Drosophila Centrosomin Protein is Required for Male Meiosis and Assembly of the Flagellar Axoneme

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Abstract. Centrosomes and microtubules play crucial roles during cell division and differentiation. Spermatogenesis is a useful system for studying centrosomal function since it involves both mitosis and meiosis, and also transformation of the centriole into the sperm basal body. Centrosomin is a protein localized to the mitotic centrosomes in *Drosophila melanogaster*. We have found a novel isoform of centrosomin expressed during spermatogenesis. Additionally, an anticentrosomin antibody labels both the mitotic and meiotic centrosomes as well as the basal body. Mutational analysis shows that centrosomin is required for spindle organization during meiosis and for organization of the sperm axoneme. These results suggest that centrosomin is a necessary component of the meiotic centrosomes and the spermatid basal body.

MICROTUBULES play important roles in cell proliferation and differentiation. In living cells, microtubules are usually nucleated from a discrete site in the cell called the microtubule-organizing center (MTOC; for review see Kellogg et al., 1994).[1] A major form of the MTOC in animal cells is the centrosome, which is composed of a pair of centrioles surrounded by amorphous electron-dense pericentriolar material (PCM; Rattner and Phillips, 1973; Borisy and Gould, 1977; Gould and Borisy, 1977). The centrosome undergoes periodic changes in its molecular composition and microtubule nucleation ability over each cell cycle. In interphase cells, the centrosomes that have relatively small amounts of PCM nucleate microtubules that are oriented with their fast-growing plus ends extending into the cytoplasm. During mitosis, the centrosomes duplicate, accumulate more PCM, and organize the bipolar spindle, which is composed of dense and dynamic microtubules (for review see Kellogg et al., 1994). In addition to this cell cycle control, the function and morphology of the centrosomes are also modified according to the differentiation state of cells. In ciliated epithelial cells, the centriole-derived basal bodies assemble morphologically different MTOCs that nonetheless share at least some common components with centrosomes (Muresan et al., 1993). In the unicellular alga *Chlamydomonas*, the flagellar basal bodies are incorporated into the centrosomes at each mitotic division, and revert to flagellar basal bodies during interphase (Cavalier, 1974). The morphological and functional changes of the centrosome in response to the cell cycle and developmental controls suggest that a full understanding of the centrosome and its molecular components requires in vivo analysis within specific developmental backgrounds.

Spermatogenesis is a useful system for study of the centrosome and microtubule organization since it involves different types of cell divisions and dramatic morphological changes of the centrosome. In *Drosophila melanogaster*, a germline stem cell divides at the tip of the testis to produce another stem cell and a spermatogonial cell. Each spermatogonial cell is amplified in four mitotic divisions to produce 16 interconnected spermatocytes. After an extensive growth period, the spermatocytes go through two meiotic divisions to produce haploid spermatids, which then undergo dramatic morphological changes to produce mature sperm. The dramatic changes in cellular morphology that occur during spermatocyte growth, the meiotic divisions, and spermatid differentiation are accompanied by dramatic modifications of the centrosomes. During the spermatocyte growth period, the centrosomes dissociate from the nuclear membrane and become associated with the spermatocyte cell membrane (Tates, 1971). At the onset of the meiotic divisions, the centrosomes migrate back to the nuclear membrane and assemble the meiotic spindles. Although the morphology of meiotic spindles is very similar to that of mitotic spindles, many structural compo-

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1. *Abbreviations used in this paper*: MTOC, microtubule-organizing center; PCM, pericentriolar material.

nents are specific to male meiosis. For example, β2-tubulin is expressed only in the meiotic and postmeiotic regions in the testis (Kemphues et al., 1982; Fuller et al., 1988; Hoyle and Raff, 1990). It has also been suggested that during meiosis, distribution of microtubule-organizing activities may be different from normal mitotic divisions (Fuller, 1993). CP190, a centrosomal antigen tightly associated with centrosomes during mitosis, is only diffusely localized to the regions surrounding the male meiotic spindle poles (Casal, 1990). Postmeiotic spermatid differentiation involves more dramatic changes in centrosomal composition. The centriole inserts into the base of the nucleus to become the basal body, which then seeds growth of the sperm axoneme (Tates, 1971). The transition between the centriole and the basal body is poorly characterized.

The unique features of spermatogenesis prompted us to examine the behavior and function of the centrosomin protein during spermatogenesis. Centrosomin is an integral component of the mitotic centrosomes (Heuer et al., 1995; Li and Kaufman, 1996). It is localized to mitotic centrosomes during multiple stages of development, and is required for assembly of other centrosomal proteins into the centrosome during the syncytial cleavage divisions (Li and Kaufman, manuscript submitted for publication). Centrosomin is a protein of 1,148 amino acids with a predicted molecular mass of 130 (it migrates aberrantly in SDS-PAGE at 150 kD). Sequence analysis predicts that large proportions of the centrosomin protein are composed of α-helical coiled-coil structures similar to those found in myosin and kinesin. There are also three distinct leucine zipper domains. Previous analyses have shown that centrosomin can be detected in the centrosomes during male meiosis (Li and Kaufman, 1996). However, accurate functional characterization of the protein during spermatogenesis was lacking. We have carried out biochemical and further immunocytochemical analyses of centrosomin during spermatogenesis as well as isolated mutations that affect centrosomin function during spermatogenesis. We have found that centrosomin is expressed as a testis-specific form during spermatogenesis, and is closely associated with the centrioles both during the mitotic and meiotic divisions, and with the basal bodies of early-stage spermatids. Mutations that disrupt centrosomin function not only affect meiotic spindle organization and result in failure of karyokinesis and cytokinesis, but also affect the growth of the axoneme; particularly formation of the central pair of microtubules. Therefore, centrosomin is an essential component of both the meiotic centrosomes and the spermatid basal body.

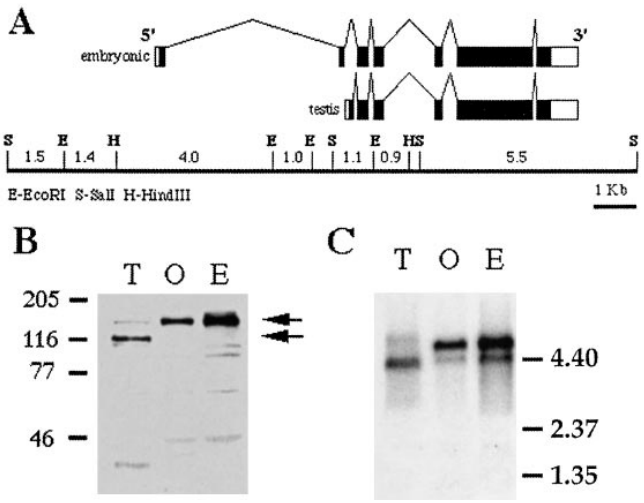

Figure 1. Identified isoforms of centrosomin. (*A*) Alternative splicing of Cnn-encoding transcript during embryogenesis and spermatogenesis. The embryonic transcription unit covers ~12 kb of genomic DNA. The exons are diagrammed as boxes, with the shaded areas representing coding sequences. The exon diagram on top of the figure represents the major form of messenger RNA expressed during embryogenesis; the diagram below represents the major transcript expressed during spermatogenesis. (*B*) Two prominent protein species that migrate at 120 kD and 150 kD were detected in the testis extracts (*T*). Only one major protein band at 150 kD is detected in the ovarian extract (lane *O*, *arrow*) and embryonic extract (lane *E*, *arrow*). We interpret the faintly staining bands running below the major bands as either tissue-specific degradation products or cross-reacting proteins to the polyclonal sera used in the analysis. The positions of molecular weight standards from top to bottom are shown to the left of the gel as follows: 205 kD, 116 kD, 77 kD, and 46 kD. (*C*) 10 μg of RNA were run in each lane, and an embryonically derived cDNA (*A*) was used as a probe. In the testis (lane *T*), one prominent band at 4.3 kb and a faint band at 5.0 kb were detected. In the ovary (lane *O*), two major transcripts of 4.5 kb and 5.0 kb were detected. In embryos, a prominent band of 5.0 kb and a minor band of 4.5 kb can be seen.

Materials and Methods

Immunofluorescence Staining of Testis Squashes

Testes were dissected in Ringer's solution and squashed on Superfrost/Plus™ (Fisher Scientific Co., Fairlawn, NJ) slides. These slides were frozen in liquid nitrogen, the coverslip was removed with a razor blade, and the squashed testes were fixed in −20°C methanol (Ashburner, 1989; Pisano et al., 1993). To disassemble microtubules, dissected testes were cultured in 50 μg/ml colchicine for 15 min before fixation and immunostaining. Anti-α-tubulin antibody was purchased from Amersham and used at a 1:100 dilution. Affinity-purified centrosomin polyclonal antibody has been described in Heuer et al. (1995) and was used at 1:500. This antiserum was raised to the COOH terminal portion of the protein encoded by the two 3′-most exons (Fig. 1 *A*). Anti-γ-tubulin antibody was a gift from Dr. M. Moritz and Dr. B. Alberts, and was used at 1:200. Fluorescent secondary antibodies were purchased from the Jackson Laboratories, and used at 1:200. Images were collected on a confocal scanning microscope (Bio-Rad Laboratories, Hercules, CA) and were processed using NIH Image and Adobe Photoshop software.

Northern Hybridization

RNA was extracted from about 50 testes using the TRIzol reagent according to manufacturer's recommendations (GIBCO BRL, Gaithersburg, MD). Approximately 10 μg of total RNA was loaded into each lane. RNA was electrotransferred to Nylon filters (Schleicher & Schuell, Inc., Keene, NH) in 0.5× TAE, UV–cross-linked, and prehybridized in hybridization buffer (5× SSC, 5× Denhart's solution, 20 mM Tris HCl, pH 7.6, 0.1% SDS, 1 mM EDTA, 1 mg/ml yeast RNA) for 1 h. Probe was added directly to the hybridization solution, and hybridization was carried out at 65°C for 12–16 h. The filter was washed in 0.2× SSC and exposed to BioMax film (Eastman Kodak Co., Rochester, NY) A 4.3-kb cDNA identified from an embryonic cDNA library was used as probe.

