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HSP70-2 is part of the synaptonemal complex in mouse and hamster spermatocytes

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Abstract. Mouse spermatogenic cells are known to express HSP70-2, a member of the HSP70 family of heat-shock proteins. The purpose of the present study was to characterize further the expression and localization of HSP70-2 in meiotic cells of mice and hamsters. After separating mouse spermatogenic cells into cytoplasmic and nuclear fractions, proteins were separated by two-dimensional gel electrophoresis and detected with HSP-specific antibodies. Of several HSP70 proteins identified in the cytoplasm, only HSC70 and HSP70-2 were also detected in the nucleus. Immunocytological analyses of spermatocyte prophase cells revealed that HSP70-2 was associated with the synaptonemal complex. Surface-spread synaptonemal complexes at pachytene and diplotene stages labeled distinctly with the antiserum to HSP70-2. Synaptonemal complexes from fetal mouse oocytes failed to show any evidence of HSP70-2. Reverse-transcriptase-polymerase chain reaction (RT-PCR) analyses of gene expression confirmed this sex specificity; *Hsp70-2* mRNA was detected in mouse testes, but not ovaries. These findings are suggestive of a previously unsuspected sexual dimorphism in structure and/or function of the synaptonemal complex.

Introduction

HSP70-2, a member of the M_r 70,000 heat-shock protein (HSP70) family, is developmentally regulated and expressed at high levels during meiosis in male mice (Allen et al. 1988; Zakeri et al. 1988), rats (Raab et al. 1995) and humans (Bonnycastle et al. 1994). The function of HSP70-2 in germ cells is unknown, but may involve the folding, sorting and/or structural assembly of other proteins, i.e., "chaperone" activities, that have been demonstrated for other HSPs (reviewed in Georgopoulos and Welch 1993; Parsell and Lindquist 1993). The appearance of HSP70-2 coincides with entry of cells into meiotic prophase, raising the possibility of a direct role for HSP70-2 in metabolism of meiotic chromosomes. Zakeri et al. (1988) speculated that this protein is associated with the synaptonemal complex (SC).

SCs form along the axes of meiotic chromosomes during synapsis of homologs and may facilitate recombination and subsequent segregation processes (Moses 1968; Hawley and Arbel 1993; Moens 1994). The SC is apparently composed of a number of proteins synthesized de novo during meiotic prophase (Heyting et al. 1988; Smith and Benavente 1992; Meuwissen et al. 1992; Dobson et al. 1994) and may include proteins that serve broader roles in chromosomal metabolism (Moens and Earnshaw 1989). Autoimmune sera from humans and mice have been found to label SCs (Dresser 1987; Haaf et al. 1989), as have polyclonal antibodies raised against SC components (Moens et al. 1992). To examine possible relationships of HSP70-2 with the SC and/or other nuclear structures, we have used biochemical and immunochemical methods to examine nuclear-cytoplasmic partitioning of HSP70-2 in germ cells and to identify the proteins and structures with which it is associated. Following isolation of cytoplasmic and nuclear fractions of spermatogenic cells, various HSP70 proteins were characterized by immunoprecipitation, two-dimensional gel electrophoresis and immunoblotting. Immunocytological analyses were used to localize HSP70-2 in spread nuclear preparations of mouse and hamster spermatocytes, and also from mouse oocytes. Finally, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was used to evaluate expression of *Hsp70-2* mRNA in mouse testes and ovaries.

Our results indicate a close association of HSP70-2 with chromosomal axes in mouse and hamster sperma-

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tocytes. Analyses in mice indicate that this association begins at the time axial elements form, and persists until SCs disassemble. Surprisingly, we find no evidence for the presence of this protein in oocytes, suggesting a hitherto unsuspected sexual dimorphism in SC structure and/or function.

Materials and methods

Mice and Armenian hamsters. Male C57Bl/6 mice (The Jackson Laboratory, Bar Harbor, Me.), male and female CD-1 mice (Charles River Laboratories, Raleigh, N.C.) and male Armenian hamsters (*Cricetulus migratorius*; Cytogen Research and Development, West Roxbury, Mass.) were used. These animals, which were 3 to 6 months of age, were housed at US Environmental Protection Agency (USEPA) and National Institute of Environmental Health Sciences (NIEHS) animal facilities under laminar flow conditions and were fed Purina rodent chow and water ad libitum. The animals were treated and housed in accordance with approved guidelines (DHEW Guidelines for the Care and Use of Laboratory Animals), and approved project reviews (Animal Care and Use Committees of the USEPA and NIEHS).

Antibodies. HSP70-2 was detected with antiserum 2A (Rosario et al. 1992), which was produced by immunizing rabbits with a synthetic oligopeptide based on the predicted sequence of HSP70-2 (Zakeri et al. 1988). HSC70 was detected with mouse monoclonal antibody 13D3 (Maekawa et al. 1989) and by rat monoclonal anti-

