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

Shawna Clark-Maguire and Paul E. Mains
Department of Medical Biochemistry, University of Calgary, Calgary, Alberta T2N 4N1, Canada

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). 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., 1992a,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, 1992a, 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 kip4 (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., 1990a,b; Clandinin and Mains, 1993). Meiosis fails in the absence of mei-1 function. In contrast, a domi-
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., 1990a).

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) 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 mei-26 functions to prevent MEI-1 assembly into mitotic structures. MEI-1 may be specialized for the organization of theacentriolar 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., 1990b). 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 mei-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 homoygotes. The nomenclature of Horvitz et al. (1979) is employed and the following genes and alleles were used:

- Linkage group I: mei-1(ct98 and cs102), mei-1(c466, c93, b284, c466ct82, c466ct100, and c466ct101), mei-26(ct26), daf-8(e1393), unc-29(ct93 or ct072).
- Linkage group II: zyg-9(g244).
- Linkage group III: glp-1(q231).
- Linkage group IV: fem-1(hcl7), fem3(q20).

A population enriched for animals homozygous for the maternal-effect inviable allele mei-1(ct466ct101) was isolated from a strain that included the free duplication gapDp, 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 gapDp, mei-1(ct466ct101) 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 survivals were shifted to the permissive temperature (15°C) where they emerge from dauers. 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 (gap-1, fem-1, and fem-3) were from the same frozen preparations used for the RNAse 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 EcolR fragment of a mei-1 cDNA, which extended from the tenth amino acid to the end of the coding region, was inserted into the NdeI site of pT7-7. This construct is 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 electrophoresed, concentrated using a centrprep-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 EcolR fragment into pGEX-3X (Smith and Johnson, 1988) at the EcoRI 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 L-498. Meioadult loading buffer without shrinking 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 polyvinyldiene difluoride (PVDF) (Millipore, Bedford, MA, probed with anti-MEI-1 diluted 1/5000, 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 paraformaldehyde in 90% glycerol. Specimens were viewed with a Zeiss Axioplan microscope equipped with epifluorescence and photographed with Kodak Technic film exposed at ASA 200-400 and developed at ASA 100. Corresponding views of the same embryos were photographed in the same

1. Abbreviations used in this paper: DAPI, diamidino-phenylindole; 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., 1990a,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., 1990a). 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., 1990a). 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., 1990a).

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., 1990a) and that mei-1 mRNA is 10-fold more abundant in this tissue 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(q20gf) (lane 3) animals. However, a mutant that produced oocytes but not sperm (fem-1(hcl7); 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(ct46ct101) (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., 1990b).

**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 5. Anti-ME1-1 and anti-tubulin staining in meiotic-defective embryos. The first column represents anti-tubulin, the middle column is anti-ME1-1 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(ctl02), a strong allele; (G-I) mei-2(ct98), a weak allele. A small amount of ME1-1 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., 1990a). Also consistent with the genetic analysis was the absence of anti-ME1-1 staining during mitotic cleavages in the embryo (Fig. 4, D and E). The only anti-ME1-1 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 ME1-1 present in the embryo binds chromatin. As mentioned above, ME1-1 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 ME1-1 staining were abolished by preincubation of ME1-1 antiserum with the glutathione-S-transferase-ME1-1 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-ME1-1. 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., 1990a), 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 ME1-1 staining was apparent. Heterozygotes appeared normal. The meiotic and mitotic phenotypes and the ME1-1 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-ME1-1 sera showed that this is indeed the case. The pattern of ME1-1 staining during meiosis was indistinguishable from wild type (not shown), but staining was present thereafter. Ectopic ME1-1 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-ME1-1 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, ME1-1 staining was present throughout the spindle, showing a pattern similar to that of tubulin (Fig. 6, C and D). During subsequent cell cycles, ectopic ME1-1 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-ME1-1 Immunocytochemistry

| Maternal genotype | Meiosis | Mitosis |
|-------------------|---------|---------|
|                   | Spindle formation* | ME1-1 staining | Spindle formation* | ME1-1 staining |
| Wild type         | Normal   | +       | Normal   | -       |
| mei-1(null or dn) | Abnormal  | -       | Abnormal  | -       |
| mei-1(null or dn)+| Normal   | +       | Normal   | -       |
| mei-1(ct46) or mei-1(ct46)+ | Normal | +       | Abnormal  | +       |
| mei-2(ct102)      | Normal   | +       | Normal   | -       |
| mei-2(ct98)       | Abnormal  | -       | Normal   | -       |
| mei-2(ct102) +/+ mei-1(ct46) | Slightly abnormal† | ±       | Normal   | -       |
| zyg-9             | Normal   | +       | Normal   | -       |