Western Blot Analysis

Testes from 20 males or ovaries from 3 females were dissected and boiled in SDS sample buffer, and one-third of the protein sample was fraction-

ated on a 7.5% polyacrylamide gel and blotted onto nitrocellulose membrane for immunodetection. The anti-centrosomin antibody was used at 1:2,000 dilution. For detection, we used HRP-conjugated goat anti–rabbit secondary antibody (Bio-Rad Laboratories) preabsorbed against 0–24 h *Drosophila* embryos, and also the ECL chemiluminescent system (Nycomed Amersham, Princeton, NJ). Males and females carrying the *mfs* mutant alleles were obtained by crossing *b cu mfs sp*/CyO males to *Df*(2R)*Cnn*/CyO females. The Cy^+ male progeny were collected and maintained apart from females for 4 d before dissection. Virgin Cy^+ females were collected and mated to wild-type males. These crosses were maintained for 5 d, and eggs were collected at the end of that period. The eggs were dechorinated and treated as the testes and ovaries above.

Library Screen

The testis cDNA library was a gift from Dr. T. Hazelrigg, and 0–2, 2–4, and 4–6-h embryonic cDNA libraries were purchased from Novagen, Inc. (Madison, WI). The 4.3-kb cDNA originally isolated from a 0–20 h embryonic cDNA library was used as probe. The library screen was performed according to Heuer et al. (1995). DNA sequencing was performed using the ABI sequencing kit (Perkin Elmer).

Immunoelectronmicroscopic Procedures

Testes from newly eclosed males were dissected in PBS and fixed for 1 h in 1% glutaraldehyde or in 4% paraformaldehyde + 0.1% glutaraldehyde in PBS at room temperature. Fixed tissues were rinsed in PBS, dehydrated in a graded ethanol series, and embedded in Lowicryl K4M. UV polymerization was carried out at 4°C. Thin sections of embedded materials were collected on uncoated 300 mesh nickel grids. Staining was performed at room temperature. Primary antibody was used at 1:25, and goat anti–rabbit 2° antibody conjugated with 10 nm gold particles (Nycomed Amersham) was used at 1:20 dilutions. Grids were poststained with 7.55 uranyl magnesium acetate diluted 1:1 with 95% ethanol for 15 min, followed by lead citrate staining for 1min.

Results

A Testis-specific Isoform Centrosomin

Prior immunocytological studies detected centrosomin expression during spermatogenesis (Li and Kaufman, 1996). To see if the same form of centrosomin is expressed during different developmental processes, we examined protein extracts from testes, ovaries, and embryos using SDS-PAGE and Western blots (Fig. 1 *B*). The expected 150-kD isoform was detected in both the ovarian (lane *O*) and embryonic extracts (lane *E*) by affinity-purified centrosomin antisera. However, in the testis extract, an additional 120-kD protein as well as the 150-kD protein were recognized (Fig. 1 *B;* lane *T*). The 120-kD protein appears to be the major form of centrosomin in the testis, and only a relatively small amount of the larger isoform is accumulated.

To investigate the origin of the different isoforms of centrosomin in the testis, we performed Northern blot analysis. In total RNA isolated from ovaries, two major transcripts (4.5 kb and 5.0 kb in length) were detected using probes made from an embryonically derived cDNA (Fig. 1 *C*; lane *O*). Transcripts of similar sizes were detected in RNA prepared from 0–4 h embryos (Fig. 1 *C*, lane *E*). However, in testis RNA preparations, we detected a prominent transcript of 4.3 kb (Fig. 1 *C*; lane *T*).

To rationalize the differences in the transcript sizes, we screened a testis cDNA library and an embryonic cDNA library and isolated multiple cDNAs (Fig. 1 *A*; see Materials and Methods). The embryonic cDNAs isolated were similar to those reported in Heuer et al. (1995), and are diagramed in Fig. 1 *A*. Several cDNAs were isolated from the testis cDNA library, and they differed from the embryonic cDNA at their 5′ ends. Through comparison with the genomic sequences, we found that these different cDNAs were derived from mRNAs that had been generated by alternatively spliced 5′ exons (Fig. 1 *A*). The alternatively spliced testis and embryonic RNAs conceptually produce centrosomins, which differ at the NH_2 terminus. Sequence analysis of the testis cDNA predicts a protein of 1,106 amino acids with a predicted molecular mass of 124. This novel cDNA isolated from the testis library may therefore represent the transcript that codes for the 120-kD protein detected on Western blots, while the 150-kD protein in the testis extract may be similar to the embryonic isoform of centrosomin.

Localization of Centrosomin in Spermatogonial Cells and Primary Spermatocytes

To examine the localization pattern of centrosomin during spermatogenesis, we performed immunofluorescent microscopic analysis on fixed tissues. Using chromosomal morphology and cell size as staging landmarks, we have determined that centrosomin is expressed in both the mitotic and meiotic regions of the testis (Fig. 2 *A*), and is closely associated with the centrosomes in a cell cycle–dependent manner throughout spermatogenesis.

During spermatogonial cell mitoses, centrosomin can be strongly detected at the spindle poles (*arrow*, Fig. 2 *B*; *b1*). During interphase of these divisions, centrosomin is still present in the centrosome, but at much lower levels (*arrowheads*, Fig. 2 *B*; *b1*). Higher levels of cytoplasmic centrosomin can be detected in these interphase cells (Fig. 2 *A*; *b2*).

After spermatogonial cell mitoses, primary spermatocytes undergo a period of rapid growth that results in a 25-fold increase in volume (for review see Fuller, 1993). The growth process is accompanied by distinct changes in nuclear morphology and centriolar behavior that can be used in staging the spermatocytes (Tates, 1971; Cenci et al., 1994). Centrosomin is in close association with the centriole throughout the spermatocyte growth phase (Fig. 2 *B*; *b2–b3*). Two dots of centrosomin staining at the cell membrane can be seen in each spermatocyte (Fig. 2, *b2*, *b3*), representing the duplicated centrosomes. Late during the spermatocyte growth period as cells approach the meiotic divisions, the centrosomes migrate back to the nuclear membrane, and higher levels of centrosomin can be detected in the centrosomes by immunostaining (Fig. 2 *B*; *b4*).

Besides the centrosomal staining, the centrosomin antisera also weakly labels the nuclear membrane in primary spermatocytes (e.g., Fig. 2 *C*; *c4*) and onion stage spermatids (see Fig. 8, *D* and *E*). We could not determine with certainty whether the nuclear membrane staining is an intrinsic property of the centrosomin protein, or if it reflects the staining patterns of another protein that cross-reacts with the centrosomin antibody. The latter explanation seems likely given that our Western blots detected other proteins, and that mutations that abolish centrosomal staining of centrosomin have no effect on the nuclear membrane staining (see below). This nuclear membrane staining, however, provided a useful way to follow nuclear behavior during our analysis of microtubule organization and nuclear divisions in mutants.