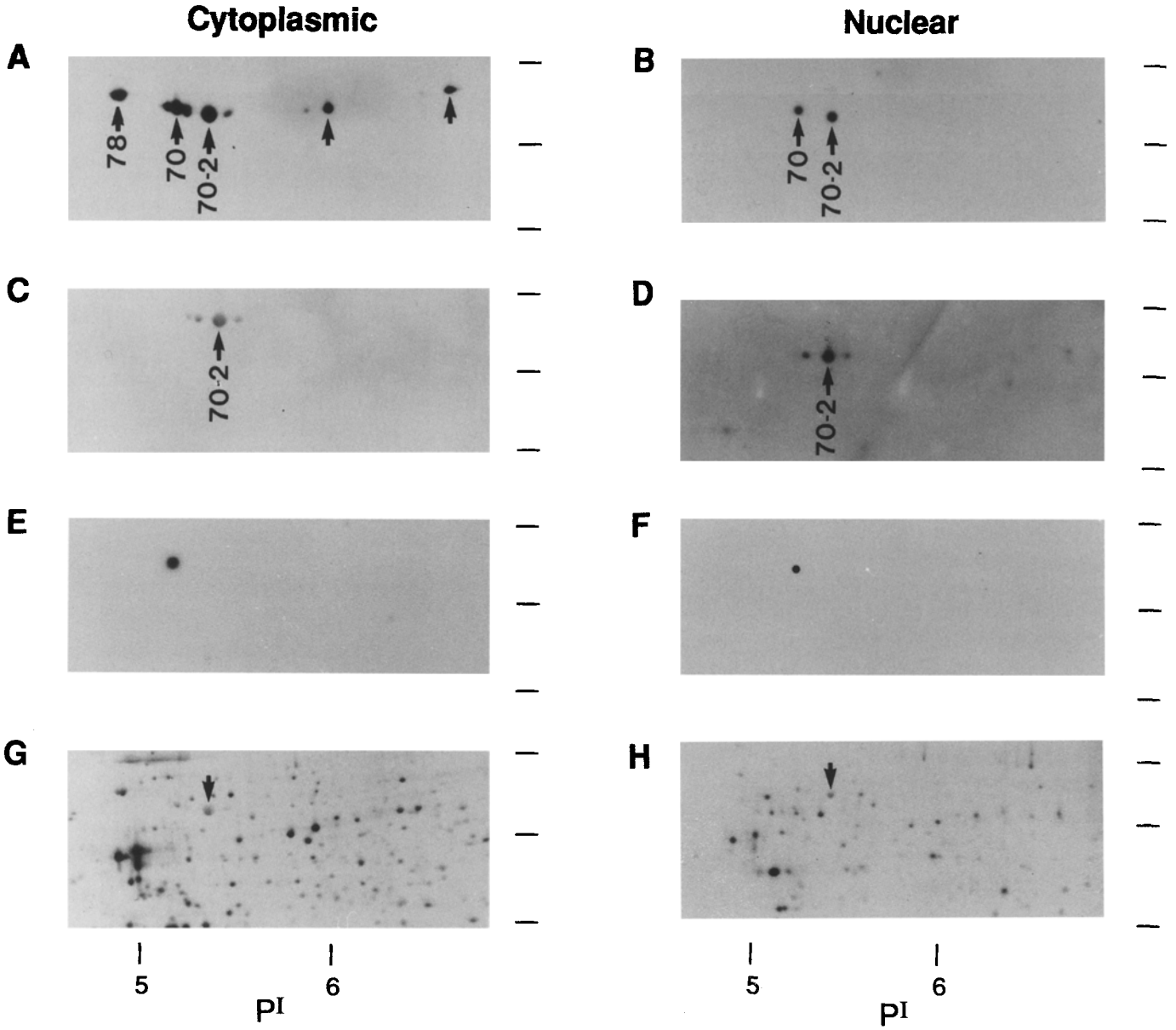

Fig. 1A–H. Two-dimensional polyacrylamide gel electrophoresis of nuclear and cytoplasmic proteins from male germ cells transferred to nitrocellulose and immunodetected. **A, C, E** and **G** Proteins from the cytoplasmic fraction. **B, D, F** and **H** Proteins from the nuclear fraction. Proteins in **A** and **B** are reacted with an antibody (BRM-22) that detects multiple HSP70s, including GRP78 (78), HSC70 (70) and HSP70-2 (70-2). Proteins in **C** and **D** are reacted with an antisera (2A) that recognizes HSP70-2. Those in **E** and **F** are reacted with an antibody (13D3) that recognizes HSC70. **G** and **H** Autoradiographic exposures of [^{35}S]methionine-labeled proteins from male germ cells (*arrow* indicates position of HSP70-2)

body 1B5 (Laszlo and Li 1985; supplied by StressGen Biotechnologies Corp., Victoria, B.C., Canada). The 13D3 antibody also recognizes HSC70t (Maekawa et al. 1989), a testis-specific protein that is synthesized during spermiogenesis. Multiple HSP70s, including HSP70-2 and HSC70, were detected by an HSP70 family-specific mouse antibody, BRM-22 (Sigma Chemical Co., St. Louis, Mo.). Anti-kinetochore antiserum (CREST) and fluorescein-conjugated goat anti-human antibody were obtained from Chemicon International (Temecula, Calif.).

Cell culture, radiolabeling and subcellular fractionation. Spermatogenic cells were dissociated from the testes of >10 week old CD-1 mice and cultured according to the procedures of O'Brien (1987, 1993). Cells at 3–4×10⁶/ml media were cultured in 10 cm dishes at 32° C, and radiolabeled by addition of 1 mCi [^{35}S]-methionine (>9,000 Ci/mmol) per plate for 3 h. Nuclear and cytoplasmic lysates were prepared as previously described (Selkirk et al. 1994). Detergents in nuclear lysates were dialyzed against 500 vol. of 1 M NaCl followed by two changes of 1,000 vol. of water. Cytoplasmic lysates were dialyzed in two changes of 1,000 vol. of water. Protein content was determined by fluorescamine reaction using BSA as standard. Samples were lyophilized and stored at −80° C until analysis.

