Localization of the *mei-1* Gene Product of *Caenorhabditis elegans*, a Meiotic-specific Spindle Component

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Abstract. Genetic evidence suggests that the product of the *mei-1* gene of *Caenorhabditis elegans* is specifically required for meiosis in the female germline. Loss-of-function *mei-1* mutations block meiotic spindle formation while a gain-of-function allele instead results in spindle defects during the early mitotic cleavages. In this report, we use immunocytochemistry to examine the localization of the *mei-1* product in wild-type and mutant embryos. During metaphase of meiosis I in wild-type embryos, *mei-1* protein was found throughout the spindle but was more concentrated toward the poles. At telophase I, *mei-1* product colocalized with the chromatin at the spindle poles. The pattern was repeated during meiosis II but no *mei-1* product was visible during the subsequent mitotic cleavages. The *mei-1* gain-of-function allele resulted in ectopic *mei-1* staining in the centers of the microtubule-organizing centers during interphase and in the spindles during the early cleavages. This aberrant localization is probably responsible for the poorly formed and misoriented cleavage spindles characteristic of the mutation. We also examined the localization of *mei-1*(+) product in the presence of mutations of genes that genetically interact with *mei-1* alleles. *mei-2* is apparently required to localize *mei-1* product to the spindle during meiosis while *mel-26* acts as a postmeiotic inhibitor. We conclude that *mei-1* encodes a novel spindle component, one that is specialized for the acentriolar meiotic spindles unique to female meiosis. The genes *mei-2* and *mel-26* are part of a regulatory network that confines *mei-1* activity to meiosis.

CELL division necessitates the coordinate expression of the gene products required for the proper localization and organization of the spindle (McIntosh and Koonce, 1989; Kuriyama and Nislow, 1992; Rose et al., 1993; Strome, 1993). While tubulin forms the backbone of the structure, numerous accessory proteins control microtubule polymerization and spindle morphogenesis, ensuring that the appropriate structure forms at the correct time and in the proper position within the cell.

Recent genetic analysis and antibody inhibition experiments have identified a number of spindle-associated proteins that are required for the organization and function of the mitotic apparatus. These include members of the kinesin superfamily of microtubule-associated motor proteins (Goldstein, 1993; Sawin and Endow, 1993). Antibody inhibition experiments have demonstrated the requirement for vertebrate kinesin-like proteins both in vitro and in vivo (Nislow et al., 1992; Sawin et al., 1992*a*,*b*; Wright et al., 1993). In *Drosophila*, products of the kinesin-like genes *ncd* and *nod* may generate opposing forces in female meiotic spindles (Zhang et al., 1990; Hatsumi and Endow, 1992*a*, *b*; Theurkauf and Hawley, 1992) and *KLP61F* is essential for mitosis (Heck et al., 1993). Fungal members of the *bimC* subfamily of kinesins (which includes *CIN8*, *KIP1*, and *CUT7*) may provide forces pushing spindle poles apart, in opposition to compressive forces generated by *KAR3* and *klpA* (Enos and Morris, 1990; Meluh and Rose, 1990; Hagan and Yanagida, 1992; Hoyt et al., 1992; Roof et al., 1992; Saunders and Hoyt, 1992; O'Connell et al., 1993). Dynein is present at the kinetochore and spindle poles (Pfarr et al., 1990; Steuer et al., 1990; Verde et al., 1991) and is required for mitotic progression (Vaisberg et al., 1993). NuMA is required for spindle integrity and the reformation of the nuclear envelope (Kallajoki et al., 1991; Yang and Snyder, 1992; Compton and Cleveland, 1993) and γ-tubulin may nucleate microtubules at the centrosome (Oakley et al., 1990; Stearns et al., 1991; Zheng et al., 1991). Together, these proteins play critical roles in the organization and function of the mitotic apparatus.

Spindles often differ markedly in morphology and orientation in different types of cells within an organism, but little is known about the products responsible for these specializations. The gene *mei-1* (*meiosis*-1) of *Caenorhabditis elegans* may define a new type of spindle component, one that is specifically required for meiosis in the female germline (Mains et al., 1990*a*,*b*; Clandinin and Mains, 1993). Meiosis fails in the absence of *mei-1* function. In contrast, a domi-

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nant mutation of *mei-1*, which is apparently refractory to normal postmeiotic inactivation, expresses the meiotic function during the subsequent mitotic cleavages, disrupting mitosis. The properties of the dominant allele demonstrate that postmeiotic inhibition of meiotic-specific activities such as *mei-1* is necessary so that the fertilized embryo can support mitosis after meiosis is completed. This highlights a unique problem in spindle morphogenesis faced by the fertilized embryo: the same cytoplasm must support two very different types of division, often within a short time of one another. Parts of the regulatory network that confines *mei-1* activity to the appropriate spindle may include the genes *mei-2*, *mei-26*, and *zyg-9*, mutations of which show complex genetic interactions with each other and with *mei-1* alleles (Mains et al., 1990*a*).

We recently cloned the *mei-1* gene (Clark-Maguire and Mains, 1994). While the *mei-1* sequence is not similar to any known spindle components, it is a member of a recently described family of ATPases with diverse roles, including transcription, membrane function, proteolysis, and cell cycle regulation. In this paper, we describe the immunolocalization of the *mei-1* product (MEI-1) in wild-type and mutant *C. elegans* embryos. MEI-1 is normally a component of the meiotic spindle, but the product of the dominant, mitotic defective mutation ectopically assembles into interphase microtubule-organizing centers (MTOC)[1] and mitotic spindles. In addition, immunolocalization of MEI-1(+) in mutant backgrounds indicates that *mei-2* activity is required for the assembly of MEI-1 into the meiotic spindle and that *mel-26* functions to prevent MEI-1 assembly into mitotic structures. MEI-1 may be specialized for the organization of the acentriolar spindle poles unique to female meiosis.

Materials and Methods

Genes and Alleles

C. elegans (Bristol variety) was cultured under standard conditions (Wood, 1988; Mains et al., 1990*b*). Animals were grown overnight at 25°C before fixation; this is the restrictive temperature for all of the heat-sensitive mutations used. Nonconditional mutations were maintained as balanced heterozygous stocks with appropriate morphological markers in *trans*. Most *mei-1*-, *mei-2*-, and *mel-26*-bearing chromosomes included an *unc-29* mutation in *cis* in order to recognize the appropriate homozygous segregant. Wild-type animals referred to in this work are usually *unc-29* homozygotes. The nomenclature of Horvitz et al. (1979) is employed and the following genes and alleles were used:

Linkage group I: *mei-2(ct98* and *ct102)*, *mei-1(ct46, ct93, b284, ct46ct82, ct46ct100,* and *ct46ct101)*, *mel-26(ct61)*, *daf-8(e1393)*, *unc-29(e193* or *e1072)*.
Linkage group II: *zyg-9(b244)*.
Linkage group III: *glp-1(q231)*.
Linkage group IV: *fem-1(hc17)*, *fem3(q20)*.

A population enriched for animals homozygous for the maternal-effect inviable allele *mei-1(ct46ct101)* was isolated from a strain that included the free duplication *gaDp1*, which covers both *mei-1* and *daf-8*. The recessive *daf-8* mutation results in temperature-sensitive (*ts*) dauer formation at 25°C. A strain of *gaDp1; mei-1(ct46ct101) daf-8* hermaphrodites was grown at 25°C where progeny that lose the duplication form dauers. The dauers, which are also homozygous for *mei-1*, were purified by treatment with SDS, which kills wild-type animals but not dauers (see Wood, 1988). The survivors were shifted to the permissive temperature (15°C) where they emerge from dauer. After growth into gravid adults, animals were washed and resuspended in M9 buffer (Wood, 1988) and flash-frozen in liquid nitrogen.

Staged *C. elegans* populations and animals carrying *ts* mutations affecting germline development (*glp-1*, *fem-1*, and *fem-3*) were from the same frozen preparations used for the RNAase protection assays described in Clark-Maguire and Mains (1994). These *ts* mutations were grown at the restrictive temperature prior to harvesting.