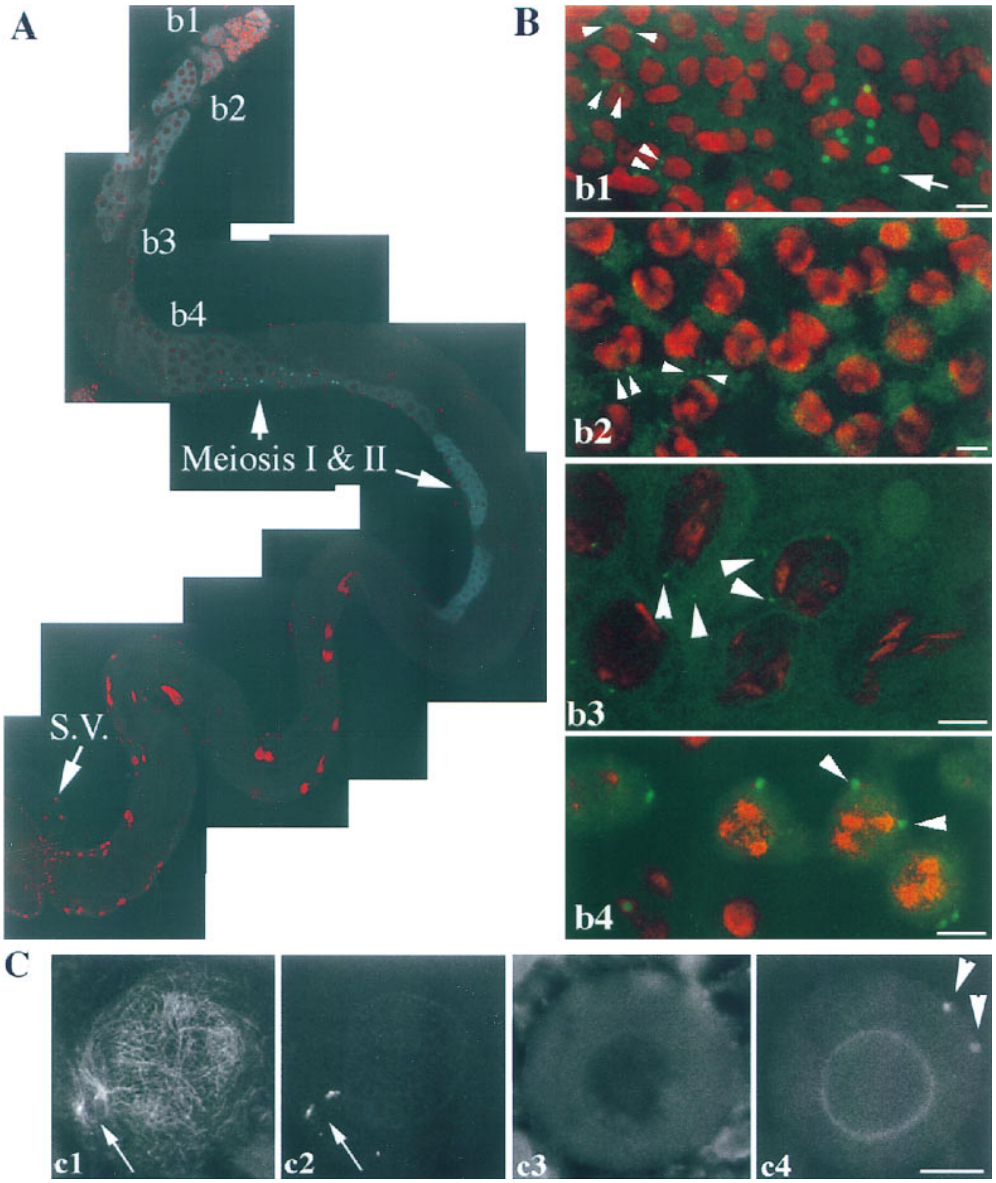

Figure 2. Centrosomin expression during spermatogenesis. (*A*) An overview of centrosomin expression during spermatogenesis. A whole testis stained for DNA (with propidium iodide; *red*) and centrosomin (anticentrosomin; *blue*) is shown. Images were collected separately and assembled in Adobe Photoshop. Centrosomin is expressed at high levels during both mitotic and meiotic divisions. Note that there are high levels of centrosomin in the cytoplasm during the interphase of mitotic divisions, and in the cytoplasm of postmeiotic spermatids. During the M phase of both mitotic and meiotic divisions, the cytoplasmic levels of centrosomin decrease, and the centrosomin antibody predominantly labels the centrosomes. Shortly after the meiotic divisions, centrosomin levels dramatically decrease. b1–b4 represent spermatocytes, and corresponding labels in *B* are higher magnifications of cells at similar stages from different preparations (S.V., seminal vesicle). (*B*) Centrosomin localization at different stages of spermatocyte development. All preparations were double-labeled with propidium iodide for DNA (*red*) and anticentrosomin (*green*). (*b1*) Mitotic region near the tip of the testis. Centrosomin is present in the centrosomes at higher levels during M phase (*arrow*), and at much lower levels during interphase (*arrowheads*). (*b2*) Early stages of spermatocyte growth period. The aggregates of three major chromosomes are associated with the nuclear membrane; each spermatocyte has two centrosomes (*arrowheads*, stained with centrosomin) associated with the cell membrane (not labeled) in addition to high levels of cytoplasmic staining. (*b3*) Mature spermatocytes also have prominent staining of centrosomin in the centrosomes (*arrowheads*). (*b4*) At the prometaphase of meiosis I, the intensity of centrosomin staining in the centrosome significantly increases. (*C*) The centrosomal and nuclear membrane staining of the centrosomin antibody are independent of microtubules. *c1* shows a mature spermatocyte labeled with an α-tubulin antibody. A dense microtubule network exists in the cytoplasm (more prominently around the nucleus), and two prominent asters start to form at the cell membrane (*arrow*). (*c2*) Same cell as in *c1,* double-labeled with anticentrosomin. Centrosomin is present in the center of the asters (*arrow*). (*c3*) Culturing of dissected testes in Ringer's solution containing 50 μg/ml colchicine eliminated detectable microtubule fibers in this spermatocyte. (*c4*) Same cell as in *c3*. In the absence of detectable microtubules, centrosomin could still be detected in the centrosome (*arrowheads*). Note the nuclear membrane staining by the anticentrosomin antibody. Bar, 10 μm.

Accumulation of centrosomin in the spermatocyte centrosome is not dependent on the presence of microtubules. Culture of dissected testes in 50 μg/ml colchicine before fixation and immunolabeling eliminated detectable microtubules, but did not affect the centrosomin staining pattern (Fig. 2 *C*; *c3*, *c4*). This result suggests that the testis form of centrosomin is an intrinsic component of the centrosome, rather than being passively transported into the centrosome along the microtubules.

Centrosomin is Localized to Meiotic Spindle Poles

The onset of meiotic division I is marked by disassembly of the microtubule network around the nucleus and incorporation of microtubules into the asters (Fig. 2 *C*; *c1*). During both meiotic divisions, centrosomin can be detected in the centrosomes (Fig. 3). At the onset of meiotic division I, the centrosomes migrate to opposite ends of the nucleus as the chromosomes condense (Fig. 3 *A*). As the cell

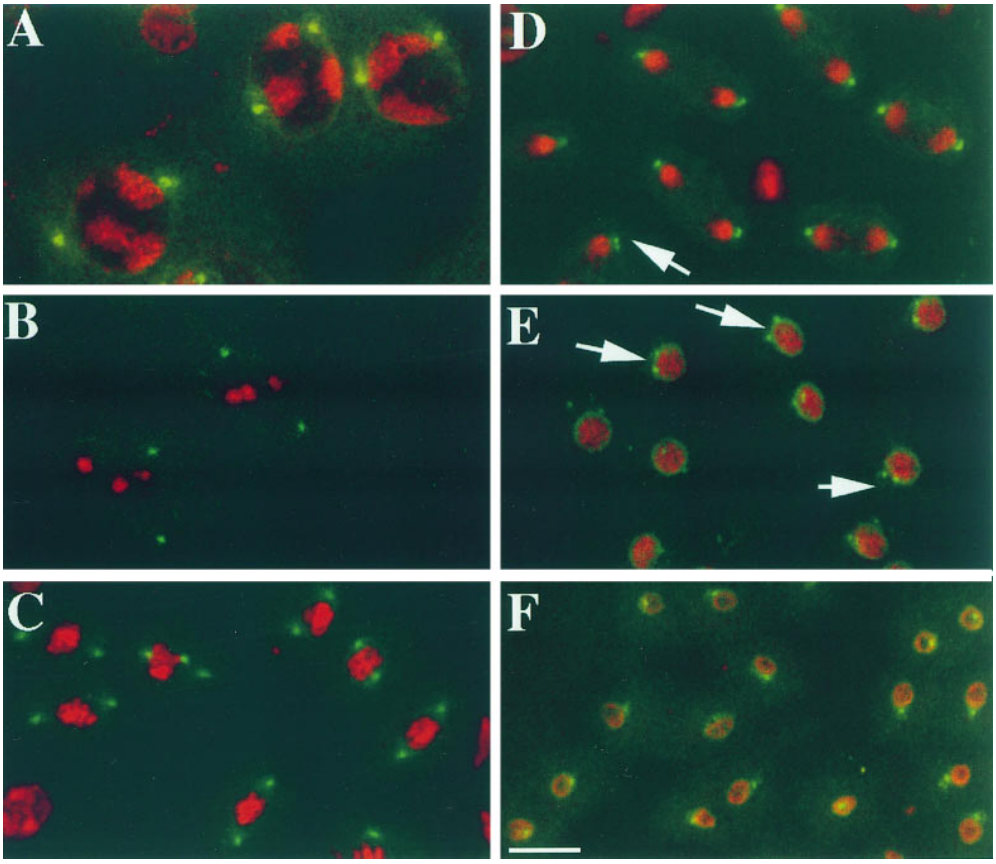

Figure 3. Centrosomin is closely associated with the centrosomes during both meiotic divisions. Dividing spermatocytes were labeled with propidium iodide (*red*) and anticentrosomin (*green*). (*A*) At the onset of meiotic division I, the centrosomes occupy opposite ends of the spermatocytes. The chromosomes start to condense, and are still associated with the nuclear membrane. (*B*) Prometaphase of meiosis I. Chromosomes have condensed, and bivalents of three major chromosomes start to align at the spindle equator. Centrosomes occupy opposite poles of each spermatocyte. (*C*) Metaphase of meiosis I: chromosomes align at the spindle equator, and some have started to separate. (*D*) Telophase of meiosis I: chromosomes separate, and in some cases the centrosomes have duplicated (*arrow*). (*E*) Interphase between meiosis I and meiosis II. Duplicated centrosomes start to migrate away from each other (*arrows*). Note there is weak staining of the nuclear membrane that may be nonspecific. (*F*) Telophase of meiosis II. Centrosomin is associated with the centrioles. Bar, 10 μm.

cycle progresses, the chromosomes gather at the metaphase plate and fuse to form one tight chromosome mass (Fig. 3 *C*). Homologous chromosomes quickly separate during anaphase, and each spermatocyte is divided into two interconnected cells through incomplete cytokinesis (Fig. 3 *D*). Meiosis II follows immediately. The two centrioles at each pole split, and the single centrioles with associated centrosomin migrate to opposite poles of the nuclei (*arrows*; Fig. 3, *D* and *E*).

Meiosis II is similar to meiosis I both in terms of microtubule organization (Cenci et al., 1994) and centrosomin behavior. One feature that is unique to meiosis II in *Drosophila* spermatogenesis is that a single centriole exists in each centrosome. Meiosis I centrosomes and mitotic centrosomes both have two centrioles. Centrosomin is also in close association with the meiosis II centrosomes (Figs. 3, *E* and *F*).

In postmeiotic cells, the single centriole inserts into the nucleus and becomes the basal body. Centrosomin can be detected in association with the basal body up to the early stages of axonemal elongation (Fig. 3 *F*; see Fig. 8 *D*). This is the latest stage at which centrosomin can be detected at the light microscopic level.

Isolation of Sterile Mutations

We have shown that centrosomin is required for proper centrosomal function during the embryonic syncytial cleavage divisions (Li and Kaufman, 1996). Its close association with the centrosome during spermatogenesis suggests that centrosomin may also be an essential component of the meiotic centrosomes. To test this hypothesis, we carried out mutational analysis of centrosomin by screening for lesions defective in spermatogenesis.