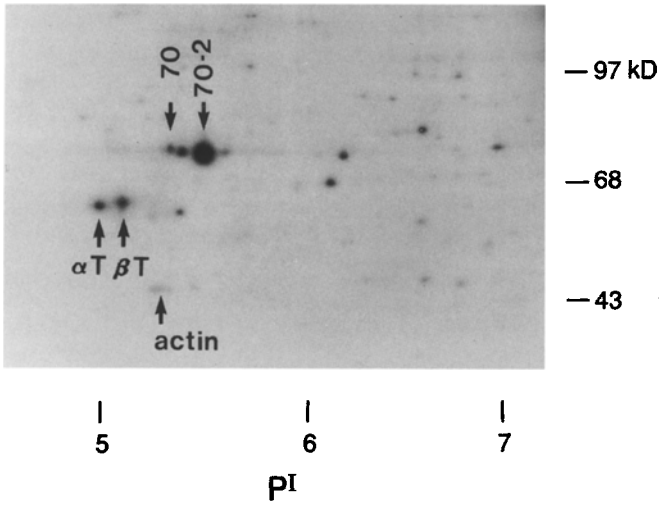

Fig. 2. Two-dimensional polyacrylamide gel electrophoresis of immunoprecipitated [^{35}S]methionine-labeled proteins from both the cytoplasm and nucleus. Immunoprecipitation was done with antiserum 2A, which recognizes HSP70-2 (70-2; *arrow*). Other proteins that complex with HSP70-2 and were thus immunoprecipitated include α- and β-tubulin (*arrows*)

Protein electrophoresis. Separation of proteins by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was conducted as previously described (Merrick et al. 1992). Proteins were separated by charge using isoelectric focusing from pH 4–8, and then by mass in a 10%–16% polyacrylamide linear gradient. Radiolabeled protein was detected by film fluorography after fixation and vacuum drying.

Immunoblotting. After electrophoretic separation, proteins were electrotransferred to nitrocellulose using a semidry blotter as previously described (Merrick et al. 1992). Membranes were blocked with 3% fatty acid-free BSA for 2 h followed by overnight incubation with primary antibody. For some blots, a 1:5,000 dilution of anti-HSP70 antibody (Clone BRM-22) was used. After removal of primary antibody by washing, blots were incubated with a 1:50,000 dilution of anti-mouse IgG (H+L) horseradish peroxidase (HRP)-conjugated secondary antibody (Boehringer Mannheim, Indianapolis, Ind.). For other blots, the primary antibody was HSP70-2 rabbit antisera 2A diluted 1:5,000, detected with HRP-conjugated goat anti-rabbit IgG at a 1:2,000 dilution (Kierkegaard-Perry Laboratories, Gaithersburg, Md.), or HSC70/HSC70t mouse monoclonal 13D3 diluted 1:1,000, and detected with HRP-conjugated goat anti-mouse IgG diluted 1:2,000. Proteins were visualized by chemiluminescent detection (ECL, Amersham, Arlington Heights, Ill.).

Immunoprecipitation. Immunoprotein complexes were formed in whole-cell lysates (see Selkirk et al. 1994) with 6.5 µg of rabbit antiserum 2A at 4° C using gentle rotation overnight. Complexes were immunoprecipitated with goat anti-rabbit IgG (H+L chains)

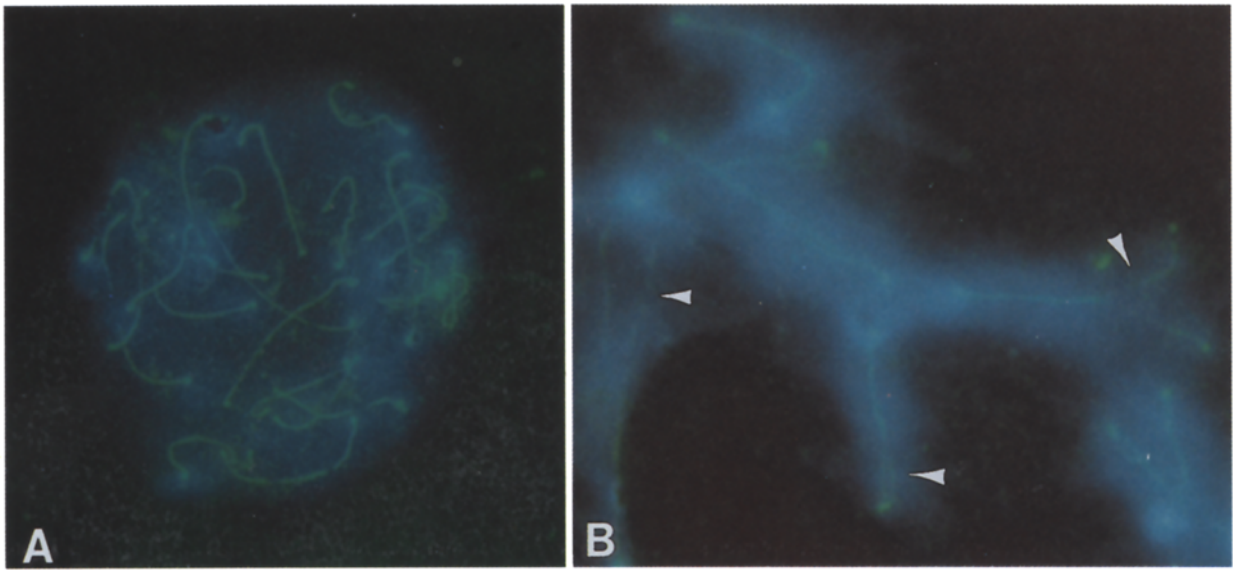

Fig. 3A, B. Immunodetection of synaptonemal complexes (SCs) of mouse spermatocytes with antiserum 2A. SCs stain green with fluorescein against blue 4,6-diamidino-2-phenylindole (DAPI)-stained DNA at **A** pachytene and **B** diplotene stages. In **B**, selected SCs are photographically enlarged. Staining of axial elements (*arrowheads*) and fully synapsed regions is evident. Magnification of cell in **A** is 1,000-fold