Antisera Production

Recombinant *mei-1* product was produced in *Escherichia coli* using the pT7-7 expression system (Studier et al., 1990). An *Eco*RV fragment of a *mei-1* cDNA, which extended from the tenth amino acid to the end of the coding region, was inserted into the *Sma*I site of pT7-7. This construct encoded the shorter of the two potential *mei-1* isoforms, which differ by the presence of three additional internal amino acids in the longer form. The plasmid was transformed into BL21(DE3) and the synthesis of *mei-1* protein was induced under standard conditions. Large scale preparations of the expressed protein were prepared from whole cell extracts by SDS-PAGE, staining with Coomassie in water and cutting out the appropriate band. Protein was electroeluted, concentrated using a centriprep-30 (Amicon, Beverly, MA), and precipitated with 4 vol of cold acetone (Harlow and Lane, 1988). The pellet was resuspended in PBS. Protein (300 μg in complete Freund's adjuvant) was injected subcutaneously into three male New Zealand White rabbits. Three boosts were done at 3-wk intervals with 300 μg MEI-1 protein in incomplete Freund's adjuvant. Serum was prepared as described in Harlow and Lane (1988). All three rabbits produced antibodies that detected the same bands on Western blots and the described meiotic and mitotic patterns of staining. Preimmune sera did not detect the putative MEI-1 protein on Western blots and did not stain embryos.

A *mei-1* glutathione-S-transferase fusion protein was made by inserting the same *Eco*RV fragment into pGEX-3X (Smith and Johnson, 1988) at the *Eco*RI site, which had been filled in with klenow polymerase. Due to insolubility, the product could not be column purified and so it was gel purified as described above. The product was used to neutralize the anti-MEI-1 sera to demonstrate the specificity of the antibody (Harlow and Lane, 1988) in immunoblots and immunostaining. The pT7-7 and glutathione-S-transferase-MEI-1 fusions ran at apparent molecular masses of 51 and 80 kD, respectively, and so the contaminating *E. coli* proteins in each gel-purified preparation are likely to be different.

Immunoblotting

Staged populations of various strains (described above) were prepared as described in Wood (1988) and flash frozen in liquid nitrogen. Worms were thawed and an equal volume of Laemmli loading buffer without tracking dye was added. Samples were boiled for 10 min and briefly sonicated at 80W. Protein was precipitated in 10 vol of cold acetone, dried, resuspended in Laemmli loading buffer, and subjected to SDS-PAGE. Equal amounts of total protein, as determined by the method of Bradford (1976), were loaded in each lane. The gel was electroblotted onto Immobilon polyvinylidene difluoride (PVDF) (Millipore, Bedford, MA), probed with anti-MEI-1 diluted 1/5,000, and detected using a horseradish peroxidase secondary antibody and a chemiluminescent substrate (ECL Western blotting system, Amersham Corp., Arlington Heights, IL).

Microscopy and Immunofluorescence

Gravid hermaphrodites were placed in a drop of water on a slide freshly coated with polylysine and embryos were extruded by the application of gentle pressure on a cover slip. Samples were then frozen on dry ice, fixed, and stained using the methanol-acetone procedures described by Albertson (1984) as modified by Kemphues et al. (1986). Primary antibodies were a mixture of rabbit anti-MEI-1 at 1/100–200 and a mouse monoclonal anti-α-tubulin at 1/100–400 (Piperno and Fuller, 1985). Secondary antibodies included a mixture of rhodamine-conjugated goat anti-mouse IgG and fluorescein-conjugated goat anti-rabbit IgG (Tago, Burlingame, CA or Jackson Immunoresearch, West Grove, PA) diluted to 1/100–200. The samples were also stained in diamidinophenylindole (DAPI) and mounted in paraphenyldiamine in 90% glycerol. Specimens were viewed with a Zeiss Axioplan microscope equipped with epifluorescence and photographed with Kodak Techpan film exposed at ASA 200–400 and developed at ASA 100. Corresponding views of the same embryo were photographed in the same

1. *Abbreviations used in this paper*: DAPI, diamidinophenylindole; *dn*, dominant-negative; *gf*, gain-of-function; PVDF, polyvinylidene difluoride; MTOC, microtubule-organizing center; *ts*, temperature-sensitive.

focal plane. Males were prepared by cutting animals in half with a scalpel in a drop of M9 buffer to allow extrusion of the gonad and then were processed for immunofluorescence as described above. Living embryos were viewed by Nomarski differential interference microscopy and photographed as described in Clandinin and Mains (1993).

Results

Summary of Genetic Interactions

Genetic evidence suggests that *mei-1* is specifically required for spindle formation during female meiosis and that its activity must be eliminated prior to mitosis. However, the precise role of *mei-1* in spindle formation is not clear; for example, it could be a structural component of the meiotic spindle or it could regulate entry into meiosis. The mutations of *mei-2*, *mel-26*, and *zyg-9* show complex genetic interactions with alleles of *mei-1*. The precise manner in which the products of these genes interact with *mei-1* is unknown; possible roles include positive or negative regulation of *mei-1* activity, or these genes might represent mitotic analogues of *mei-1*. In this paper we address these questions by determining the immunolocalization of the MEI-1 in wild-type and mutant embryos. We will first summarize the genetic and phenotypic properties of these genes.

The embryonic defects and the genetic interactions of *mei-1*, *mei-2*, *mel-26* (Mains et al., 1990*a*,*b*; Clandinin and Mains, 1993), and *zyg-9* (Wood et al., 1980; Albertson, 1984; Kemphues et al., 1986) are summarized in Fig. 1. Mutations of these genes, all of which show strict maternal effects, disrupt either meiosis or mitosis. Meiosis in wild-type embryos results in the formation of two small polar bodies in the anterior of the embryo. The first mitotic cleavage follows within 20 min, with the spindle aligned along the anterior-posterior axis, positioned slightly posterior of center (Nigon et al., 1960; Hirsh et al., 1976; Strome and Wood, 1983; Albertson, 1984; Albertson and Thomson, 1993).

The mitotic-defective mutations, which include *ts*, dominant gain-of-function (*gf*) alleles of *mei-1(ct46)* and *mel-26(ct61)*, and recessive loss-of-function alleles of *zyg-9*, are characterized by a shortened mitotic spindle in the posterior of the embryo, often with a dorsal-ventral orientation (Fig. 1; Mains et al., 1990*a*). A pseudocleavage takes place in the anterior. Cleavage furrows are sometimes incomplete and often result in blastomeres fusing with one another. At a semipermissive temperature, pairwise combinations of *ts* mutations of these genes show a strong enhancement (exacerbation) of the lethal phenotype, implying that the gene products interact. Meiosis is normal in these mutants.

The meiotic-defective mutations were identified as dominant suppressors of *mei-1(ct46gf)* (Mains et al., 1990*a*). These include extragenic mutations of *mei-2* and intragenic revertants of *mei-1* that were induced in *cis* to *ct46* to eliminate the dominant "poison" activity. Recessive phenotypes of these new *mei-1* and *mei-2* alleles include the failure to form meiotic spindles, followed by the generation of either abnormally large polar bodies (as shown in Fig. 1) or no polar bodies at all. The subsequent mitotic cleavages, which include chromosomes from the sperm pronucleus and occasional maternal chromosomes that join with it, proceed with their normal orientation and rhythm. Double heterozygotes [i.e., *mei-2 +/+ mei-1(rec)*] show an enhancement of the otherwise recessive meiotic defects (Clandinin and Mains, 1993).