* Judged by anti-tubulin staining and Nomarski microscopy.
† Spindles differed from wild-type, but most were functional (see text).
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., 1990b] 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, *ct61b4* [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 +/+ 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 (F) 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.
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 mei-2(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 mei-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., 1990a). 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., 1990a). 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 mei-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., 1990a; 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-l(ct46) results in the persistence of MEI-1 activity after meiosis (Mains et al., 1990a). The earliest defect observed in embryos from mei-l(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-l(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, 1992a,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-l is unique because its product must be inactivated after meiosis. Immunolocalization and genetic evidence suggest that mei-26 is required for the prevention of postmeiotic MEI-1 assembly into microtubule-based structures. Mutations of mei-26 result in the same pattern of postmeiotic MEI-1 mis-localization as seen for mei-l(ct46) (Fig. 6). Furthermore, epistasis experiments indicate that mei-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-l(ct46) and mei-26 contribute to ectopic MEI-1 expression. In addition, mei-l(dn)/+ results in less MEI-1 activity for mei-26 to inactivate, and so mei-l(dn) acts as a dominant suppressor of mei-26.

Mutations in mei-2 cause recessive meiotic phenotypes similar to mei-l 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-l(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 post-meiotic mutations. Finally, mei-2/++ would further limit the decreased MEI-1 activity in mei-l(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-l 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-l(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-l(ct46) and postmitotic mutations. Finally, mei-26 may inhibit mei-l function indirectly, for example through mei-2 or some as yet unidentified gene. Further molecular analysis on mei-26 and mei-2 is needed to resolve these issues.

What could be the biochemical function of mei-l? mei-l 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-l activity. The times of meiosis and mitosis are indicated at the top. mei-2 has a positive effect on mei-l activity while mei-26 has a negative effect. The wild-type activities are indicated for each gene, except for mei-l(dn) (in brackets), which antagonizes mei-l(+) or mei-l(gf) activities. In this scheme, the gf mutation mei-l(ct46) would not be sensitive to negative regulation by mei-26.
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. Candinin, 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. K. Lee and L. Edgar for antimony chemistry 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.

References

Albertson, D. G. 1984. Formation of the first cleavage spindle in nematode embryos. Dev. Biol. 101:61-72.

Albertson, D. G., and J. N. Thomson. 1993. Segregation of holocentric chromosomes at meiosis in the nematode, Caenorhabditis elegans. Chromosome Res. 1:15-26.

Austin, J., and J. K. Kimble. 1987. Gip-1 is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell. 51:589-599.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Barnard, M. K., T. B. Schedl, and J. K. Kimble. 1987. Gain-of-function mutations of fem-3, a sex-determination gene in Caenorhabditis elegans. Genetics. 115:107-119.

Candinin, T. C., and P. E. Mains. 1993. Genetic studies of mei-1 gene activity during the transition from meiosis to mitosis in Caenorhabditis elegans. Genetics. 134:199-210.

Clark, S. W., and D. I. Meyer. 1992. Centrin acts as an actin homologue associated with the centrosome. Nature (Lond.). 359:246-250.

Clark-Maguire, S., and P. E. Mains. 1994. mei-1, a gene required for meiotic spindle formation in Caenorhabditis elegans, is a member of a family of ATPases. Genetics. 136:533-546.

Compton, D. A., and D. W. Cleveland. 1993. NuMA is required for the proper completion of mitosis. J. Cell Biol. 120:947-957.

Enos A. P., and N. R. Morris. 1990. A mutation of a gene that encodes a kinesin-like protein blocks nuclear division in A. nidulans. Cell. 60:1019-1027.

Fröhlich, K. U., H. W. Fries, M. Rüdiger, R. Erdmann, D. Botsstein, and D. Mecke. 1991. Yeast cell cycle protein CDC48 shows full-length homology to the mammalian protein VCP and is a member of a protein family involved in secretion, peroxisome formation, and gene expression. J. Cell Biol. 114:443-453.

Gard, D. L. 1992. Microtubule organization during maturation of Xenopus oocytes: Assembly and rotation of the microtubule spindles. Dev. Biol. 151:516-530.

Ghislain, M., A. Ulvardly, and C. Mann. 1993. S. cerevisiae 26S protease mutants arrest cell division in G2/m-phase. Nature (Lond.). 366:358-362.

Goldstein, L. S. B. 1993. With apologies to Scheherazade: tails of 1001 kinesin proteins. Annu. Rev. Genet. 27:319-351.

Gordon, C. G., M. Curcur, P. D. Wellings, and N. D. Hastiie. 1993. Defective mitosis due to a mutation in the gene for a fission yeast 26S protease subunit. Nature (Lond.). 366:355-357.

Hagan, I., and M. Yangudia. 1992. Kinesis-related cut7 protein associates with mitotic and meiotic spindles in fission yeast. Nature (Lond.). 356:74-76.

Harlow, E., and D. Lane. 1988. Antibodies, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 726 pp.