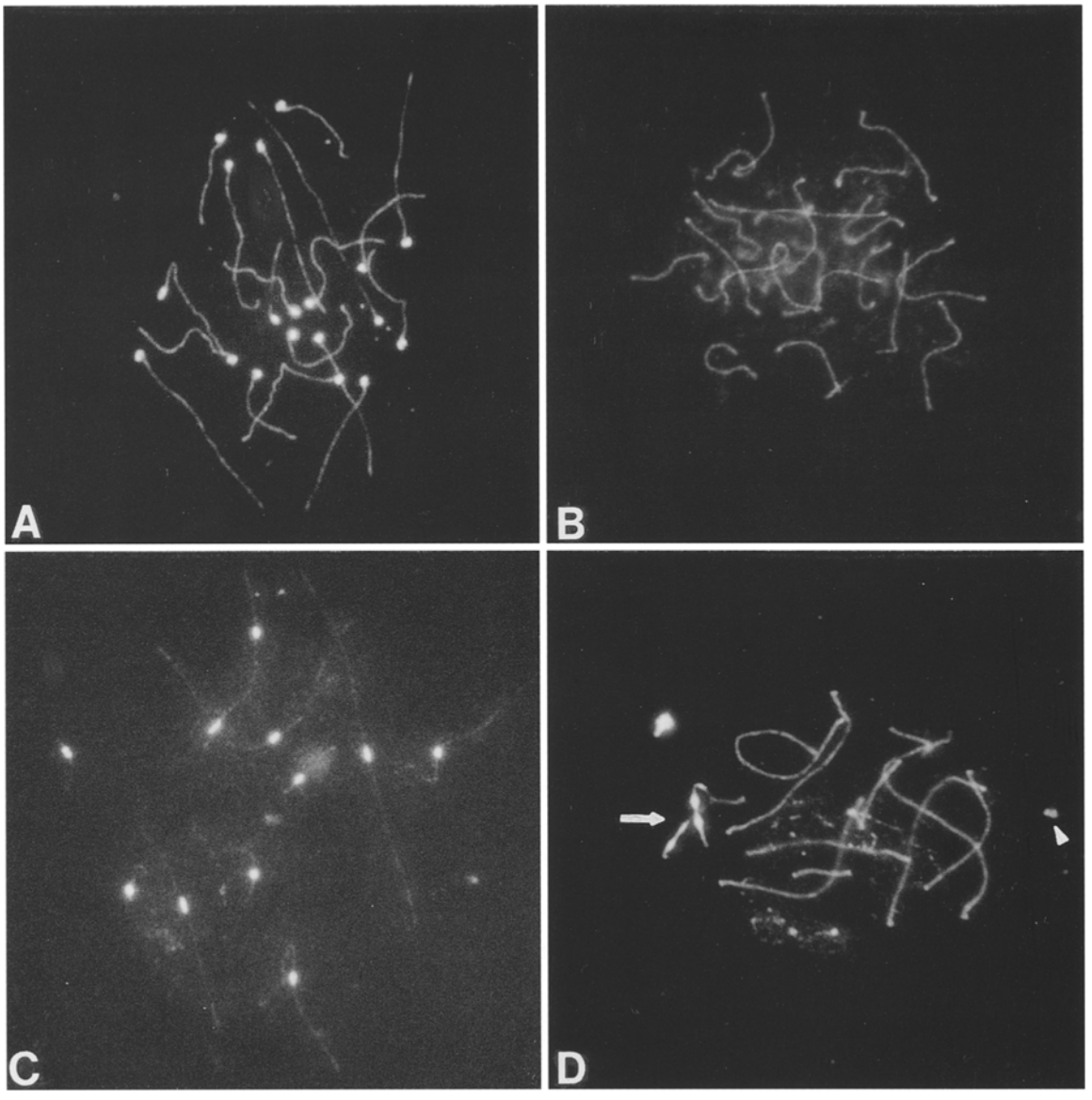

Fig. 4A–D. Immunodetection of SCs with anti-kinetochore (CREST) antiserum, or with antiserum 2A, in spermatocytes from mice and hamsters. **A** Mouse SC immunofluorescence following labeling with anti-kinetochore (CREST) antiserum. **B** Mouse SC immunofluorescence following labeling with antiserum 2A to HSP70-2. **C** Armenian hamster SC immunofluorescence following labeling with anti-kinetochore (CREST) antiserum. **D** Armenian hamster SC immunofluorescence following labeling with antiserum 2A to HSP70-2. Hamster X, Y chromosomal axes reveal intense HSP70-2 staining (*arrow*). Other bodies commonly observed in spermatocytes to exhibit HSP70-2 staining (*arrowhead*) may be centrosomes. Magnification is 1,000-fold

attached to Protein G-coated agarose beads (Oncogene Science, Cambridge, Mass.) and nonspecifically bound protein was removed by seven washes with an isotonic NP-40 buffer. After removal of washing buffer, proteins were solubilized in urea lysis buffer and frozen at –80° C. The negative control for the HSP70-2 antiserum was preimmune rabbit serum.