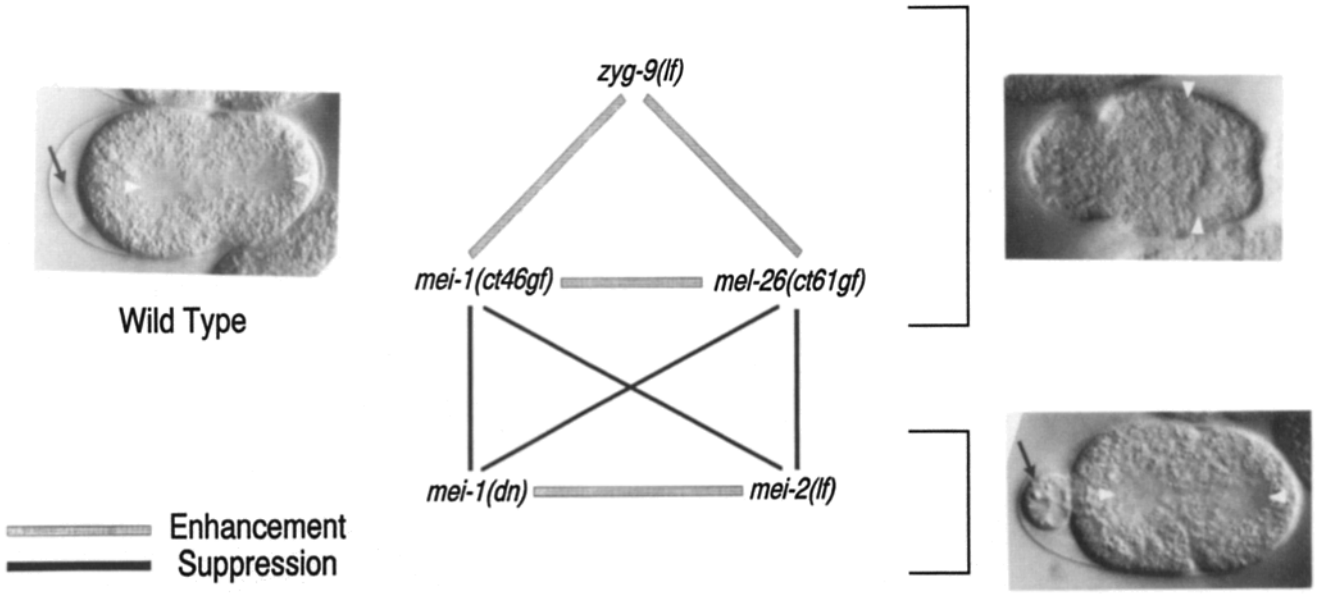

Figure 1. Summary of the phenotypes and genetic interactions of *mei-1* and related genes. The genetic interactions are as indicated and are explained in detail in the text. Nomarski photomicrographs of representative embryos at the first mitotic cleavage displaying evidence of previous meiotic defects [*mei-2 (ct102)*, *lower right*] or undergoing an abnormal mitosis [*mei-1(ct46)*, *upper right*] are shown and a wild-type embryo is included for comparison (*upper left*). The spindles are in the area of clear cytoplasm between the triangles. Arrows indicate polar bodies, that in wild-type is slightly below the focal plane while the polar body in the mitotic-defective embryo is not in focus. Only one polar body is produced by the meiotic-defective embryos and we have not investigated the timing of its formation with respect to normal extrusion of the first or second polar body. Embryos are oriented with anterior to the left.

Two unusual interactions between the mitotic- and meiotic-defective classes are shown in Fig. 1. First, most recessive *mei-1* mutations act in a dominant–negative (*dn*) fashion to suppress (alleviate) the *mei-1(ct46)* defect in *trans*, that is the embryos from *mei-1(ct46)/mei-1(dn)* hermaphrodites show good hatching at the restrictive temperature. These *mei-1(dn)* alleles decrease the amount of *mei-1(ct46)* activity present at both meiosis and mitosis (Clandinin and Mains, 1993). This lowered level is sufficient for meiosis but is not enough to cause mitotic defects. Under appropriate conditions (e.g., +/*dn*/*dn*), it can be shown that these *dn* mutations also inhibit wild-type function in *trans*. True null alleles of *mei-1* do not suppress *ct46* in trans. The second unusual interaction indicated in Fig. 1 is that *mei-1(dn)* and *mei-2* mutations are also dominant suppressors of *mel-26*, even though they were selected as suppressors of *mei-1(ct46)* (Mains et al., 1990*a*).

Generation of MEI-1 Antisera

Rabbit polyclonal antisera were raised against recombinant MEI-1 produced using the pT7-7 expression system as described in Materials and Methods. On Western blots of wild-type gravid hermaphrodites, the antisera detected a doublet at ~59 and 55 kD (Fig. 2, lane *5*). Neither band was present when the serum was preincubated with gel-purified MEI-1 expressed as a glutathione-S-transferase fusion, although other background bands remained (data not shown). *mei-1* encodes two potential isoforms of 51.7 and 52.1 kD (Clark-Maguire and Mains, 1994). Several lines of evidence indicate that the lower 55-kD band represents one or both forms of MEI-1. Previously, we showed that *mei-1* gene function is essential only in the female germline (Mains et al., 1990*a*) and that *mei-1* mRNA is 10-fold more abundant in this tissue

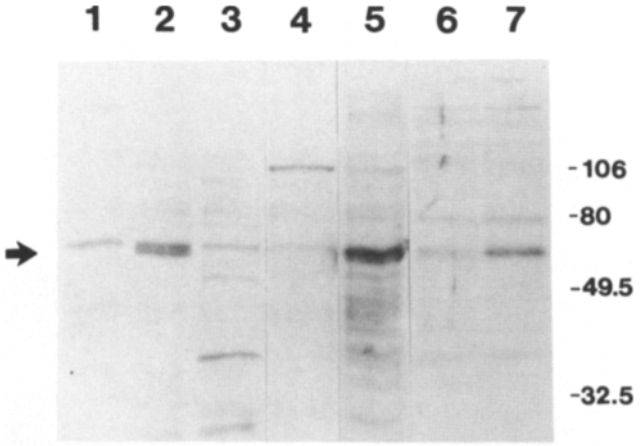

Figure 2. Immunoblots of wild-type and mutant *C. elegans* homogenates probed with anti–MEI-1. Lane *1*: *glp-1(q231)*, which have a few sperm but no mitotic germ cells or oocytes; lane *2*: *fem-1(hc17)*, which have oocytes but no sperm; lane *3*: *fem-3(q20gf)*, which have sperm but no oocytes; lane *4*: *mei-1(ct46ct10l)*, a null (nonsense) mutation; lane *5*: wild-type gravid hermaphrodites; lane *6*: freshly isolated wild-type embryos; lane *7*: wild-type embryos isolated and incubated 3 h before harvest. Equal amounts of total protein were loaded in each lane and the same level of exposure was used for all lanes. The putative MEI-1 band is indicated by the arrow on the left and molecular masses (in kD) are as indicated on the right.

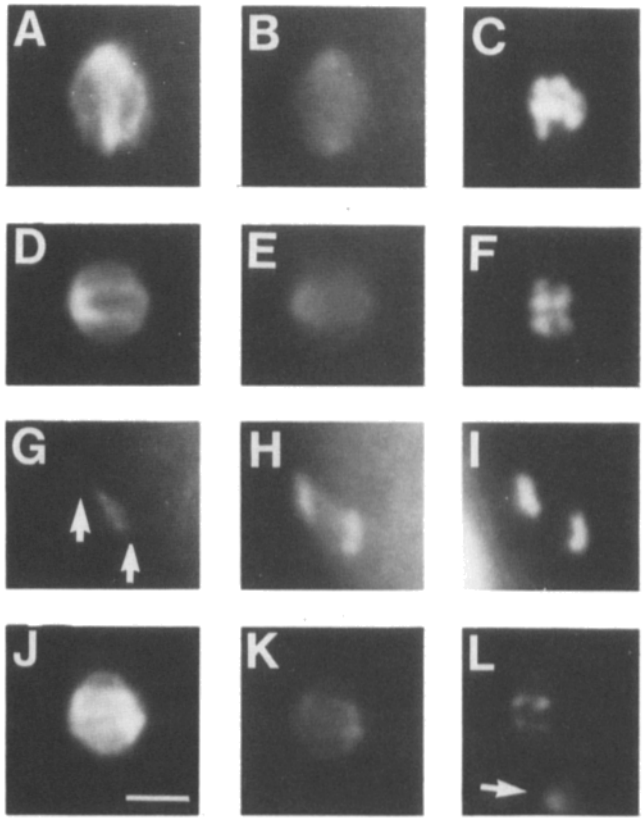

Figure 3. Photomicrographs of meiosis in wild-type embryos. Corresponding indirect immunofluorescence images are of anti-tubulin (*left column*), anti–MEI-1 (*center column*) and DNA stained with DAPI (*right column*). (*A–C*) early meiosis I metaphase, (*D–F*) late meiosis I metaphase, note that the chromosomes have congressed to the metaphase plate and one pole is now juxtaposed to the cortex, which is immediately to the left. (*G–H*) meiosis I telophase. Arrows in *G* show the location of the poles, which stain brightly with anti–MEI-1 and DAPI in *H* and *I*, respectively. (*J–L*) late meiosis II metaphase. Note the chromosomes aligned on the metaphase plate in (*L*) and the first polar body (*arrow*). The anterior of the embryo is to the left in all panels. Bar, 3 μm.

