![Fig. 5. Phosphorylation status of CDC2 protein.](image)

**Fig. 5.** Phosphorylation status of CDC2 protein. Proteins extracted from the testis of wild-type (lanes 1) and Hsp70-2−/− (lane 2) mice were resolved by SDS-PAGE. The blot was probed with the antiserum to CDC2. Three different variants of the CDC2 protein are indicated.

**Fig. 6.** In vitro reconstitution of the CDC2/cyclin B1 complexes. (A) Proteins extracted from the testis of wild-type mice without immunoprecipitation were used as a control (lane 1). Proteins extracted from the testis of Hsp70-2−/− mice were mixed with increasing amounts of His6-HSP70-2 (0 ng, lane 2; 100 ng, lane 3; 200 ng, lane 4; 300 ng, lane 5; 400 ng, lane 6 and 500 ng lane 7). The mixtures were immunoprecipitated with antisera to CDC2 and subjected to western blot analysis with antisera to cyclin B1. (B) CDC2 kinase activity was assayed using the immunoprecipitated CDC2/cyclin B1 protein from the reconstitution experiments (0 ng, lane 1; 100 ng, lane 2; 200 ng, lane 3; 300 ng, lane 4; 400 ng, lane 5 and 500 ng, lane 6) as described in Materials and Methods. Immunoprecipitates from the testis of wild-type mice were used as a control (lane 7). Immunoprecipitates with antisera to CDC2 and cyclin B1 were used as a positive control (lane 9).
Inducible HSP70 was involved in determining the duration of the G2-phase arrest caused by doxorubicin in murine fibrosarcoma WEHI-S cells (Karlseder et al., 1996).

**HSP70-2 is required for formation of the CDC2/cyclin B1 complex and CDC2 kinase activity in testis**

CDC2 kinase activity was substantially lower in the testis of Hsp70-2−/− mice than in wild-type mice (Fig. 3), indicating that HSP70-2 is necessary for the generation of CDC2 kinase activity in mouse spermatocytes. The absence of HSP70-2 could affect synthesis or stability of CDC2 and cyclin B1, the assembly of the CDC2/cyclin B1 complex, and/or the phosphorylation status of CDC2, conditions which have key roles in controlling the G2/M-phase transition (Morgan 1995). Immunoprecipitation and western blot analysis showed that CDC2 and cyclin B1 proteins were present in the testis of Hsp70-2−/− mice (Fig. 4), but that CDC2/cyclin B1 complexes were not present as in the testis of wild-type mice (Fig. 5A, lane 1). Furthermore, CDC2/cyclin B1 complexes were not only present but also had CDC2 kinase activity after purified recombinant His6-HSP70-2 was added to freshly prepared testis extracts from Hsp70-2−/+ mice (Fig. 7). Although other HSP70 proteins are expressed in the spermatocytes of Hsp70-2−/+ mice, they do not associate with CDC2 (data not shown) and do not compensate for loss of HSP70-2. It is possible that the interaction between HSP70-2 and CDC2 involves additional proteins. Nevertheless, these data strongly suggest that CDC2 requires direct or indirect interaction with HSP70-2 to be able to complex with cyclin B1 and to acquire kinase activity in mouse spermatocytes.

Our findings are consistent with those from prior studies indicating that HSPs and other proteins are important in the assembly of protein complexes. For example, HSP70 was required for assembly of the rat glucocorticoid (Hutchison et al., 1994) and progesterone receptors (Smith et al., 1992) into heterocomplexes with HSP90. Other studies have shown recently that HSPs can serve as auxiliary proteins in formation of CDK/cyclin complexes. Cdc37 is required for assembly of CDC2/cyclin complexes in Saccharomyces cerevisiae (Gerber et al., 1995; Morgan et al., 1996) and the mammalian homologue (p50(Cdc37)) is the subunit of HSP90 that acts as a molecular chaperone to stabilize CDK4/cyclin D complexes in mouse fibroblasts (Stepanova et al., 1996). This HSP90 subunit has also been implicated in the stabilization of the Raf-1 (Stancato et al., 1993; Nathan and Lindquist 1995), Wee1 (Alique et al., 1994), pp60src (Boschelli 1993; Xu and Lindquist 1993), and Cdk4 protein kinases (Stepanova et al., 1996). In addition, MAT1 is a RING finger protein that has been identified recently as an assembly factor for CDK7 and cyclin H to form CAK (cyclin-dependent kinase activating kinase) complexes in human HeLa and EW-1 cells (Yee et al., 1995), mouse 3T3 cells (Fisher et al., 1995) and starfish and Xenopus oocytes (Devault et al., 1995). MAT1 becomes a subunit of the stable CAK assembly, but neither CDK7 nor cyclin H alone binds MAT1 (Tassan et al., 1995). However, since HSP70-2 was found in association with CDC2 but not with cyclin B1 or the CDC2/cyclin B1 complex, the protein apparently assists in CDC2/cyclin B1 complex formation through interaction with CDC2, rather than becoming part of the complex. We hypothesize that HSP70-2 establishes and/or maintains the CDC2 protein in a conformation that is competent for cyclin B1 binding in mouse spermatocytes.