Analysis of synaptonemal complexes. Slides were prepared and stained using methods described by Dresser et al. (1987). Slides of oocyte SCs were prepared using methods described by Moses and Poorman (1984), and were stained by the same procedures used for SCs of spermatocytes. Rabbit antiserum 2A to HSP70-2, in dilutions from 1:100 to 1:2,000, was applied to slides and incubated in a humidified chamber at room temperature in the dark for 30 min. The slides were rinsed three times, 5 min each., with PBS, and then incubated as above with fluorescein-conjugated goat anti-rabbit IgG (Cappel/Organon Teknika, Durham, N.C.). After a final rinsing in PBS, the slides were washed briefly with distilled water, placed in 0.4% Photoflo (Kodak, Rochester, N.Y.) for 10 s and allowed to air-dry. They were mounted in antifade solution (Johnson and Nogueira Araujo 1981) containing 0.5–1.0 μg/ml DAPI (4′,6-diamidino-2-phenylindole dihydrochloride; Sigma Chemical Co., St. Louis, Mo.) and viewed with a Nikon FXA fluorescence microscope. Negative control slides, to which the primary antibody was not applied (all other conditions remained the same), were prepared concurrently. As a positive control for SC immunostaining, the anti-kinetochore (CREST) antibody (1:15 dilution of commercial solution) and fluorescein-conjugated goat anti-human antibody (1:50 dilution) were used for staining (as described for the antiserum to HSP70-2).

For electron microscopic (EM) analyses, slides were incubated with the primary antibody in a humidified chamber at 37° C for 45 min. Gold-conjugated goat anti-rabbit IgG (BioRad, Melville, N.Y.) was applied undiluted, and incubated for 45 min at 37° C.

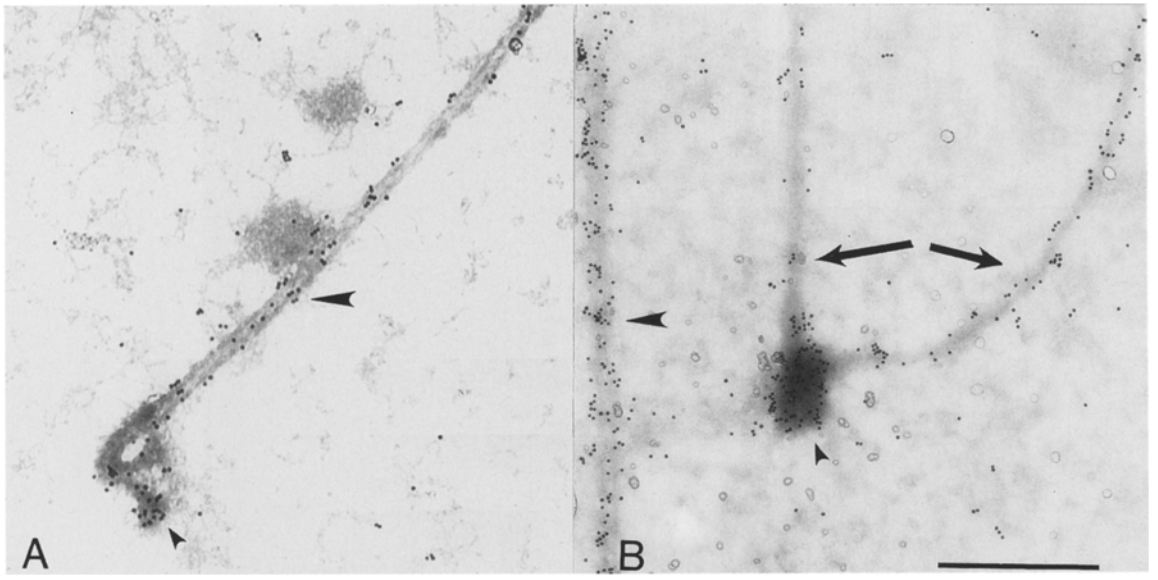

Fig. 5A, B. Electron micrographs of immunogold images of mouse spermatocyte SCs following labeling with antiserum 2A, at **A** pachytene and **B** diplotene. Gold particles are continuous along the length of synapsed regions (*large arrowheads*) and axial elements (*arrows*), and appear clustered at attachment plaque regions (*small arrowheads*). Bar represents 1 μm

Following rinses, slides were fixed again in 4% paraformaldehyde, dipped in 0.4% Photoflo, and air-dried. Immediately after drying, slides were stained for 30 min in phosphotungstic acid (Counce and Meyer 1973), rinsed, dried and prepared for EM viewing.

As a control for specificity, antiserum 2A was preincubated with the HSP70-2 oligopeptide at a concentration of 1 mg/ml for 4 h at 4° C. The solution was then applied to slides, which were processed as above. To determine whether HSP70-2 was binding nonspecifically to SCs, surface-spread preparations were treated with high-salt lysis buffer composed of 300 mM NaCl, 5 mM $MgCl_2$, 0.5% NP-40, 0.1% sodium deoxycholate, and 20 mM HEPES buffer, pH 7.4. The slides were placed in lysis buffer for 30 s, 1 min, 2 min, 5 min, 10 min, or 20 min in a water bath either with no shaking or with shaking (150 rpm) immediately before the paraformaldehyde treatments.