than in the soma (Clark-Maguire and Mains, 1994). Consistent with this, the 55-kD band was absent from adult hermaphrodites lacking oocytes. The mutation *glp-1(q231)* produces about 1% the normal number of mitotic germ cells, all of which differentiate into sperm prior to adulthood (Austin and Kimble, 1987). *fem-3(q20gf)* adult hermaphrodites do have mitotic germ cells and make sperm but lack oocytes (Barton et al., 1987). The 55-kD band was not detected in either *glp-1(q231)* (Fig. 2, lane *1*) or *fem-3(gf)* (lane *3*) animals. However, a mutant that produced oocytes but not sperm (*fem-1(hc17)*; Kimble et al., 1984) did express the 55-kD band (Fig. 2, lane *2*). Furthermore, this band was not detected in animals homozygous for the nonsense allele *mei-1(ct46ct10l)* (Fig. 2, lane *4*), providing additional evidence that this band represents MEI-1. We do not know the origin of the upper cross-reacting band, but *C. elegans* does have several other *mei-1*-related genes (Clark-Maguire and Mains, 1994). It is possible that the 59-kD band includes both the somatic MEI-1 isoform and a cross-reacting protein. The in-

tensity of this band did decrease in the presence of the null allele (Fig. 2, lane *4*) and in animals that lack the female germline (Fig. 2, lanes *1* and *3*). However, this interpretation might be questioned because somatic MEI-1 is the shorter isoform. In addition, the gel system employed is unlikely to resolve the two MEI-1 isoforms, which differ by only three amino acids.

The 55-kD *mei-1* band decreases to undetectable levels during embryogenesis. Fig. 2 shows immunoblots of wild-type gravid hermaphrodites (lane *5*), freshly harvested embryos (lane *6*) and embryos incubated for three additional hours after harvesting (lane *7*). MEI-1 was only apparent in gravid hermaphrodites, the only stage that included meiotic cells (cells in meiosis are destroyed by the alkaline hypochlorite procedure used to isolate embryos). Therefore, MEI-1 apparently disappears some time after meiosis. Since only 21% of the freshly harvested embryos in lane *6* were ≤2 h postfertilization, it is not clear how rapidly MEI-1 protein disappears after meiosis is completed. There must be some translational or posttranslational mechanism to eliminate MEI-1 during embryogenesis since we previously showed by RNAase protection assays that equal amounts of *mei-1* mRNA were present in aliquots of the samples corresponding to lanes *5–7* (Clark-Maguire and Mains, 1994). The need to eliminate MEI-1 during embryogenesis is perhaps consistent with genetic observations showing that ectopic *mei-1* activity is lethal during the early cleavages (Mains et al., 1990*b*).

Immunolocalization of mei-1 During Meiosis

Albertson and Thomson (1993) recently used anti-tubulin immunocytochemistry to assess the pattern of microtubule localization in the meiotic spindles of *C. elegans*. We found that the distributions of MEI-1 and tubulin staining were similar but not identical. Embryos were examined by indirect immunofluorescence after double staining with anti–MEI-1 and anti-α tubulin. During early meiosis I metaphase, MEI-1 was found throughout the spindle but was more concentrated at the poles (Fig. 3, *A–C*). Bright points were often present at the apices of the spindles. In comparison, anti-tubulin staining was more uniformly distributed throughout the spindle. Prior to anaphase, the meiotic spindle rotates so that one pole is adjacent to the anterior cortex and the spindle shortens into a barrel-shaped structure (Fig. 3, *D–F*). At telophase, MEI-1 staining was primarily found with the chromatin at the spindle poles, while tubulin staining remained in the region between the poles (Fig. 3, *G–I*). The second meiotic division showed the same pattern of MEI-1 and tubulin staining (Fig. 3, *J–L*).

We examined anti–MEI-1 staining in other than female meiotic structures. None was visible during meiosis in males (Fig. 4, *A–C*), which is consistent with genetic evidence

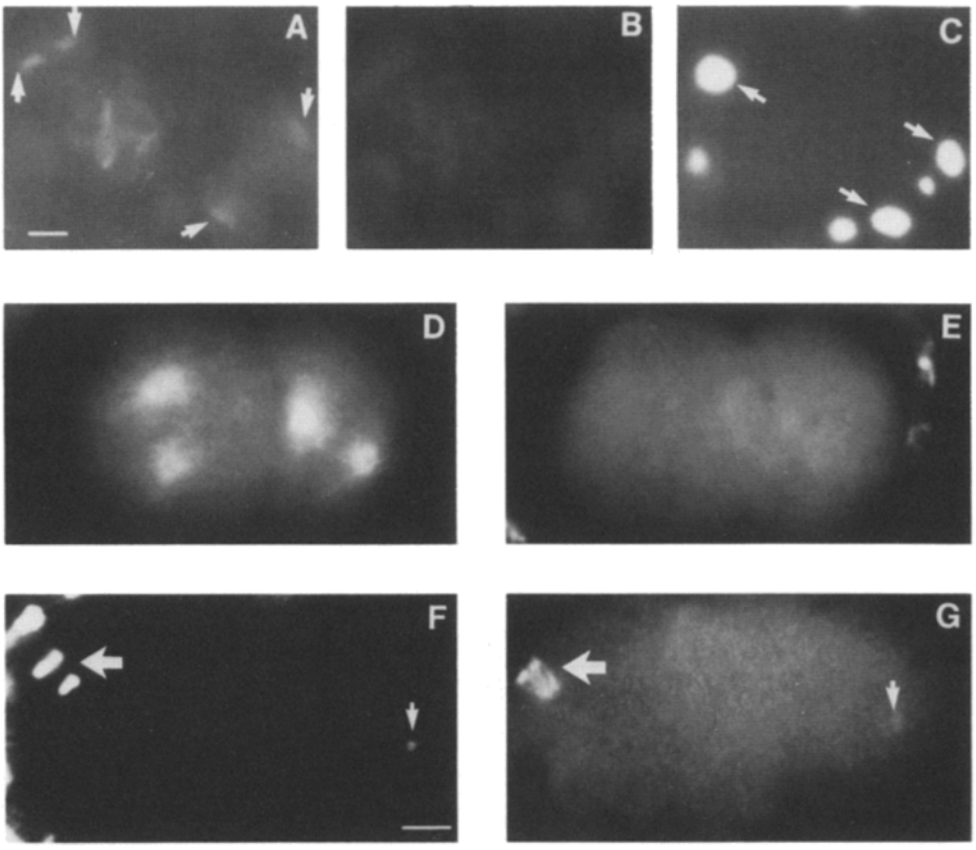

Figure 4. MEI-1 staining in wild-type males and postmeiotic embryos. (*A–C*) Meiosis in the germline of males. Arrows in *A* show poles of two meiotic figures stained with anti-tubulin. No anti–MEI-1 staining is visible in *B*. DAPI staining in *C* shows that the chromosomes in the cell in the upper left are at the metaphase plate (*arrow*, chromosomes are seen as one brightly staining mass). The chromosomes in the cell at the lower right are at the poles (*arrows*). Note the chromosome lagging at the metaphase plate in the latter cell; this is probably the unpaired X chromosome in the X0 male (see Albertson and Thomson, 1993). (*D* and *E*) Two cell embryo entering the third round of cleavage. Spindles are seen forming in *D* with anti-tubulin staining while no anti–MEI-1 staining is visible in this embryo (*E*). (*F* and *G*) Staining of the sperm nucleus. DAPI and anti–MEI-1 staining, indicated by the large arrows in *F* and *G*, respectively, shows that the embryo is in telophase of meiosis I (compare to Fig. 3, *H* and *I*). The sperm nucleus (*small arrows*) is stained in both panels. Note other regions stained by DAPI in *F* are not visible in *G*, indicating that the anti–MEI-1 staining of the sperm nucleus is not spill-over from the DAPI channel. Anterior is to the left in (*D–G*). Bars: (*A*) 3 μm; (*F*) 5 μm.