These studies also provide evidence that the phosphorylation status of CDC2 is altered in the testis of Hsp70-2−/+ mice and suggest that this is due to the failure of CDC2/cyclin B1 complex formation (Fig. 6). However, CDC2 is activated in eukaryotes by CDC25 phosphatase and CDK7/cyclin H kinase while it is associated with cyclin A or B1 (Solomon et al., 1991, 1994; Morgan 1995). CDK7 and cyclin H transcripts are abundant in human testis (Yee et al., 1995) and an alternatively spliced form of CDC25 is present in late pachytene and diplotene spermatocytes in mice (Wu and Wolgemuth 1995). It will be of interest to determine if HSP70-2 is also associated with these or related cell cycle regulatory molecules in meiosis, or if the requirement for HSP70-2 in this process is limited to CDC2/cyclin B1 complex assembly.

**HSP70-2 may have other roles in mouse spermatocytes**

Disruption of the Hsp70-2 gene also caused all pachytene spermatocytes to undergo apoptosis by the end of meiosis prophase I (Dix et al., 1996b; Mori et al., 1997). Since apoptosis and cell cycle regulation are closely linked in proliferating cells (King and Cidlowski 1995; Meikrantz and Schlegel 1995), sperma-
HSP70-2 is required for CDC2 kinase activity

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Implication of HSP70-2 association with CDC2 for cell cycle regulation

Several novel assembly factors identified recently have provided an additional regulatory pathway for controlling the activity of CDKs in somatic cells (Fisher et al., 1995; Devault et al., 1995; Yee et al., 1995). The present study suggests a similar role of HSP70-2 in the G2/M transition in meiosis I of spermatogenesis. Furthermore, these results raise questions of whether other chaperones are involved in assembly of CDK/cyclin complexes during mitosis in somatic cells (e.g., thymus in this study) or during meiosis in oocytes. Since the apparent broad specificity of HSP70 proteins may be restricted by partner proteins that customize chaperone interactions and determine specificity (Rassow et al., 1995), other proteins may remain to be identified that determine how HSP70 proteins are involved in cell cycle processes (Miliarski et al., 1989).

tocyte apoptosis might occur because of disruption of CDC2/cyclin B1 complex assembly and CDC2 function. However, previous studies are not in agreement on the role of CDC2/cyclin B in apoptosis. CDC2 activation was required for apoptosis in YAK-1 lymphoma cells (Shi et al., 1994), and cyclin A-associated CDC2 and CDK2 kinase activities increased during apoptosis in HeLa cells while cyclin B-associated kinase activity remained low (Meikrantz et al., 1994). In contrast, inactivation of CDC2 increased the level of apoptosis induced by DNA strand break-inducing drugs in a temperature-sensitive mouse CDC2-mutant cell line, suggesting that CDC2 suppressed apoptosis in G2-arrested cells (Ongkeko et al., 1995). This implies that the role of CDC2 in apoptosis varies between cell types. Further studies will be necessary to determine if CDC2/cyclin B1 kinase activity affects the level of apoptosis in mouse spermatocytes.

We have also shown that HSP70-2 is associated with the synaptonemal complex in pachytenic spermatocytes and that disassembly of the synaptonemal complex is disrupted in Hsp70-2<sup>−/−</sup> mice (Allen et al., 1996; Dix et al., 1996b), MLH1 and PMS2 have been identified recently as physiological regulators for meiotic cell cycle and are also associated with the synaptonemal complex in pachytenic spermatocytes (Baker et al., 1995; Edelmann et al., 1996). Interestingly, the testicular abnormalities observed in PMS2-deficient mice (Baker et al., 1995) and MLH1-deficient mice (Edelmann et al., 1996) were similar to those seen in Hsp70-2<sup>−/−</sup> male mice. If HSP70-2 is a molecular chaperone for those or other proteins associated with the synaptonemal complex, their functions may also be disrupted in Hsp70-2<sup>−/−</sup> mice, thereby leading to apoptosis. In addition, SCP1 is a unique component of the transverse filaments of the synaptonemal complex and contains a carboxy-terminal domain that is a potential target site for CDC2 kinase (Meuwissen et al., 1992; Dobson et al., 1994). Since protein complex disassembly often involves phosphorylation, failure of synaptonemal complex desynapsis in Hsp70-2<sup>−/−</sup> spermatocytes might be due to loss of CDC2 kinase activity. Although the relationship between the processes of meiosis, apoptosis and synaptonemal complex function is unclear, they appear to share a common need for the HSP70-2 protein in pachytenic spermatocytes.