We have previously reported the isolation of lethal alleles in the *centrosomin* (*cnn*) gene (Li and Kaufman, 1996). These mutations were subsequently found to be allelic to mutations at the previously identified *arrow* (*arr*) locus (Nüsslein-Volhard and Wieschaus, 1980). Embryonic lethality caused by the lethal *arr* alleles prevented study of centrosomin function during spermatogenesis. Moreover, we have not yet been able to affect rescue of our lethal mutations with a transgenic construct containing the transcription unit encoding Cnn. Therefore, we carried out an additional ethyl methane sulfonate mutagenesis screen to isolate sterile mutations using *Df(*2R*)Cnn*, a deficiency that removes the Cnn-encoding transcription unit as as-

sayed by in situ hybridization to polytene chromosomes (Heuer et al., 1995). Multiple sterile mutations were recovered that fall into several complementation groups. One group of mutations (*mfs1*, *mfs2,* and *mfs3*) caused both male and female sterility. Three pieces of evidence support the conclusion that these are mutations in the gene encoding Cnn. First, male and female sterility of all three mutants is rescued by a transgene carrying a 16-kb genomic fragment that covers the known Cnn-encoding transcription unit, and 3–4 kb of upstream sequences (Fig. 1 *A*). Furthermore, basal levels of expression (at 25°C) from a transgene carrying a 4.3-kb embryonically derived cDNA under the control of an *Hsp70* promoter also rescues male sterility of all three alleles. Second, all three mutant alleles produced centrosomin protein that was detectable on Western blots (Fig. 4). The proteins shown were derived from early embryos, and demonstrate that two of the alleles (*mfs2* and *mfs3*) encode proteins that are smaller than the wild-type. Additionally, the proteins encoded by all three alleles accumulate to significantly lower levels than those seen in wild-type. This is especially true of the *mfs1* allele. The apparently lower molecular weight of the protein encoded by *mfs2* and *mfs3* is consistent with sequence analysis of these two lesions (see below). We were not able to detect Cnn on either Western blots or in the centrosomes during spermatogenesis, presumably because of the diminished accumulation of the protein in the mutants coupled with the lower levels of expression in the wild-type testes as compared with the early embryo (data not shown; see below). Third, sequence analysis has identified mutations within the Cnn coding sequence in two of the three alleles. The mapped lesions are associated with stop codons in the segment of the open reading frame encoding the third leucine zipper motif. These translational stops predict production of a truncated polypeptide as is seen in the Western blots (Fig. 4). Therefore, these sterile alleles represent viable mutations that affect a centrosomal function of centrosomin. Male sterility caused by these mutations suggests that wild-type centrosomin function is required for spermatogenesis.

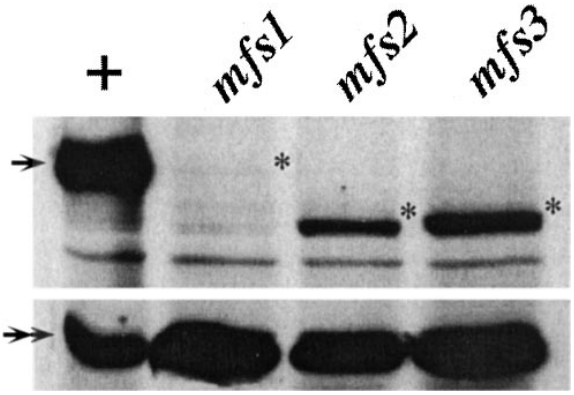

Figure 4. Centrosomin protein produced by the *mfs1, mfs2,* and *mfs3* mutant alleles. The single-headed arrow indicates the position of the normal polypeptide extracted from wild-type embryos. The stars mark the position of the proteins produced by the three mutant alleles. The mfs2 and mfs3 lanes show that the protein runs at a lower molecular weight, consistent with the fact that these two alleles have stop codons upstream of the normal translational termination signal. The mfs1 encoded protein runs at the normal molecular weight, but is barely detectable. The proteins encoded by the other two alleles are also accumulated at lower than wild-type levels (compare lane *1* + to lanes *3* and *4*). The double-headed arrows indicate the position of α-tubulin, which serves as a loading control. The blot was overexposed to reveal the presence of the mutant polypeptides. The bands running below the Cnn band most likely represent cross-reacting species to the polyclonal antisera used to detect Cnn.

Male Sterile Phenotypes of mfs Mutants

To determine which processes are affected by the *mfs* mutations, we first analyzed mutant testes using phase contrast microscopy. Production and proliferation of spermatogonial cells are not affected by these mutations. However, dramatic defects were observed in postmeiotic spermatids. In wild-type testes, postmeiotic spermatids go through a distinct transition stage (onion stage) during which mitochondria aggregate to form a phase dark body (the nebenkern) lying next to the phase light nucleus (Fig. 5 *A*). The nebenkern bodies and the nuclei are normally equal in number and approximately equal in size. Failure of cytokinesis results in a larger nebenkern associated with more than one nucleus, and failure of karyokinesis results in nuclei of unequal sizes (Castrillon and Wasserman, 1994; Eberhart and Wasserman, 1995; for review see Fuller, 1993). Mutations of the *mfs* group disrupt both cytokinesis and karyokinesis (Fig. 5 *B*). First, large proportions of the postmeiotic cells have two or four nuclei associated with each large nebenkern (Fig. 5 *B*). Second, the nuclei are often unequal in size, indicating the failure of proper chromosomal segregation during the meiotic divisions (Fig. 5 *B*, *arrows*). The percentage of aberrant spermatids varies from testis to testis (20–50%) in all three alleles, and the cytokinetic defects were always more severe than karyokinetic defects. However, all the testes examined showed defects in cytokinesis and karyokinesis, and none of them were completely wild-type.

Despite the defects associated with the meiotic divisions, the aberrant spermatids elongate their mitochondrial derivative and go through varying degrees of spermiogenesis (Fig. 5 *C*; see Fig. 9). These abnormal spermatids degenerate before individualization, and no motile sperm can be detected in the seminal vesicles (see Fig. 9).

Microtubule Organization in mfs Mutant Testes

Abnormal spindle morphology strongly suggests disruption of centrosomal function and microtubule organization in *mfs* mutants. We therefore examined centrosomin localization and microtubule organization in *mfs* mutants using immunocytochemistry.

At the primary spermatocyte stage, wild-type cells show a dense network of microtubules in the cytoplasm, and centrosomin is associated with the centrioles at the cell membrane (Fig. 6, *A* and *A′*). In *mfs* mutants, microtubules in primary spermatocytes are morphologically normal (Fig. 6 *B*), although centrosomin cannot be detected in the centrosomes (Fig. 6 *B′*). At the transition to meiotic division I, the centrosomes in wild-type spermatocytes nucleate two prominent asters and migrate to opposite ends of the nucleus (Fig. 6, *C* and *C′*). In *mfs* mutants, these as-

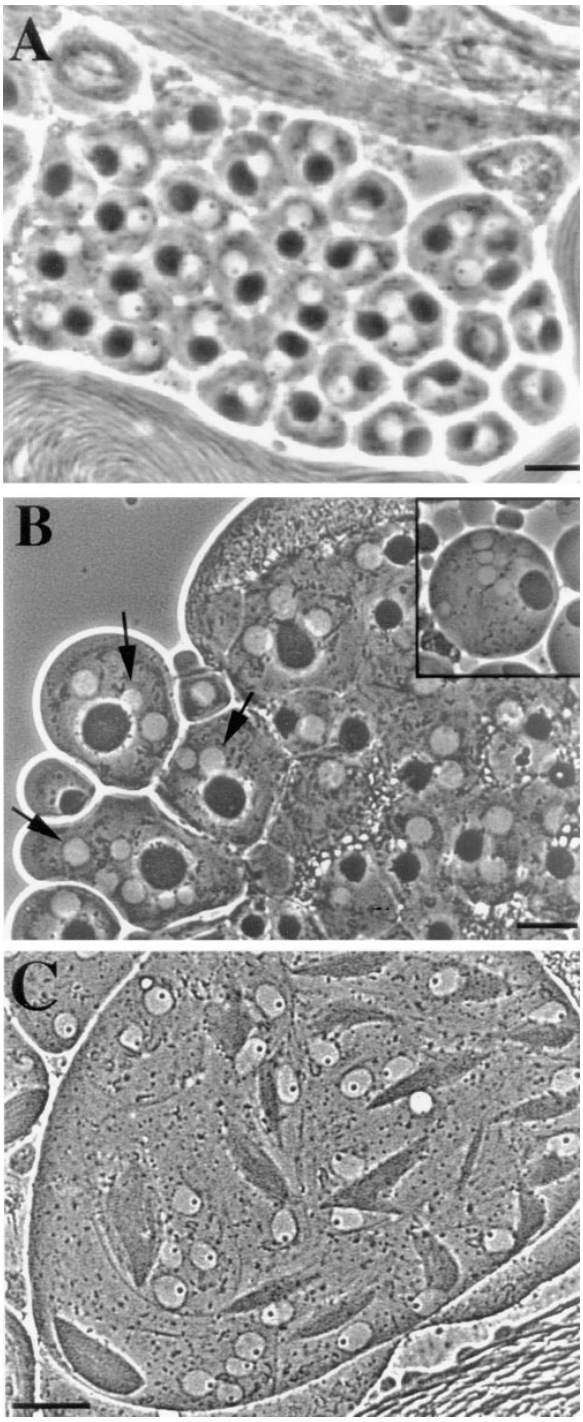

Figure 5. Cnn *mfs* mutations affect cytokinesis and karyokinesis during male meiotic divisions. Live testis squashes were observed under phase contrast optics. (*A*) A wild-type onion stage cyst. The phase dark nebenkerns and the phase light nuclei are of equal numbers, and are approximately equal in size. (*B*) Onion stage spermatids from a *mfs1/Df(*2R*)Cnn* male. Various numbers of nuclei are associated with nebenkern, suggesting failure of cytokinesis during the meiotic divisions. Some cysts have nuclei of unequal sizes (*inset* and *arrows*), suggesting failure of karyokinesis. (*C*) Despite the meiotic defects, aberrant spermatids elongate and undergo morphogenesis. A cyst from a *mfs2/Df(*2R*)Cnn* male is shown. Bars, 10 μm.

ters do not form. Although regions of high microtubule densities are seen in some mutant spermatocytes, these asters are never as dense as in wild-type, and do not have detectable centrosomin in the center (Fig. 6, *D–E′*). Many primary spermatocytes have only one microtubule focus, a phenomenon rarely seen in wild-type spermatocytes. Since duplication of the centrosomes occurs early during the spermatocyte growth period, by the time microtubule asters can be clearly detected, the centrosomes should have already separated from each other. Formation of single asters in *mfs* mutants may represent failure of centrosomal duplication or separation (Fig. 7).

The microtubule organization defects become more dramatic during the meiotic divisions. Male meiotic spindles are composed of two cup-shaped half spindles organized by the centrosomes (Fig. 7 *A*; Cenci et al., 1994). In *mfs* mutants we frequently observe spindles with one focused pole and one diffuse pole (Fig. 7 *B*, spindles a, b, and d). In many cases, the spindles are apparently monopolar (Fig. 7 *B*, spindle a; Fig. 7 *D*). Additionally, cysts with multipolar spindles and disorganized microtubules are also frequently seen (Fig. 7 *C*, *arrowheads*; Fig. 7 *E*).