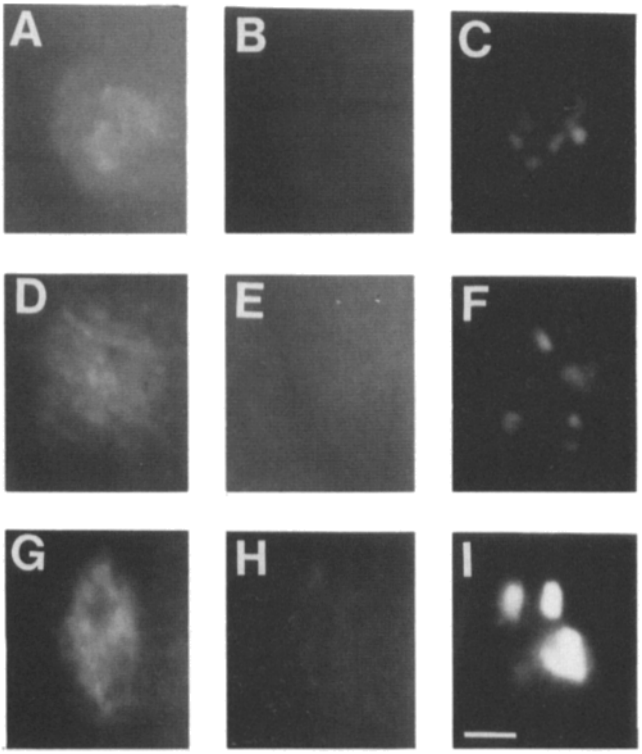

Figure 5. Anti-MEI-1 and anti-tubulin staining in meiotic-defective embryos. The first column represents anti-tubulin, the middle column is anti-MEI-1 and the right column is DNA stained with DAPI. All embryos are from hermaphrodites homozygous for each mutation. (*A–C*) *mei-1(ct46ct101)*, a null (nonsense) allele; (*D–F*) *mei-2(ct102)*, a strong allele; (*G–I*) *mei-2(ct98)*, a weak allele. A small amount of MEI-1 staining may be present in *H*. Anterior is to the left. Bar, 3 μm.

showing that neither dominant nor recessive *mei-1* mutations affect male fertility (Mains et al., 1990*a*). Also consistent with the genetic analysis was the absence of anti-MEI-1 staining during mitotic cleavages in the embryo (Fig. 4, *D* and *E*). The only anti-MEI-1 staining that we found other than in female meiotic spindles was in the polar bodies (not shown) and in the sperm nucleus during meiosis (Fig. 4, *F* and *G*). This may indicate that MEI-1 present in the embryo binds chromatin. As mentioned above, MEI-1 also colocalizes with chromatin at meiotic telophase. The embryo shown in Fig. 4, *F* and *G*, shows staining of both meiotic telophase chromosome and the sperm nucleus.

All forms of MEI-1 staining were abolished by preincubation of MEI-1 antiserum with the glutathione-S-transferase–MEI-1 fusion, although staining with anti-tubulin was not affected (not shown).

Embryos from animals homozygous for meiotic-defective mutations were stained with anti-tubulin and anti–MEI-1. Included were *mei-1(null)* mutations (*ct46ct99* and *ct46ct101*) and *mei-1(dn)* alleles (*b284*, *ct46ct82*, *ct46ct100*, and *ct93*). As described in an earlier report (Mains et al., 1990*a*), meiotic spindles did not form in these mutants, but instead an amorphous cloud of anti-tubulin staining surrounded the maternal chromosomes (Fig. 5, *A–C*). No MEI-1 staining was apparent. Heterozygotes appeared normal. The meiotic and mitotic phenotypes and the MEI-1 staining pattern of these mutations, and those described later, are summarized in Table I.

The Mutation mei-1(ct46) Results in Ectopic Assembly into Mitotic Structures

Genetic evidence suggests that the dominant allele *mei-1(ct46)* results in the ectopic expression of otherwise normal meiotic *mei-1* activity during mitosis (Clandinin and Mains, 1993). Immunocytochemistry of embryos from *mei-1(ct46)* and *mei-1(ct46)/+* hermaphrodites using the anti–MEI-1 sera showed that this is indeed the case. The pattern of MEI-1 staining during meiosis was indistinguishable from wild type (not shown), but staining was present thereafter. Ectopic MEI-1 localization was first observed in the asters adjacent to the sperm pronucleus (Fig. 6, *A* and *B*; Table I; centrioles are contributed by the sperm in *C. elegans* [Albertson, 1984]). Anti–MEI-1 weakly stained microtubules that radiated from the asters, but a much stronger signal was apparent in the microtubule-free centers of the structures. During the first mitotic cleavage, MEI-1 staining was present throughout the spindle, showing a pattern similar to that of tubulin (Fig. 6, *C* and *D*). During subsequent cell cycles, ectopic MEI-1 was seen in the centers of the interphase MTOCs and throughout the mitotic spindles; this pattern continued until about the beginning of gastrulation (2 h after fertilization when there are 28 cells). Treatment of *mei-1(ct46)* embryos

Table I. Summary of Anti–MEI-1 Immunocytochemistry

	Meiosis		Mitosis	
Maternal genotype	Spindle formation*	MEI-1 staining	Spindle formation*	MEI-1 staining
Wild type	Normal	+	Normal	−
mei-1(null or *dn)*	Abnormal	−	Normal	−
mei-1(null or *dn)/+*	Normal	+	Normal	−
mei-1(ct46) or *mei-1(ct46)/+*	Normal	+	Abnormal	+
mel-26(ct61) or *mel-26(ct61)/+*	Normal	+	Abnormal	+
mei-2(ct102)	Abnormal	−	Normal	−
mei-2(ct98)	Slightly abnormal‡	±	Normal	−
mei-2(ct102) +/+ mei-1(ct46)	Normal	+	Normal	−
zyg-9	Normal	+	Abnormal	−

* Judged by anti-tubulin staining and Nomarski microscopy.
‡ Spindles differed from wild-type, but most were functional (see text).