Reverse transcriptase-PCR. Total RNAs were extracted using an RNA isolation kit from Stratagene Cloning Systems, La Jolla, Calif., and treated with RNase-free DNase I (Life Technologies, Gaithersburg, Md.). cDNAs were produced with an RNA PCR kit using random hexamer primers (Perkin-Elmer Cetus, Norwalk, Conn.). RT-PCR products were then generated with oligonucleotide primers corresponding to sequences in the first and second exons of *Hsp70-2* (5′CACCGGCTGGTCACTCC3′ and 5′GGTCTTCATCTCCCCTTTAT3′, respectively). Control reactions to confirm cDNA integrity were performed with primers specific for the 3-UTR of *Hsc70* (5′GAGCATCAGCAGAAAGAAC3′ and 5′GCCTGAAGAAGCACCAC3′). PCR amplifications were for 35 cycles (94° C, 1 min; 57° C–65° C, 2 min; 72° C, 1 min). RT-PCR products were analyzed on 2% agarose gels stained with ethidium bromide.

Results

Identification of HSP70-2 in the nucleus of spermatogenic cells

The distribution of HSP70s in spermatogenic cells was analysed by preparing cytoplasmic and nuclear fractions, separating the proteins in each fraction by 2-D PAGE, and identifying them with HSP70-specific antibodies. Figure 1 presents a series of immunoblots from the cytoplasmic and nuclear protein fractions. Figure 1A, B demonstrates that while there are at least five different HSP70s in the cytoplasm of germ cells detected by antibody BRM-22 to the HSP70 protein family, only two of these HSP70s are detectable in the nucleus of these same cells. One of the HSP70s found in both the cytoplasm and nucleus of spermatogenic cells is HSP70-2. Figure 1C, D reveals immunoblots of cytoplasmic and nuclear proteins, respectively, reacted with antiserum 2A to HSP70-2. The protein detected by antiserum 2A has the same electrophoretic migration pattern as one of the HSP70s identified in Fig. 1A, B. The second nuclear HSP70 is identified as HSC70 by immunoblotting with monoclonal antibody 13D3 (Fig. 1E, F). HSP70-2 and HSC70 are relatively abundant proteins in spermatogenic cells, and can be seen without immunodetection on blots of cytoplasmic and nuclear proteins labeled with [^{35}S]methionine (Fig. 1G, H).

Antiserum 2A was used to immunoprecipitate [^{35}S]methionine-labeled, unfractionated proteins from spermatogenic cells, and then the proteins were characterized by 2-D PAGE (Fig. 2). HSC70 coprecipitated with HSP70-2. Two other major proteins recognizable by their migration patterns in Fig. 2 are α- and β-tubulin, which appear here as proteins in the cytoplasmic and nuclear fractions from spermatogenic cells (Fig. 1G, H). None of the other major proteins in Fig. 2 correspond either to identifiable nuclear proteins (see Fig. 1H) or other HSP70s. Control immunoprecipitations with normal rabbit sera were negative for the labeled proteins from whole spermatogenic cells (data not shown).

Localization of HSP70-2 to the synaptonemal complex

The present studies were focused on localization of HSP70-2 within meiotic prophase nuclei. Surface-spread