In *mfs* mutants, the midzone microtubules do not form (Fig. 7 *C, arrow*). These results suggest that the cytokinesis and karyokinesis defects observed in *mfs* mutants are caused by the disruption of microtubule spindles.

mfs Mutations Affect Assembly of the Axoneme

At the completion of meiosis, the centriole inserts into the spermatid nucleus and becomes the basal body (Fig. 8 *A*). Anticentrosomin antibody brightly labels the basal bodies in onion stage spermatids (Fig. 8 *B′*, *arrows*). In *mfs* mutant spermatids, no basal body staining of centrosomin can be detected, although the nuclear membrane staining remains (Fig. 8 *C′*). Thus, the *mfs* alleles provide a reagent to determine if the centrosomin protein associated with the basal body performs any functional role during spermatid morphogenesis. We were able to examine postmeiotic development in *mfs* mutants in spite of the meiotic defects because defective spermatids undergo various degrees of elongation and differentiation before they degenerate (Fig. 5 *C*; Fig. 9).

Fig. 9, *A–C* shows transverse sections of stage 17 spermatid tails from *mfs1/Df(*2R*)Cnn* males (staged according to Tates, 1971) showing multiple defects. In wild-type spermatids, each axoneme is associated with two mitochondrial derivatives, and together they are surrounded by a double membrane (Tates, 1971). In *mfs* mutants, multiple axonemes (Fig. 9 *A*, *arrowheads*) with various numbers of mitochondrial derivatives (Fig. 9 *A*, *open arrowheads*) occupy one membrane system, reflecting failure of cytokinesis during meiosis. In addition, the axonemes themselves are structurally abnormal. Wild-type axonemes are composed of one central pair of single microtubules surrounded by nine doublet microtubules (similar to those in Fig. 9 *C*; Tates, 1971). Later during sperm differentiation, nine single accessory microtubules grow out of the β-subunit of the outer double microtubules (Fig. 9 *E*). In *mfs* mutants, formation of the central pair of microtubules is frequently affected. Although ~40% of the axonemes have a pair of central microtubules (Fig. 9 *C*) as in the

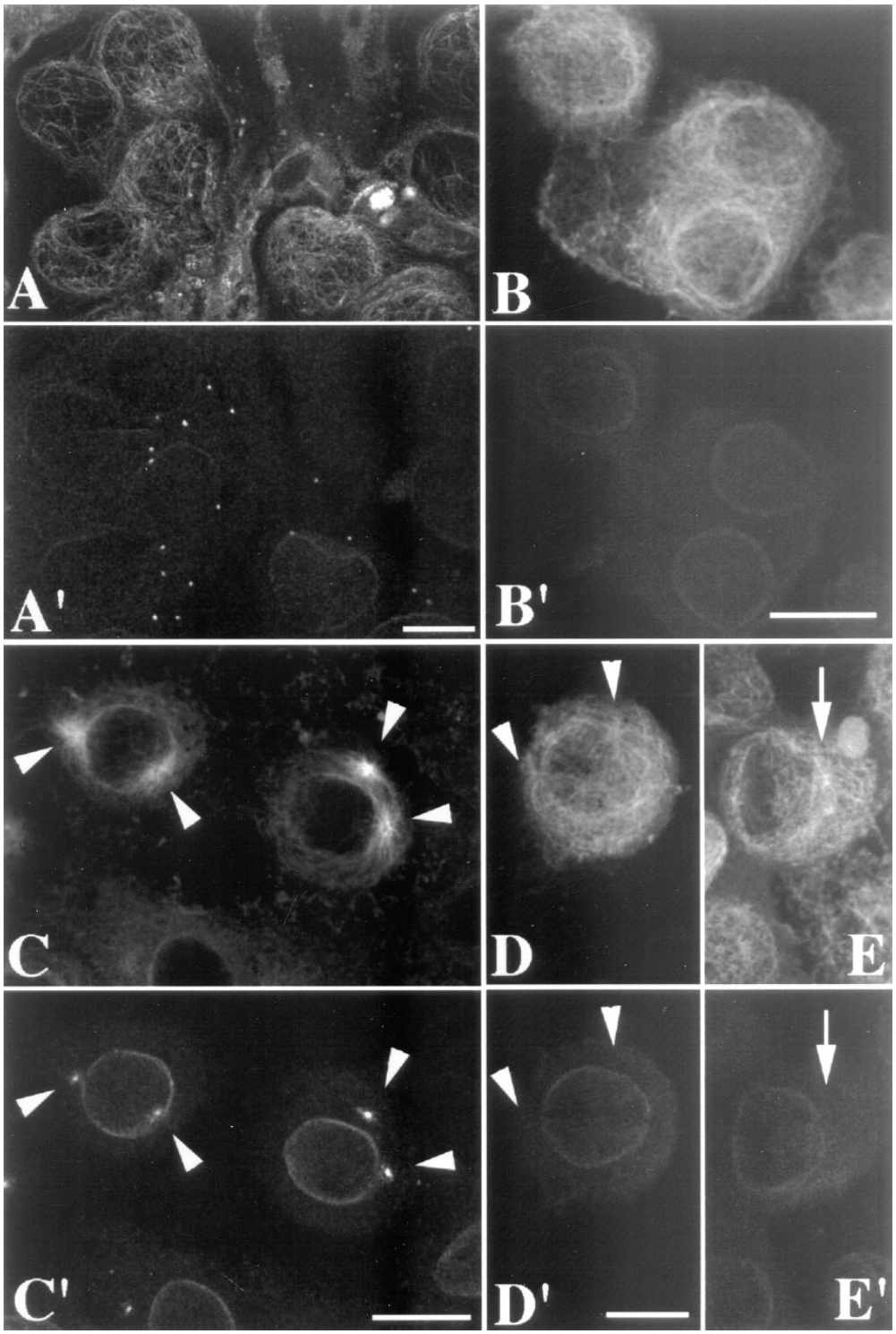

Figure 6. (*A* and *A′*) Comparison of microtubule organization in wild-type and *mfs* mutants during premeiotic stages. In wild-type testis, mature spermatocytes have a dense array of cytoplasmic microtubules (*A*). Centrosomin antibody brightly labels the centrosomes and weakly labels the nuclear membranes (*A′*). (*B* and *B′*) The microtubule network in spermatocytes from a *mfs1/Df(*2R*)Cnn* male is morphologically normal (*B*), yet no centrosomin staining is detected in the centrosomes. The nuclear membrane staining is not affected, suggesting that it may be nonspecific staining. (*C* and *C′*) In wild-type spermatocytes during prometaphase of meiotic division I, the duplicated centrosomes organize microtubules around them and migrate to opposite poles (*C*). These centrosomes have an increased amount of centrosomin staining (*C′*). In spermatocytes from *mfs1/Df(*2R*)Cnn* (*D* and *D′*), or *mfs2/Df(*2R*)Cnn* (*E* and *E′*), prominent microtubule asters cannot be detected. Some regions of the spermatocytes have a higher density of microtubules (*D*, *arrowheads*), but they do not have associated centrosomin staining (*arrowheads*, *D′*). In some cases, only one focus of higher microtubule density can be distinguished (*E*). No centrosomin signal can be detected in these asters (*E′*). Bar, 10 μm.

wild-type, 40% of the axonemes have no central pair (Fig. 9 *A*), while 20% have only one central microtubule (Fig. 9 *B*). The nine doublet microtubules and the accessory microtubules are not severely affected by the *mfs* mutations (Fig. 9 *D*, *arrow*), although we do occasionally see axonemes with incomplete outer microtubules (Fig. 9 *D*, *arrowhead*). The spokes that connect the outer microtubules with the central pair form normally, even in the absence of the central pair of microtubules (Fig. 9 *A* and *D*).

Disruption of axonemal organization in the mfs mutants suggests that centrosomin may be closely associated with centrioles and basal bodies, and may affect the internal organization of these organelles. Indeed, using immunoelectron microscopy, we consistently observe close association of centrosomin with the centrioles, either in primary spermatocytes where there are a pair of centrioles (Fig. 9, *F–H*), or in postmeiotic cells when the single centriole is beginning to insert into the nucleus (Fig. 9 *I*).