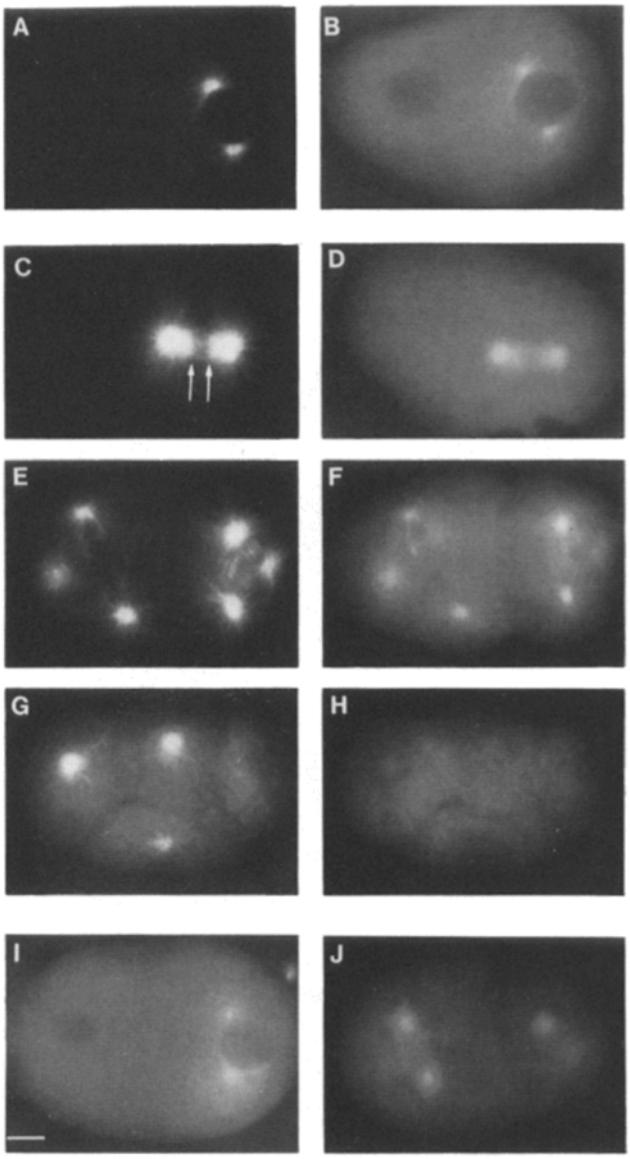

Figure 6. Anti-MEI-1 and anti-tubulin staining in mitotic-defective embryos. For *A–H*, the left column is anti-tubulin and the right column is anti-MEI-1. (*A* and *B*) Pronuclear migration in embryos from *mei-1(ct46)/+*. The maternal pronucleus is to the left (anterior) and the sperm pronucleus, with its accompanying asters, is to the right. Note the anti-tubulin-stained regions in *A* have dark centers while the anti-MEI-1-stained structures have bright centers in *B*. (*C* and *D*) First mitotic cleavage *mei-1(ct46)/+*. The spindle is aligned along the anterior-posterior axis but is smaller and more posterior than in wild type and ectopic MEI-1 staining is present. DAPI staining (not shown) indicated that this cell was in anaphase with the chromosomes positioned as indicated by the arrows in *C*. MEI-1 can be seen colocalizing with both kinetochore to pole and pole to pole microtubules. (*E* and *F*) Early cleavage in embryos from *mel-26(ct61)*. Ectopic anti-MEI-1 staining is apparent. (*G* and *H*) Phenotypically normal four cell embryo from a + *mei-1(ct46)/mei-2(ct102)* + hermaphrodite. Ectopic anti-MEI-1 staining that would otherwise result from the *ct46* mutation is not visible in the centers of the MTOCs. Central spindle and astral microtubules do not show well in *A*, *C*, *E*, and *G* because printing the photographs to better show these structures would have overexposed the MTOCs, whose dark centers we wished to emphasize. Ectopic anti-MEI-1 staining in *B*, *D*, and *F* does not result from spill-over of the rhodamine, which was used to detect tubulin, into the fluorescein channel, which was used to detect MEI-1. The anti-tubulin primary and rhodamine-conjugated secondary were not included in *I* and *J*, but anti-MEI-1 staining is visible in the asters adjacent to the sperm pronucleus (*I*) and in the second cleavage spindles (*J*) of embryos from *mei-1(ct46)* hermaphrodites. Note that the posterior blastomere in *J* is smaller than normal (compare to Fig. 4 *D*) and that one spindle pole is out of the focal plane. Anterior is to the left. Bar, 5 μm.

with nocodazole blocked formation of meiotic spindles, mitotic spindles, and interphase microtubule arrays. No anti-MEI-1 staining remained (not shown), indicating that accumulation of visible MEI-1 depended on microtubule-based structures.

The anti-MEI-1 staining did not result from spill-over from the rhodamine channel, which was used to visualize the anti-tubulin antibody, into the fluorescein channel, which was used to detect anti-MEI-1. When the fluorochromes used with the secondary antibodies were reversed, the staining patterns were not altered (data not shown). In addition, when the primary and secondary antisera used to detect tubulin were not included, the MEI-1 staining pattern was not altered (Fig. 6, *I* and *J*).

mel-26 Behaves as a Postmeiotic Inhibitor of MEI-1

The mutations *mel-26(ct61)* and *mei-1(ct46)* result in similar dominant mitotic defects and the mutations enhance one another's phenotypes (Fig. 1), suggesting that the two genes lie in the same genetic pathway. Immunolocalization of MEI-1(+) in *mel-26(ct61)* and *mel-26(ct61)/+* backgrounds supports this idea: the interphase MTOCs and mitotic spindles of embryos from these hermaphrodites showed ectopic MEI-1 staining identical to that of *mei-1(ct46)* (Fig. 6, *E* and *F*; Table I).

This pattern of anti-MEI-1 staining in a *mel-26* mutant suggests that *mel-26*(+) may function to limit MEI-1 expression to meiosis, preventing MEI-1 from localizing to MTOCs and spindles. If this is the case, then the *mei-1(null)* meiotic-specific phenotype should be epistatic to the mitotic defects of *mel-26*. That is, if there is no MEI-1 for *mel-26* to inhibit, then the mitotic defects of *mel-26* should be eliminated. This is indeed the case: *mei-1(ct46ct101) mel-26(ct61)* double mutants produced embryos that underwent an aberrant meiosis (as indicated by abnormally large polar bodies) followed by normal mitotic cleavages (Fig. 7). (The *mel-26* allele *ct61* is a *gf* mutation, which could confuse the interpretation of *mel-26*(+) activity. However, genetic results [Mains et al., 1990*b*] are consistent with *mel-26(ct61)* antagonizing wild-type *mel-26* activity in a dominant-negative manner, and so the observed *ct61* phenotype likely resembles the loss of *mel-26* function. In addition, we have isolated a putative *cis*-linked loss-of-function revertant, *ct61sb4* [T. R. Clandinin, unpublished results], which showed the same pattern of anti-MEI-1 staining as did *ct61* [data not shown].)

mei-2 Is Required for MEI-1 Assembly into the Meiotic Spindle

The genetic evidence summarized in Fig. 1 suggests that *mei-2* is also in the same pathway as *mei-1*. Double heterozygotes for recessive mutations in both genes [i.e., *mei-2* +/+