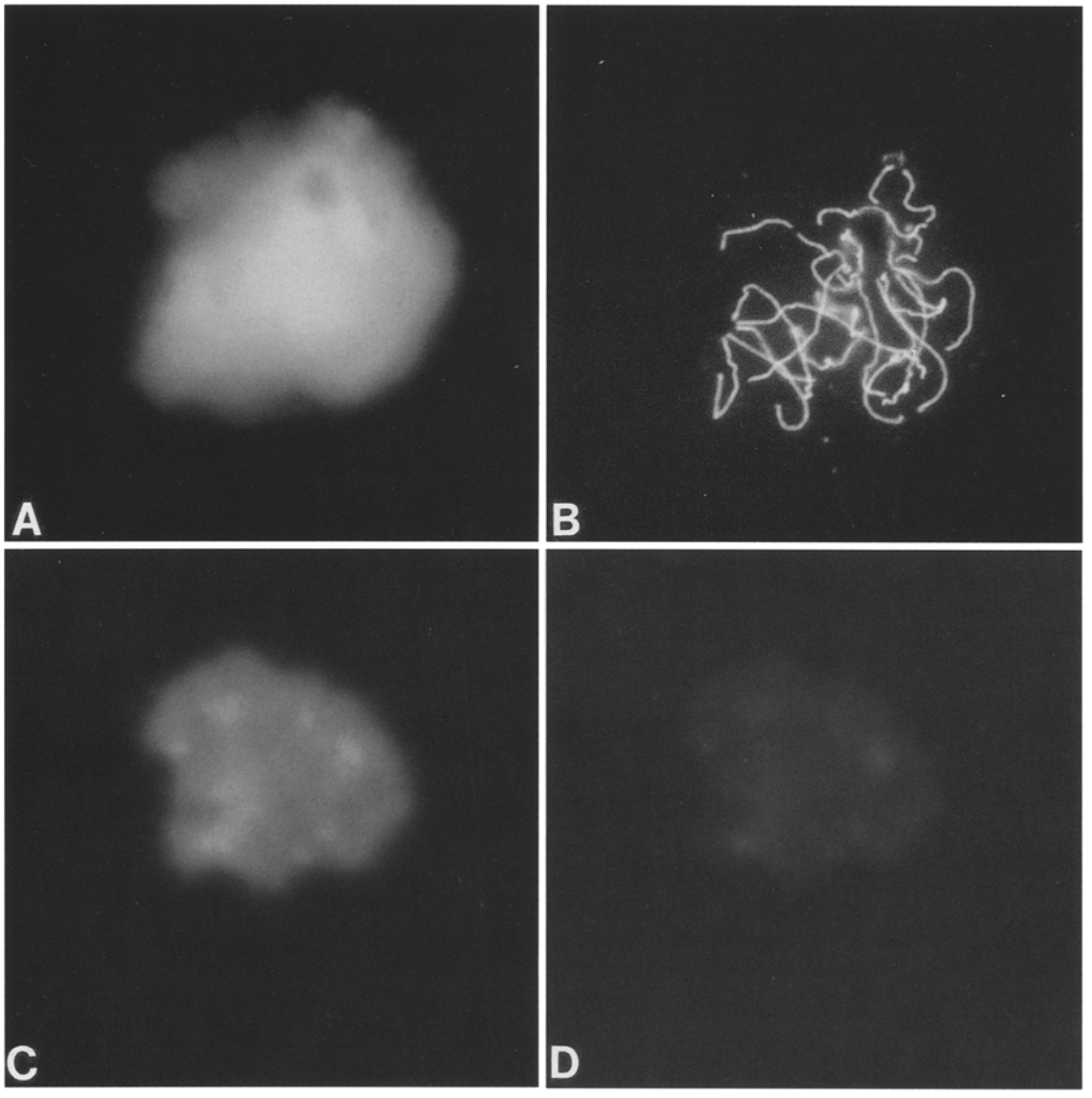

Fig. 6A–D. Mouse oocyte SCs subjected to labeling with anti-kinetochore (CREST) antiserum, or with antiserum 2A. **A** A pachytene stage oocyte is identified with DAPI staining. **B** SCs are identified in the same cell following labeling with anti-kinetochore (CREST) antiserum. **C** A different pachytene oocyte is identified with DAPI staining. **D** The same cell showing no evidence of SC labeling after treatment with antiserum 2A to HSP70-2. Magnification is 1,000-fold

zygotene nuclei (staged using phase contrast microscopy prior to immunostaining), immunolabeled with antiserum 2A and a secondary antibody tagged with fluorescence or gold, showed HSP70-2 staining to be heavier in nucleoli than in faintly labeled axial elements of the forming SC. As cells progressed through later stages, HSP70-2 staining increased in SCs and decreased in nucleoli. Fully formed SCs at middle to late pachytene stained intensely with antiserum 2A to HSP70-2, in male mice and hamsters (Figs. 3–5). There was no indication that nucleoli or dense bodies in late pachytene cells stain with antiserum 2A. Axial elements in disassembling SCs at diplotene also showed clear HSP70-2 immunofluorescence and gold labeling (Figs. 3B and 5B).

In spermatocytes from mice and hamsters, HSP70-2 staining appears continuous along SCs with increased intensity of label in telomeric regions and in X, Y chromosomal axes. This pattern is different from that seen with the anti-kinetochore (CREST) antiserum in which staining is limited to synapsed regions and kinetochores, and not X, Y chromosomal axes (Fig. 4). The difference in antiserum staining of X, Y chromosomal axes is especially striking in the Armenian hamster (Fig. 4C, D).

EM analysis of localization of HSP70-2 in SCs of mouse spermatocytes reveals a uniform pattern of labeling with antiserum 2A and secondary antibody tagged with colloidal gold, except that concentrations often appear especially heavy in attachment plaque (telomeric) regions (Fig. 5).

The addition of the oligopeptide used to generate antiserum 2A completely blocked staining of surface-spread SCs in pachytene spermatocytes (data not shown). Treatment of surface spreads of these cells with high-salt lysis buffer failed to disassociate HSP70-2

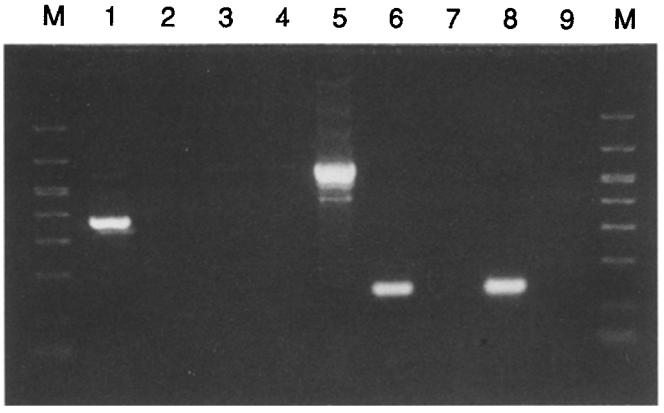

Fig. 7. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of *Hsp70-2* mRNA expression in mouse adult testis and fetal ovary. *Lanes 1–5* RT-PCRs with primers specific for *Hsp70-2*. *Lane 1* Testis RNA treated with reverse transcriptase, resulting in a 370 bp mRNA amplicon. *Lane 2* No RT control for genomic DNA contamination in testis RNA. *Lane 3* RT-PCR of ovary RNA. *Lane 4* No reverse transcriptase control for ovary RNA. *Lane 5* Ovary RNA not treated with DNase or reverse transcriptase resulting in a 609 bp genomic DNA. The genomic DNA amplicon is 239 bp shorter than the mRNA amplicon because the RT-PCR primers bracket the *Hsp70-2* intron. *Lanes 6–8* RT-PCRs with primers specific for *Hsc70*. Both testis (*lane 6*) and ovary (*lane 8*) RNA yield a 143 bp mRNA amplicon that is absent when reverse transcriptase is left out of the RT-PCRs (*lanes 7 and 9*)

from the SCs, leaving antiserum 2A immunostaining patterns unchanged (data not shown).

Mouse oocyte SCs did not stain with antiserum 2A. Oocyte SCs did stain with the anti-kinetochore (CREST) antiserum in a pattern comparable to that observed in spermatocytes, although kinetochores per se did not stain distinctly with this particular antibody (Fig. 6).