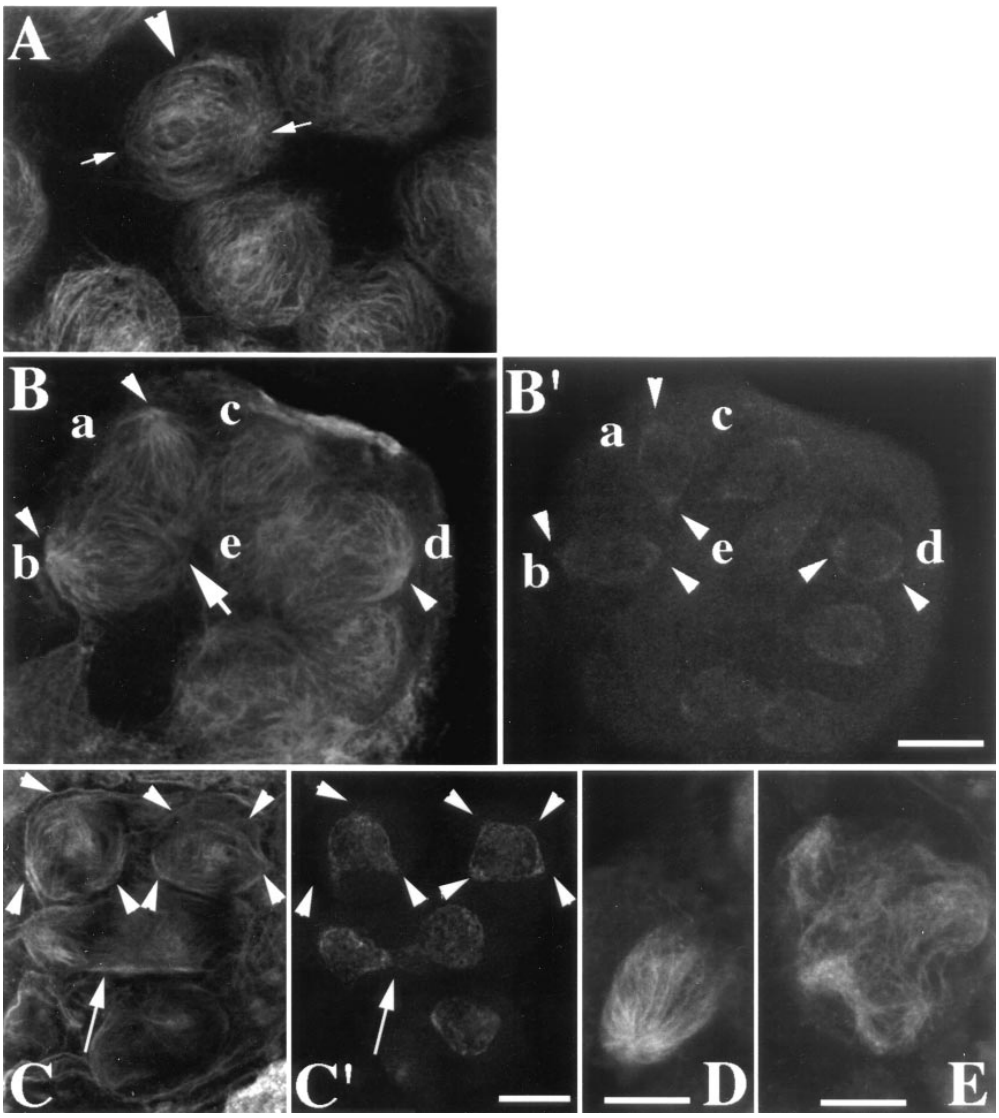

Figure 7. Meiotic spindle defects in *mfs* mutants. (*A*) Wild-type meiotic spindle at anaphase of meiosis I. The spindles are bipolar (*arrows*), and high densities of microtubules are apparent at the spindle midzone (*arrowhead*). (*B* and *B′*) Abnormal meiotic spindles in *mfs1/Df(*2R*)Cnn*. A meiotic cyst was double-labeled with anti-α-tubulin (*B*) and anticentrosomin (*B′*). Several spindles in this cyst clearly displayed spindle organization defects. Note that both poles of spindles a, b, and d are in the focal plane judged by the nuclear membrane staining of anticentrosomin (*arrowheads* in *B′*). However, only one microtubule pole can be clearly defined for these spindles (*arrowheads* in *B*). (*C* and *C′*). Divisions in *mfs* mutants are often seen to be multipolar, as suggested by both microtubule staining (*arrowheads*, *C*) and by shaping of the nuclear membrane system revealed by the centrosomin antibody (*arrowheads*, *C′*). The midzone microtubules and the cytoplasmic constriction cannot be detected at telophase of meiosis in mutants (*arrow* in *C*), although separation of the nuclei appears normal (*arrow*, *C′*). A monopolar spindle (*D*) and tangled microtubules (*E*) in meiosis of a *mfs1/Df(*2R*)Cnn* male. Bars, 10 μm.

Discussion

Spermatogenesis is a highly specialized and evolutionarily conserved process of gamete production. Two qualitatively different forms of cell division–mitosis and meiosis–and elaborate morphological changes are required to produce a mature sperm from a stem cell. Centrosomes are involved in these processes as microtubule-organizing centers, but the extent of that role and the molecular composition of these centrosomes is largely unknown. This study has examined the role of centrosomin, a core protein of the embryonic centrosome (Li and Kaufman, 1996), in mitotic, meiotic, and postmeiotic (as a basal body) cells of the *Drosophila* testis.

Alternate Isoforms of Centrosomin

Meiosis is a specialized form of cell division that differs from mitosis in several respects. It not only has a unique cell cycle control mechanism that allows reduction of chromosome number, but also uses many specialized structural components. Several genes have been identified that function principally if not exclusively during the meiotic divisions. For example, two homologs of the yeast *S. pombe CDC25* gene exist in *Drosophila*: *string* encodes a mitotic specific form (Edgar and O'Farrell, 1990), and *twine* encodes a meiotic specific form (Courtot et al., 1992). During spermatogenesis, *string* is expressed at the mitotic region, while *twine* is expressed at the meiotic region (Courtot et al., 1992). Additionally, there is a testis-specific isoform of β2-tubulin that is expressed exclusively in the postmitotic cells of the testis (Kemphues et al., 1982; Fuller et al., 1988; Hoyle and Raff, 1990).

An alternate isoform of centrosomin is detected in the testis, and is generated via alternative splicing of five exons (Fig. 1). Although both the embryonic form (150 kD) and the testis form (120 kD) of centrosomin can be detected in testis extracts, the 120-kD protein appears to be the predominant form. Isoform-specific antibodies are needed to reveal the distribution pattern of these isoforms

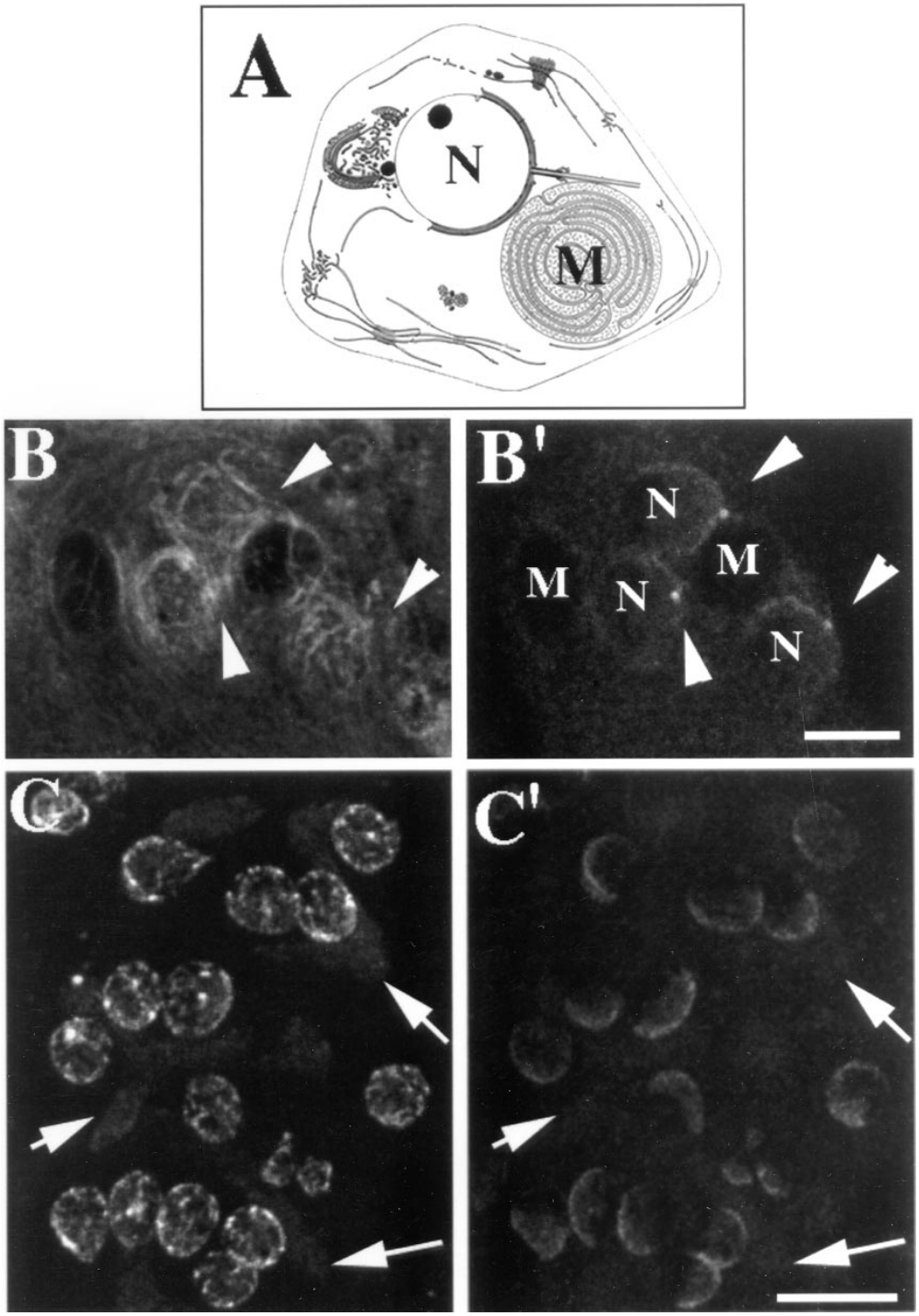

Figure 8. Centrosomin localization in postmeiotic spermatids. (*A*) Schematic diagram of an onion stage spermatid showing the relative positions of the nucleus (*N*), the nebenkern (*M*), and the centriole. Drawing modified from Tates (1971). (*B*) Anti-α-tubulin staining of postmeiotic spermatids. Arrowheads point to the position of the basal bodies. (*B′*) Anticentrosomin staining of the same field of cells as in (*B*). Centrosomin is detected in association with the centrioles (*arrowheads*). (*C*) DNA staining of postmeiotic spermatids from *mfs1/Df(*2R*)Cnn*. Multiple nuclei are associated with each mitochondrial derivative (*arrows*; mitochondria have started to elongate in these spermatids). (*C′*) Same field of cells as in *C*. No centrosomin can be detected in association with the basal body, although the nuclear membrane staining remains. Arrows point to the position of mitochondrial derivatives. Bars, 10 μm.

during spermatogenesis. The significance of the testis isoform, however, is unclear because the embryonic isoform of centrosomin (via a cDNA rescue construct) can restore fertility to *mfs* mutant males, suggesting possible functional redundancies of the isoforms.

Centrosomin is a Core Component Required for Normal Centrosomal Function During Spermatogenesis

Immunolocalization of centrosomin shows that it is a core component of the centrosome throughout spermatogenesis. It remains closely associated with the centrosome, even during the spermatocyte growth period; a phase of the cell cycle when the centriole loses most of its pericentriolar material and migrates to the cell membrane (Tates, 1971). During the meiotic divisions, it is present at the centrosomes at elevated levels. In postmeiotic stages, centrosomin is found associated with the basal body of the maturing spermatid.