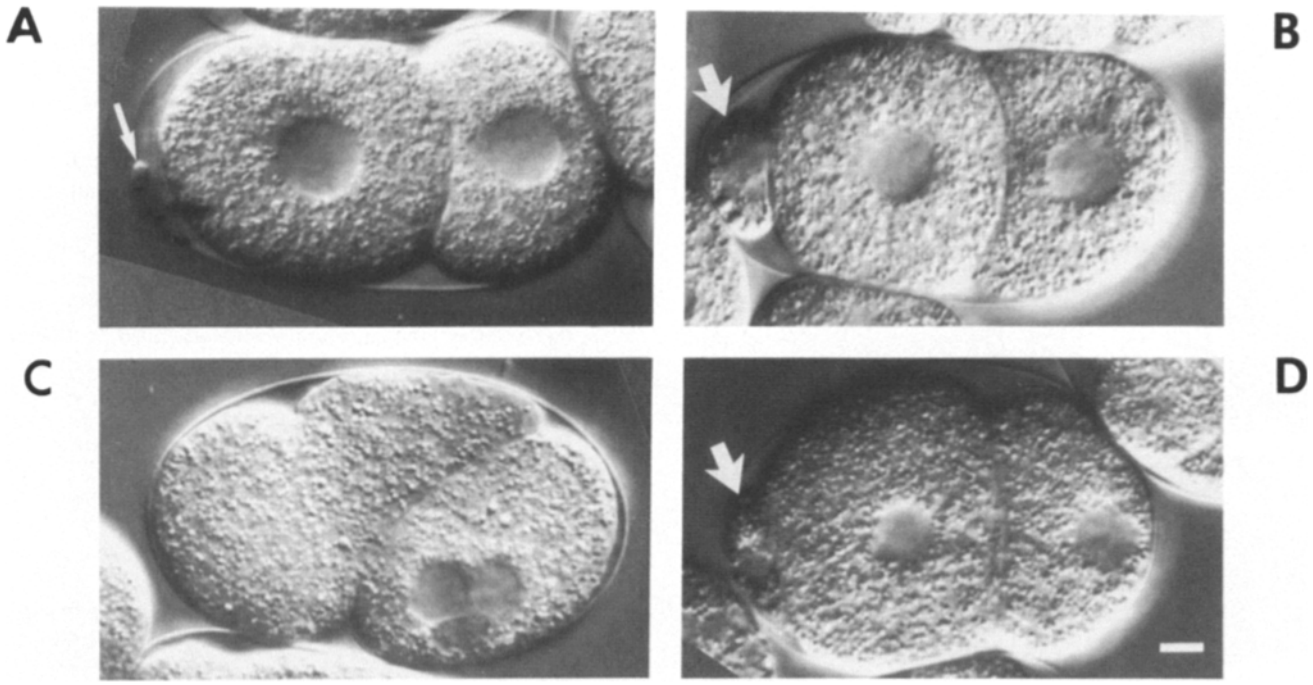

Figure 7. Epistasis between *mei-1(null)* and *mel-26(ct61)*. Nomarski photomicrographs of living embryos at the two cell stage are oriented with anterior to the left. (*A*) Wild type, arrow, polar body. (*B*) Embryo from *mei-1(ct46ct101)*, a null (nonsense) mutation. The embryo appears normal except for the large polar body indicated by the arrow. (*C*) Embryo from *mel-26(ct61)*. The cleavage furrow is incomplete and is displaced toward the anterior-posterior axis and an anterior cytoplast is forming. Mitosis has failed and multiple nuclei are present in one cell. (*D*) Embryo from a *mei-1(ct46ct101) mel-26(ct61)* double mutant with an enlarged polar body indicated by the arrow. The embryo resembles that in *B* rather than *C*. Anterior is to the left. Bar, 10 μm.

mei-1(dn)] enhance one another's meiotic defects and single heterozygotes of each can suppress the mitotic phenotypes of *mei-1(ct46)* and *mel-26(ct61)*. There are two alleles of *mei-2*. *mei-2(ct102)* causes complete recessive maternal-effect lethality. The anti-tubulin and anti–MEI-1 staining patterns in embryos from homozygous *mei-2(ct102)* hermaphrodites were similar to those characteristic of the homozygous *mei-1(null)* mutants: no MEI-1 product was apparent within an indistinct cloud of anti-tubulin staining (Fig. 5, *D–F*; Table I). However, the absence of MEI-1 staining in a *mei-2(ct102)* background might simply result from the lack of an organized spindle in which MEI-1 can assemble, rather than indicating an interaction between the two gene products.

The second *mei-2* allele, *ct98*, may be more informative since it only partially limits *mei-2* activity. Even though three-quarters of the embryos from homozygous *mei-2(ct98)* mothers hatch, 80% of the viable embryos have abnormal polar bodies (Mains et al., 1990*a*). Thus, *mei-2* activity in *ct98* hermaphrodites is probably barely adequate. Anti-tubulin staining revealed meiotic spindles that were more diffuse than in wild type, but functional spindles into which MEI-1 could potentially assemble were clearly present. Nevertheless, little or no MEI-1 was visible in these spindles (Fig. 5, *G–I*; Table I). It thus appears that limited *mei-2* activity in turn limited MEI-1 recruitment to the meiotic spindle. Since genetic evidence shows that *mei-1* is essential for meiosis, it is probable that small amounts of MEI-1 were nevertheless present in the *mei-2(ct98)* spindles.

The interpretation that *mei-2* is necessary for localization of MEI-1 to spindles is strengthened by examining the interaction between *mei-2* and *mei-1(ct46)*. *mei-2* mutations act as dominant suppressors of *mei-1(ct46)*; over 75% of embryos from *mei-2(ct102) +/+ mei-1(ct46)* hermaphrodites hatch (Mains et al., 1990*a*). Little or no ectopic MEI-1 mitotic staining was visible (Fig. 6, *G* and *H*; Table I), implying that the allele *mei-2(ct102)* can prevent MEI-1 assembly into mitotic structures.

A third genetically interacting locus that results in mitotic defects, *zyg-9*, did not alter the pattern of meiotic or mitotic MEI-1 localization (Fig. 1; Table I).

Discussion

The newly fertilized *C. elegans* embryo must support two different modes of cell division, meiosis and mitosis. Prior to fertilization, the oocyte arrests at diakinesis of the first meiotic division. Upon sperm entry, the embryo rapidly completes the two meiotic divisions and the first mitotic cleavage follows within 20 min (Nigon et al., 1960; Hirsh et al., 1976; Strome and Wood, 1983; Albertson, 1984; Albertson and Thomson, 1993). The meiotic and mitotic spindles differ in a number of respects, including their morphologies and positions within the cell, the pairing of homologous chromosomes, and the presence of centrioles in mitosis but not meiosis. The activities of gene products responsible for these differences must be carefully regulated to ensure that they function only in the appropriate spindle. The immunolocalization results reported here, in combination with the previous genetic characterization, show that *mei-1* encodes one such specialized spindle component, required for

meiosis but not mitosis. *mei-2* is necessary for MEI-1 assembly into the meiotic spindle while *mel-26* inhibits MEI-1 function or promotes its inactivation after the completion of meiosis (Fig. 8). As outlined below, this simple model is consistent with the complex genetic interactions previously described among these genes (Fig. 1; Mains et al., 1990*a*; Clandinin and Mains, 1993).

Genetic evidence shows that MEI-1 is essential for meiotic spindle formation. Consistent with this, MEI-1 is present in the meiotic spindle, concentrated in the polar regions (Fig. 3). In the absence of MEI-1 function, anti-tubulin staining detects a diffuse cloud that does not coalesce into an organized spindle (Fig. 5; Table I). Albertson and Thomson (1993) recently reported that in wild-type *C. elegans* anti-tubulin staining initially detects an amorphous cloud around the meiotic chromosomes, after which the structure elongates into a bipolar spindle. A similar pattern is seen in early meiosis of other organisms (Schatten et al., 1985; Sawada and Schatten, 1988; Gard, 1992; Theurkauf and Hawley, 1992). Perhaps MEI-1 is involved in an early step of meiotic spindle organization, during the formation of the spindle poles.

The immunolocalization results confirm the genetic interpretation that the *gf* mutation *mei-1(ct46)* results in the persistence of MEI-1 activity after meiosis (Mains et al., 1990*a*). The earliest defect observed in embryos from *mei-1(ct46)* hermaphrodites is the frequent failure of the centrosomes to rotate after the maternal and paternal pronuclei meet. The centrosomes, which accompany the sperm nucleus, often remain parallel to the dorsal-ventral axis rather than migrating to an anterior-posterior alignment (Hyman and White, 1987; Hyman, 1989). The absence of these microtubule-mediated movements leads to the misalignment of the first cleavage. These defects are likely caused by the ectopic association of MEI-1 with the MTOCs (Fig. 6). In addition, the presence of *mei-1(ct46)* product in the mitotic spindles could result in their being shorter than normal. Inhibitors of microtubule polymerization phenocopy these centrosome and spindle defects (Strome and Wood, 1983; Hird and White, 1993), perhaps indicating that MEI-1 interferes, directly or indirectly, with microtubule function or polymerization.