RT-PCR analysis did not detect *Hsp70-2* mRNA (Fig. 7) in fetal ovaries of gestation day 16–18, when SCs are formed. Adult mouse ovaries were also negative for *Hsp70-2* mRNA (data not shown). In contrast, *Hsc70* mRNA was present in testis and ovary (Fig. 7). Immunofluorescence with both mouse (13D3) and rat (1B5) monoclonal antibodies against HSC70 failed to detect this protein in association with SCs.

Discussion

Previous studies of HSPs in mouse spermatogenesis have indicated that HSP70-2 is regulated developmentally and expressed at high levels in pachytene spermatocytes (Allen et al. 1988; Zakeri et al. 1988; Rosario et al. 1992). In the present study, HSP70-2 was found to be relatively abundant in both the cytoplasm and the nucleus of spermatogenic cells. As speculated by Zakeri et al. (1988), this protein was localized to the SC in mice and hamsters. Unlike certain SC antigens (Dresser 1987; Dobson et al. 1994) which are only found in SC paired regions, or in unpaired X, Y chromosomal axes, HSP70-2 is evident in fully formed SCs, apparently along lateral elements and X, Y axes. The HSP70-2 protein begins to appear in association with the axial elements at early prophase (leptotene-zygotene), increases in amount along the SCs until mid-prophase (pachytene), and then persists until SCs disassemble in late prophase (diplotene) cells. Antigenic sites and structures common to SCs and nucleoli have been reported in a number of studies (Moens et al. 1987). Whether the increased HSP70-2 signal in mid-late SC development seen here is quantitatively related to the apparently diminished signal in nucleoli is unknown.

While an artifactual association of HSP70-2 with the SC is difficult to rule out fully, there are several lines of evidence that argue against this. (1) Of several HSP70s evaluated, only HSP70-2 and HSC70 were localized to extracts of germ-cell nuclei by biochemical fractionation and immunoblot detection. (2) Rosario et al. (1992) have localized HSP70-2 to spermatocyte nuclei by immunofluorescence (using antiserum 2A) on histological sections of mouse testis. (3) Oligopeptide competition of antiserum 2A eliminated detection of HSP70-2 with immunostaining of the SC, and indicated specificity of the antibody. (4) Extraction with a high-salt buffer prior to fixation of SCs did not eliminate immunodetection of HSP70-2, and indicated its strong association with the SC structure. (5) There is biological evidence consistent with a functional role for HSP70-2 in the SC. Preliminary studies of mice homozygous for a targeted mutation in the *Hsp70-2* gene revealed males to have a lack of antiserum 2A immunostaining for this protein in SCs, SC abnormalities in late prophase spermatocytes, and depletion of subsequent spermatogenic cell stages (Dix et al. 1996). There was no discernible phenotype in female HSP70-2-deficient mice (Dix et al. 1996), which is consistent with our evidence for the lack of HSP70-2 in mouse oocytes.

While the objective of this study was to characterize HSP70-2 and other HSP70 proteins in the nucleus, it is noteworthy that HSP70-2 was found to coprecipitate with HSC70, and α- and β-tubulins, which are also detectable outside the nucleus. Whether HSP70-2 plays a functional role in the cytoplasm or localizes to cytoplasmic structures remains to be seen. The association of HSP70-2 with SCs suggests that it functions in SC assembly and/or disassembly, or other activities in meiosis. However, the chaperone activities of HSP70 protein in the cytoplasm of other cells suggests that HSP70-2 may also function in the cytoplasm. Chaperones recognize and stabilize incompletely folded polypeptides during processes of folding, assembly, rearrangement and disassembly (Gething and Sambrook 1992; Parsell and Lindquist 1993). This would not be inconsistent with HSP70-2 also having a role in the SC.

The lack of HSP70-2 in mouse oocytes is surprising, since these cells contain SCs that are comparable structurally to those found in spermatocytes. Nevertheless, *Hsp70-2* mRNA was not detected in fetal ovaries by RT-PCR, and HSP70-2 protein was not seen by immunostaining with antiserum 2A in oocyte SCs. In humans, the homolog of *Hsp70-2* mRNA is reportedly detected in adult ovaries (Bonnycastle et al. 1994). However, it is not clear whether HSP70-2 protein is significant in adult ovarian function. Mouse oocytes may express an as yet unidentified HSP70 during meiosis to substitute for HSP70-2 in the SC. In adult mice, evaluations of unfertilized oocytes for expression of HSP70 have given negative results (Hendrey and Kola 1991). *Hsc70* mRNA is

expressed in mouse fetal ovary, as well as in the testis (Fig. 7). However, we were unable to confirm the presence of HSC70 in spermatocyte or oocyte SCs using HSC70-specific antibodies. Thus, at present, it is unclear whether an alternative (to HSP70-2) HSP70 is associated with oocyte SCs.

We speculate that the lack of HSP70-2 in pachytene oocytes signifies a previously unsuspected male-female difference in SC metabolism relating to inherent differences in their environments. In spermatocytes, SCs form throughout adult life, outside the main body cavity and at several degrees below core body temperature. In oocytes, SCs form during fetal development at maternal body temperatures. Sex differences in X,Y-chromosome constitution, meiotic chromosomal methylation status and/or physical dimensions (e.g. length), could also be important factors in determining the need for HSP70-2 expression and function.

Although the significance of HSP70-2 is unknown, it is reasonable to assume that it supports fundamental meiotic processes. HSP70-2 may have a cytoprotective role, and/or it may help to stabilize structures important in achieving chromosomal recombination and segregation. The specificity of HSP70-2 for spermatocyte SCs could have implications for sex-specific germ-cell loss, aneuploidy and other possible outcomes of failed or aberrant synapsis/exchange.

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