The close association of centrosomin with the centriole throughout spermatogenesis distinguishes it from some other centrosomal proteins. It has been shown that CP190, a centrosomal protein tightly localized to the spindle poles during mitosis, is diffusely distributed in the cytoplasm

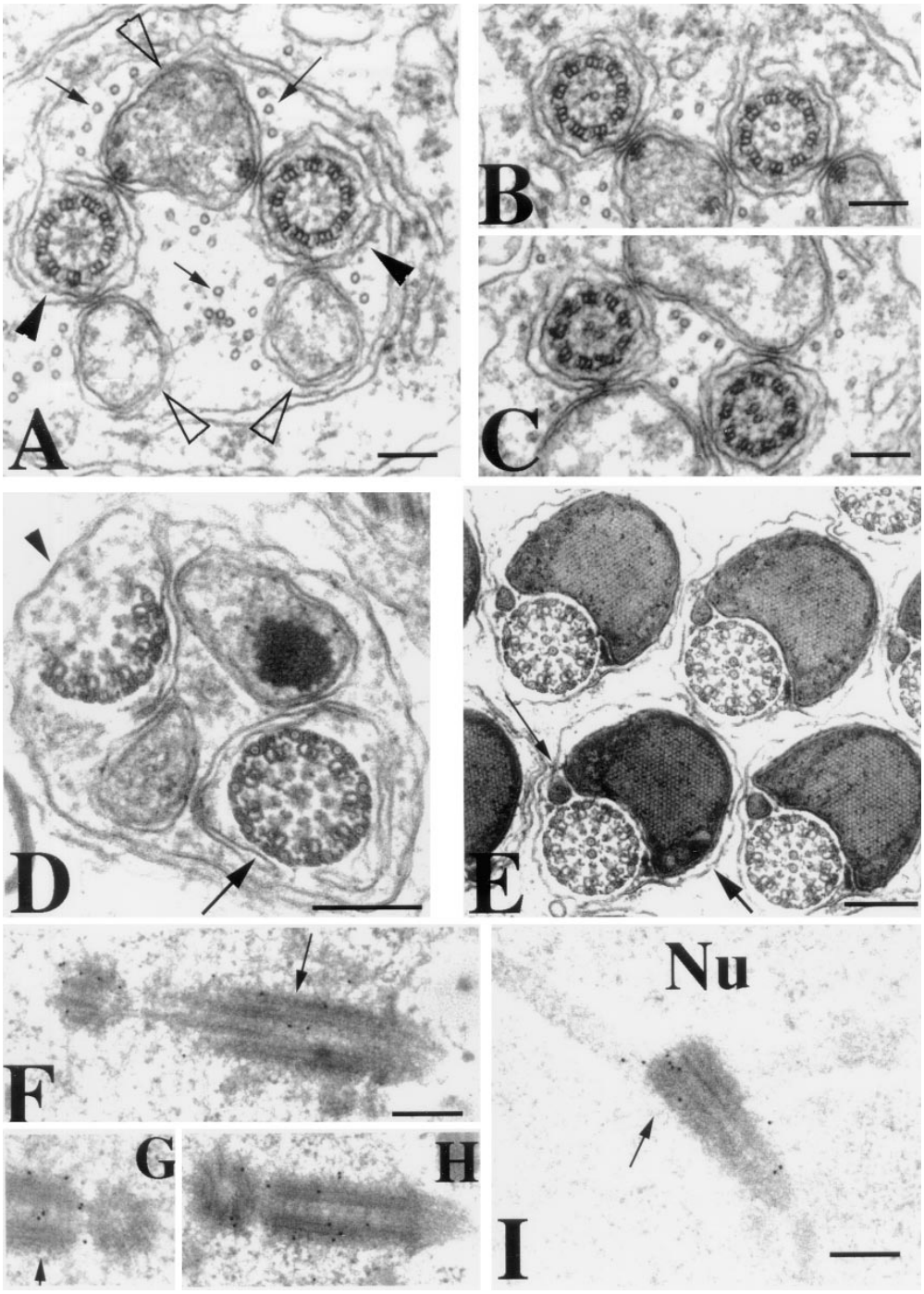

Figure 9. mfs mutations disrupt axonemal organization. (*A–C*). Transverse sections of a sperm tail from *mfs1/Df(*2R*)Cnn* at stage 17 (Tates, 1971). Multiple axonemes (*filled arrowheads*) may occupy one double-membrane system, and various numbers of mitochondrial derivatives (*open arrowheads*) may be associated with each axoneme. Some axonemes have no central pair of microtubules (*A*), some have one central microtubule (*B*), and some have two (C), as in the wild-type. The cytoplasmic microtubule appeared normal (*small arrows*). (*D*) Transverse section of a later stage sperm tail from a *mfs1/Df(*2R*)Cnn* male. One axoneme is missing the central pair (*arrow*); in the other, the ring of outer doublet microtubules is incomplete (*arrowhead*). The morphology of accessary microtubules in the complete axoneme is normal. (*E*) Cross-section of wild-type spermatids at very late stages, mainly to show the mature axonemes with fully developed accessory tubes. Large and small arrows point to the major and minor mitochondrial derivatives, respectively. (*F–I*). Immunoelectronmicroscopic analyses show that centrosomin is closely associated with the centrioles both in primary spermatocytes, which have a pair of centrioles (*F–H*), and in post-meiotic spermatids, which have single centrioles (*I*). Bars, 200 nm.

during male meiotic divisions (Casal et al., 1990). This result suggests that the meiotic centrosomes have a different composition than the mitotic centrosomes. Centrosomin appears to be a shared component of the mitotic and meiotic centrosomes, although the two isoforms may be differentially expressed in the mitotic vs. meiotic regions. Mutational analysis shows that centrosomin is required for proper functioning of meiotic centrosomes. Defective meiotic spindles and incomplete cytokinesis are evident in live squashes of mutant testes, and immunolabeling reveals severe microtubule organizational defects. We have shown that during the syncytial cleavage divisions, centrosomin is required to recruit or retain at least three other core centrosome components–γ-tubulin (Moritz et al., 1995, Zheng et al., 1995), CP60 (Kellogg et al., 1995), and CP190 (Whitfield et al., 1995)–suggesting that centrosomin plays a role in the organization and/or maintenance of centrosomes (Li and Kaufman, manuscsript in preparation). It is also possible that centrosomin may function to assemble meiotic centrosomes during male meiosis. Failure to recruit functional subunits into the meiotic centrosome could conceivably lead to failure in centrosomal separation and/or failure of microtubule nucleation. Monopolar spindles could form if one of the centrosomes failed to nucleate

microtubules, or if the centrosome fails to duplicate or separate to opposite poles. Formation of multipolar spindles in *mfs* mutants could be a consequence of the failure of cytokinesis, which places several microtubule-organizing centers in a shared cytoplasm. An understanding of the precise function of centrosomin in the meiotic centrosomes awaits further biochemical analysis of the protein and identification of more meiotic centrosome markers.

Microtubules, Cytokinesis, and Karyokinesis During Meiosis

Our results show that cytokinesis during male meiosis is more sensitive to reduced centrosomin function than is karyokinesis. This observation presumably reflects the dependence of cytokinesis on microtubule dynamics. Earlier work has established that during mitotic divisions, the position of the contractile ring is determined by the position of the spindle microtubules (Rappaport, 1975). Both during mitosis and male meiosis, the microtubule spindles reorganize during anaphase and telophase so that the centrosome to kinetochore microtubules decrease in density, while a new population of dense microtubules (called central spindles in male meiosis; Cenci et al., 1994) form in the midzone (Fig. 8 *A*). These microtubules appear to signal the position of cytokinesis. In *mfs* mutants, formation of the midzone microtubules is rarely seen, suggesting that the microtubule dynamics are affected when centrosomal function is compromised.

Nuclear divisions are more resistant to disruption of centrosomin function. Although spermatid nuclei of unequal sizes, indicative of chromosomal segregation defects, are often seen in *mfs* mutants, we rarely saw complete fusion of spermatid nuclei. This result is consistent with the finding that centrosomes are not absolutely required for chromosome segregation. During *Drosophila* female meiosis, chromosomes and motor proteins act together to organize the microtubule spindles and assure chromosome segregation, apparently in the absence of centrioles and known centrosomal proteins (Matthies et al., 1996). Although meiotically dividing cells in males contain centrosomes, it has been suggested that they are not required for nucleation of spindle microtubules. Using micromanipulation, Church et al. (1986) demonstrated that chromosome pairs relocated from the nucleus to the spermatocyte cytoplasm are capable of organizing minispindles around themselves with no centrosomes at the poles. In *ms(1)516* (Lifschytz and Hareven, 1977), both centrioles are passed to one pole at the second meiotic division, but bipolar spindles form even though one of the spindle poles lack astral membranes. We have observed similar asymmetric bipolar spindles in the meioses of *mfs* mutants (Fig. 6 *D*). These spindles may have centrosomes at only one pole. Direct observation of microtubule organization in *mfs* mutants showed that these abnormal spindles often have one confined pole and one diffuse pole. In extreme cases, spindles appear monopolar (Fig. 7 *B* and 6 *D*). The half spindles with diffuse poles may therefore be assembled by the chromosomes.