Other components required for the formation of the female meiotic spindles have been described in *Drosophila*, and these include the kinesin-like genes *nod* and *ncd* (Hatsumi and Endow, 1992*a*,*b*; Theurkauf and Hawley, 1992) and an α-tubulin isotype (Matthews et al., 1993). However, these gene products are also normally active during the early cleavages, and so *mei-1* is unique because its product must be inactivated after meiosis. Immunolocalization and genetic evidence suggest that *mel-26* is required for the prevention of postmeiotic MEI-1 assembly into microtubule-based structures. Mutations of *mel-26* result in the same pattern of postmeiotic MEI-1 mis-localization as seen for *mei-1(ct46)* (Fig. 6). Furthermore, epistasis experiments indicate that *mel-26* is not required in the absence of functional MEI-1 (Fig. 7). The pathway in Fig. 8 is consistent with the genetic interactions between the two genes outlined in Fig. 1. Mutual enhancement of similar mitotic defects occurs because both *mei-1(ct46)* and *mel-26* contribute to ectopic MEI-1 expression. In addition, *mei-1(dn)*/+ results in less MEI-1 activity for *mel-26* to inactivate, and so *mei-1(dn)* acts as a dominant suppressor of *mel-26*.

Mutations in *mei-2* cause recessive meiotic phenotypes similar to *mei-1* and result in embryos that lack detectable MEI-1(+) product in their meiotic spindles (Fig. 5). This leads to the model that *mei-2*(+) activates MEI-1(+) in some manner, enabling MEI-1 to assemble into the meiotic spindle (Fig. 8). This proposal is strengthened because it also explains the genetic interactions outlined in Fig. 1. Decreasing *mei-2* activity in *mei-2*/+ limits the amount of active MEI-1 and so suppresses excess *mei-1(ct46)* mitotic expression. Sufficient MEI-1 activity would remain for meiosis, but there would not be enough to disrupt mitosis. The limitation of MEI-1(+) activation by *mei-2*/+ would also lead to the suppression of *mel-26* mutations. Finally, *mei-2*/+ would further limit the decreased MEI-1 activity in *mei-1(dn)*/+, resulting in the observed enhancement of meiotic defects in double heterozygotes. Implicit in these arguments is that a considerable reduction (but not elimination) of the wild-type level of *mei-1* activity during meiosis is compatible with embryonic viability. This assumption is based on previous genetic observations (Clandinin and Mains, 1993).

The model presented in Fig. 8 is the simplest formal description of our observations, but the situation could be more complex. For example, Fig. 8 places *mei-2* upstream of *mei-1*, but it is certainly possible that the two genes work at the same step of the pathway. MEI-1 and MEI-2 could form multimers or MEI-2 could act as a bridge between MEI-1 and other spindle components. Referring to one as the activator of the other would be somewhat of a misnomer. More complicated models could place *mei-1* upstream of *mei-2*. For example, *mei-1(ct46)* could cause overactivation of *mei-2*, leading to ectopic *mei-2* expression in mitosis. However, this does not necessarily predict the ectopic localization of MEI-1 in mitotic structures by *mei-1(ct46)* and *mel-26* mutations. Finally, *mel-26* may inhibit *mei-1* function indirectly, for example through *mei-2* or some as yet unidentified gene. Further molecular analysis on *mel-26* and *mei-2* is needed to resolve these issues.

What could be the biochemical function of *mei-1*? *mei-1* is a member of a recently described family of ATPases (Clark-Maguire and Mains, 1994), but rather diverse cellular roles have been ascribed to different family members. These functions involve membrane activities, transcription, proteolysis and cell cycle regulation. Of the related genes, the most in-

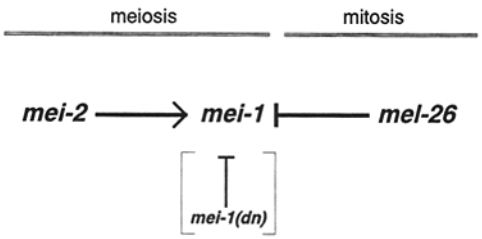

Figure 8. Proposed pathway for the regulation of mei-1 activity. The times of meiosis and mitosis are indicated at the top. *mei-2* has a positive effect on *mei-1* activity while *mel-26* has a negative effect. The wild-type activities are indicated for each gene, except for *mei-1(dn)* (in brackets), which antagonizes *mei-1*(+) or *mei-1(gf)* activities. In this scheme, the *gf* mutation *mei-1(ct46)* would not be sensitive to negative regulation by *mel-26*.

triguing phenotypic similarities are with the *CIM3* and *CIM5* genes of *Saccharomyces cerevisiae* and the *mts2* gene of *Schizosaccharomyces pombe* (Ghislain et al., 1993; Gordon et al., 1993). These encode protease subunits that are required for progression through mitosis. However, their similarity to *mei-1* might be superficial, since mutations of these fungal genes arrest cell cycle at anaphase while the *mei-1* block is at early metaphase. Perhaps the phenotype of another related gene, *CDC48* of *S. cerevisiae* (Fröhlich et al., 1991), is more similar to that of *mei-1* since mutations of this gene also block cell division in early metaphase, prior to spindle pole body separation. A note of caution in these comparisons is that of over a dozen genes that show significant sequence similarity to *mei-1*, none is more closely related to *mei-1* than any other (30–40% identity and 50–60% similarity over 200 amino acids). It is therefore difficult to draw direct inferences as to the function of *mei-1* from the sequence comparisons. Perhaps a general functional theme that could be common to many members of the family is an ATP-dependent modulation of protein-protein interactions (for example see Rothman and Orci, 1992; Pleasure et al., 1993).

The immunolocalization and the mutant phenotypes of *mei-1* might suggest an association with the microtubule-based motor proteins that are required for spindle organization. Binding to a minus-end directed motor would transport MEI-1 to the meiotic spindle poles and to the centers of the interphase MTOCs, explaining the concentration of MEI-1 to these areas. Like *mei-1*, interfering with the function of several spindle-associated kinesin-like proteins (*ncd*, *nod*, and Eg5) by mutation or by antibody inhibition disrupts spindle organization, resulting in frayed and unfocused spindles (Hatsumi and Endow, 1992*a*,*b*; Sawin et al., 1992*a*,*b*; Theurkauf and Hawley, 1992; Wright et al., 1993; see Sawin and Endow, 1993; Goldstein, 1993 for recent reviews). Members of the *bimC* family of kinesin-like genes are required for spindle formation in fungi (Enos and Morris, 1990; Hagan and Yanagida, 1992; Hoyt et al., 1992; Roof et al., 1992; Saunders and Hoyt, 1992). Mutations of these genes block cell cycle progression at the point of spindle-pole body separation, similar to the *mei-1* related gene, *CDC48*, which was mentioned above (Fröhlich et al., 1991). Another motor protein with which *mei-1* could associate is dynein, which is present at the spindle poles (Pfarr et al., 1990; Steuer et al., 1990; Vaisberg et al., 1993) and is likely required for their organization (Verde et al., 1991). The affinity of MEI-1 for chromatin might be related to the presence of dynein on kinetochores.

mei-1 shows no sequence similarity to any of the described subunits of either kinesin or dynein. Nonetheless, MEI-1 might associate with these spindle components. Alternatively, MEI-1 product might bind other products found at the spindle poles, such as centractin (Clark and Meyer, 1992), γ-tubulin (Oakley et al., 1990; Stearns et al., 1991; Zheng et al., 1991), or NuMA (Kallajoki et al., 1992; Yang and Snyder, 1992; Compton and Cleveland, 1993). Further molecular work should clarify any relationships. It is clear that *mei-1*, *mei-2*, and *mel-26* participate in a pathway of spindle formation specifically required for female meiosis, where they may specify unique properties of the meiotic spindle. An intriguing possibility is that the *mei-1* pathway is involved in organizing acentriolar spindle poles, which are only found during female meiosis.

We would like to thank T. R. Clandinin, M. R. Dow, C. H. Hsieh, A. Wissmann, and J. D. McGhee and members of his laboratory for discussion and technical advice. J. B. Rattner is thanked for help in the interpretation of the stained embryos and comments on the manuscript. We also thank K. Kemphues and L. Edgar for immunocytochemistry advice and anonymous reviewers for suggestions on the manuscript.

This work was supported by grants from the Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources.

Received for publication 12 January 1994 and in revised form 28 March 1994.

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