Nature of the mfs Mutants

The fact that the mfs mutants survive to adulthood and produce testes with no apparent Cnn protein in the centrosomes creates a two-part paradox. First, we know that Cnn is present in the stem cells and in the mitotic cyst cells where it presumably functions in the centrosome. Despite this fact, these divisions are normal in mutant animals. Second, the adult Cnn mutant flies develop through the larval and pupal stages with only the mutant protein present (the maternally supplied polypeptide is gone by midembryogenesis [Heuer et al., 1995]). Thus, the cell division that takes place postembryonically must occur in the absence of normal Cnn, but again is normal. One possible rationalization of these observations is that the centrosomes of the mitotically dividing cells in the imaginal discs and premeiotic cells of the testes are qualitatively different from the meiotic cells. This reasoning assumes that although Cnn is made and is localized to the centrosome in these cell types, it provides no essential function there. However, we have demonstrated using more extreme alleles of Cnn that the protein is necessary for cell division in the imaginal discs (Li and Kaufman, 1996). Therefore, we must seek a resolution to the paradox elsewhere. As noted above, the three male sterile mutations analyzed in this paper do accumulate Cnn protein, albeit at reduced levels, and in two cases of altered electrophoretic mobility. Thus, these alleles are hypomorphic in character. This leakiness is manifested in survival of the mutant animals to adulthood and the normal premeiotic mitotic divisions in the testes. We conclude, therefore, that our failure to detect Cnn in the centrosomes of the mutant animals is a problem of resolution rather than absolute absence of the protein from that structure. That is, the protein is in the centrosome, but at levels that are below detection. This conclusion is consistent with our Western blot results in which we are able to resolve the proteins when extracted from eggs, but not from testes. Additionally, we have been able to detect centrosomal Cnn protein in imaginal discs dissected from *mfs* mutant animals using immunofluorescence, but the levels of accumulation are clearly well below that seen in wild-type (data not shown). We would argue, therefore, that the mutations cause a reduction in the pool size of Cnn in mutant cells, and that this pool of protein is sufficient to support some, but not all Cnn-mediated centrosomal functions. Thus, only those functions that are most sensitive to Cnn levels are affected. Consistent with this conclusion, we find that meiotic cytokinesis is affected more dramatically than is karyokinesis. Moreover, that basal levels of expression from a heat shock–driven Cnn construct are capable of rescuing the steriles is also indicative of the fact that these steriles are extreme hypomorphs. Further testing will of course be required to confirm this hypothesis.

Basal Body and Axoneme Formation

The fate of centrosomal proteins in the transformation of centrosome to basal body is unknown. In many organisms, the sperm basal body is incorporated into the first functional centrosome after fertilization (for review see Schatten, 1994). Previous studies have shown that the basal body carries into the egg only a small amount/number of associated proteins necessary for recruitment of other proteins from the egg cytoplasm (Felix et al., 1994; Stearns

and Kirschner, 1994). Only a few proteins have been reported to be associated with the sperm basal bodies (e.g., the CTR2611 antigen; Felix et al., 1994; pericentrin; Stearns and Kirschner, 1994). Presumably, most of the proteins present in the meiotic centrosomes dissociate from the basal body at different time points during spermatid differentiation. Although we have not been able to determine if centrosomin is in the basal body of mature sperm, centrosomin was detected in the spermatid basal body up to the onion nebenkern stage (Fig. 8 *D*; Fig. 9 *I*). In *mfs* mutants, defects were observed in the organization of microtubules in the elongating axoneme of spermatids (Fig. 9), indicating that centrosomin plays a role in basal body function. The central pair of microtubules was the most frequently disrupted component of the axonemal structure. Mechanisms that differentially organize the central microtubules, the nine doublet microtubules, and the accessory microtubules are not known (Tokuyasu, 1974; Fackenthal et al., 1995). If centrosomin is involved in the organization of the centrosome during cell division, it is conceivable that it may also organize materials required for formation of the central pair microtubules.

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References

Ashburner, M. 1989. *Drosophila*, a Laboratory Handbook. Cold Spring Harbor Laboratory Press, Plainview, NY.

Borisy, G.G., and R.R. Gould. 1977. Microtubule-organizing centers of the mitotic spindle. *In* Mitosis Facts and Questions. M. Little, editor. Springer-Verlag. Berlin. 78–97.

Casal, J., C. Gonzalez, and P. Ripoll. 1990. Spindles and centrosomes during male meiosis in *Drosophila melanogaster*. *Eur. J. Cell Biol.* 51:38–44.

Castrillon, D.H., and S.A. Wasserman. 1994. Diaphanous is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformity* gene. *Development.* 120:3367–3377.

Cavalier, S.T. 1974. Basal body and flagellar development during the vegetative cell cycle and the sexual cycle of *Chlamydomonas reinhardii*. *J. Cell Sci.* 16:529–556.

Cenci, G., S. Bonaccorsi, C. Pisano, F. Verni, and M. Gatti. 1994. Chromatin and microtubule organization during premeiotic, meiotic, and early postmeiotic stages of *Drosophila melanogaster* spermatogenesis. *J. Cell Sci.* 107: 3521–3534.

Church, K., R.B. Nicklas, and H.P. Lin. 1986. Micromanipulated bivalents can trigger mini-spindle formation in *Drosophila melanogaster* spermatocyte cytoplasm. *J. Cell Biol.* 103:2765–2773.

Courtot, C., C. Fankhauser, V. Simanis, and C.F. Lehner. 1992. The *Drosophila* cdc25 homolog twine is required for meiosis. *Development.* 116:405–416.

Eberhart, C.G., and S.A. Wasserman. 1995. The *pelota* locus encodes a protein required for meiotic cell division: an analysis of G2/M arrest in *Drosophila* spermatogenesis. *Development.* 121:3477–3486.

Edgar, B.A., and P.H. O'Farrell. 1990. The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by *string*. *Cell.* 62:469–480.

Fackenthal, J.D., J.A. Hutchens, F.R. Turner, and E.C. Raff. 1995. Structural analysis of mutations in the *Drosophila* beta2-tubulin isoform reveals regions in the beta-tubulin molecular required for general and for tissue-specific microtubule functions. *Genetics.* 139:267–286.

Felix, M.A., C. Antony, M. Wright, and B. Maro. 1994. Centrosome assembly in vitro: role of gamma-tubulin recruitment in *Xenopus* sperm aster formation. *J. Cell Biol.* 124:19–31.

Fuller, M.T. 1993. Spermatogenesis. *In* The Development of *Drosophila melanogaster*. M. Bates and A. Martinez Arias, editors. Cold Spring Harbor Laboratory Press, Plainview, NY. pp. 71–148.

Fuller, M.T., J.H. Caulton, J.A. Hutchens, T.C. Kaufman, and E.C. Raff. 1988. Mutations that encode partially functional beta 2 tubulin subunits have different effects on structurally different microtubule arrays. *J. Cell Biol.* 107: 141–152.

Gould, R.R., and G.G. Borisy. 1977. The pericentriolar material in Chinese hamster ovary cells nucleates microtubule formation. *J. Cell Biol.* 73:601–615.

Heuer, J.G., K. Li, and T.C. Kaufman. 1995. The *Drosophila* homeotic target gene *centrosomin* (*cnn*) encodes a novel centrosomal protein with leucine zippers and maps to a genomic region required for midgut morphogenesis. *Development.* 121:3861–3876.

Hoyle, H.D., and E.C. Raff. 1990. Two *Drosophila* β-tubulin isoforms are not functionally equivalent. *J. Cell Biol.* 111:1009–1026.

Kalt, A., and M. Schliwa. 1993. Molecular components of the centrosome. *Trends Cell Biol.* 3:118–128.

Kellogg, D.R., M. Moritz, and B.M. Alberts. 1994. The centrosome and cellular organization. *Annu. Rev. Biochem.* 63:639–674.

Kellogg, D.R., K. Oegema, J. Raff, K. Schnerder, and B.M. Alberts. 1995. CP60: a microtubule-associated protein that is localized to the centrosome in a cell cycle-specific manner. *Mol. Biol. Cell.* 6:1673–1684.

Kemphues, K.J., T.C. Kaufman, R.A. Raff, and E.C. Raff. 1982. The testis-specific beta-tubulin subunit in *Drosophila melanogaster* has multiple functions in spermatogenesis. *Cell.* 31:655–670.

Li, K., and T.C. Kaufman. 1996. The homeotic target gene centrosomin encodes an essential centrosomal component. *Cell.* 85:585–596.

Lifschytz, E., and D. Hareven. 1977. Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. *Dev. Biol.* 58:276–294.

Matthies, H.J., H.B. McDonald, L.S. Goldstein, and W.E. Theurkauf. 1996. Anastral meiotic spindle morphogenesis: role of the non-claret disjunctional kinesin-like protein. *J. Cell Biol.* 134:455–464.

Moritz, M., M. Braunfeld, J. Sedat, B. Alberts, and D. Agard. 1995. Microtubule nucleation by gamma-tubulin-containing rings in the centrosome. *Nature.* 378:638–640.

Muresan, V., H.C. Joshi, and J.C. Besharse. 1993. Gamma-tubulin in differentiated cell types: localization in the vicinity of basal bodies in retinal photoreceptors and ciliated epithelia. *J. Cell Sci.* 104:1229–1237.

Nüsslein-Volhard, C., and E. Wieschaus. 1980. Mutations affecting segment number and polarity in *Drosophila. Nature*. 287:795–801.

Pisano, C., S. Bonaccorsi, and M. Gatti. 1993. The kl-3 loop of the Y chromosome of *Drosophila melanogaster* binds a tektin-like protein. *Genetics.* 13: 569–579.

Rappaport, R. 1975. Establishment and organization of the cleavage mechanism. *In* Molecules and Cell Movement. S. Inoue and R.E. Stephens. Raven Press, Ltd., New York.

Rattner, J., and S.G. Phillips. 1973. Independence of centriole formation and DNA synthesis. *J. Cell Biol.* 57:359–372.

Schatten, G. 1994. The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and it restoration during fertilization. *Dev. Biol.* 165:299–335.

Stearns, T., and M. Kirschner. 1994. In vitro reconstitution of centrosome assembly and function: the central role of gamma-tubulin. *Cell.* 76:623–637.

Tates, A.D. 1971. Cytodifferentiation during spermatogenesis in *Drosophila melanogaster*: an electron microscope study. Ph.D. thesis. J.H. Pasmans, The Hague.

Tokuyasu, K.T. 1974. Dynamics of spermiogenesis in *Drosophila melanogaster*. 3. Relation between axoneme and mitochondrial derivatives. *Exp. Cell Res.* 84:239–250.

Whitfield, W.G., M.A. Chaplin, K. Oegema, H. Parry, and D.M. Glover. 1995. The 190 kDa centrosome-associated protein of *Drosophila melanogaster* contains four zinc finger motifs and binds to specific sites on polytene chromosomes. *J. Cell Sci.* 108:3377–3387.

Zheng, Y., M.L. Wong, B. Alberts, and T. Mitchison. 1995. Nucleation of microtubule assembly by a gamma-tubulin-contraining ring complex. *Nature.* 378:578–583.