Hatsumi, M., and S. A. Endow. 1992a. Mutants of the microtubule motor protein, nonclaret disjunctional, affect spindle structure and chromosome movement in meiosis and mitosis. J. Cell Sci. 101:547-559.

Hatsumi, M., and S. A. Endow. 1992b. The Drosophila nonclaret microtubule protein is spindle-associated in meiotic and mitotic cells. J. Cell Sci. 103:1015-1020.

Heck, M. M. S., A. Pereira, P. Pesavento, Y. Yannou, A. C. Spradling, and L. S. B. Goldstein. 1993. The kinesis-like protein KLP61F is essential for mitosis in Drosophila. J. Cell Biol. 123:663-679.

Hird, S. N., and J. G. White. 1993. Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. J. Cell Biol. 121:1343-1355.

Hirth, D., D. Oppenheim, and M. Klass. 1976. Development of the reproductive system of Caenorhabditis elegans. Dev. Biol. 49:200-219.

Horvitz, H. R., S. Brenner, J. Hodgkin, and R. K. Herman. 1979. A uniform genetic nomenclature for the nematode Caenorhabditis elegans. Mol. Gen. Genet. 157:129-133.

Hoyt, M. A., L. He, K. K. Loo, and W. S. Saunders. 1992. Two Saccharomyces cerevisiae kinesin-related gene products required for mitotic spindle assembly. J. Cell Biol. 118:109-120.

Hyman, A. A. 1989. Centrosome movement in the early divisions of Caenor-
Kallajoki, M., K. Weber, and M. Osborn. 1992. Ability to organize microtubules in taxol-treated mitotic HeLa cells goes with the SPN antigen and not with the centrosome. J. Cell Sci. 102:91-102.

Kemphues, K. J., M. Wolf, W. B. Wood, and D. Hirsh. 1986. Two loci required for cytoplasmic organization in early embryos of Caenorhabditis elegans. Dev. Biol. 113:449-460.

Kimble, J., L. Edgar, and D. Hirsh. 1984. Specification of male development in Caenorhabditis elegans: the fem genes. Dev. Biol. 105:234-239.

Kuriyama, R., and C. Nislow. 1992. Molecular components of the mitotic spindle. Bioessays. 14:81-88.

Mains, P. E., K. J. Kemphues, and S. A. Sprunger, I. A. Sulston, and W. B. Wood. 1990a. Mutations affecting the mitotic and mitotic divisions of the early Caenorhabditis elegans embryo. Genetics. 126:593-605.

Mains, P. E., I. A. Sulston, and W. B. Wood. 1990b. Dominant maternal-effect lethal mutations causing embryonic lethality in Caenorhabditis elegans. Genetics. 125:351-369.

Matthews, K. A., D. Rees, and T. C. Kaufman. 1993. A functionally specialized α-tubulin is required for oocyte meiosis and cleavage mitoses in Dro sophila. Development (Camb.). 117:977-991.

McIntosh, J. R., and M. P. Koonce. 1989. Mitosis. Science (Wash. DC). 245:622-625.

Meluh, P. B., and M. D. Rose. 1990. KAR3, a kinesin-related gene required for yeast nuclear fusion. Cell. 60:1029-1041.

Nigon, V., P. Guerrier, and H. Monin. 1960. L'Architecture polaire de l'oeuf et mouvements des constituents cellulaires au cours des premières étapes du développement chez quelques nématodes. Bull. Biol. Fr. Belg. 94:132-201.

Nislow, C. V., A. Lombillo, R. Kuriyama, and J. R. McIntosh. 1992. A plus-end-directed motor enzyme that moves antiparallel microtubules in vitro localizes to the interzone of mitotic spindles. Nature (Lond.). 359:543-547.

Oakley, B. C., E. C. Oakley, Y. Yoon, and M. K. Jung. 1990. γ-Tubulin is a component of the spindle pole body that is essential for microtubule function in Aspergillus nidulans. Cell. 61:1289-1301.

O'Connell, M. J. P., B. M. Meluh, M. D. Rose, and N. R. Morris. 1993. Suppression of the bimC4 mitotic spindle defect by deletion of klpA, a gene encoding a KAR3-related kinesin-like protein in Aspergillus nidulans. J. Cell Biol. 120:153-162.

Pfarr, C. M., M. Coue, P. M. Grissom, T. S. Hayes, M. E. Porter, and J. R. McIntosh. 1990. Cytoplasmic dynein is localized to kinetochores during mitosis. Nature (Lond.). 345:263-265.

Piperno, G., and M. T. Fuller. 1985. Monoclonal antibodies specific for an acetylated form of α-tubulin recognize the antigen in cilia and flagella from a variety of organisms. J. Cell Biol. 101:2085-2094.

Pleasure, I. T., M. M. Black, and J. H. Keen. 1993. Valosin-containing protein, VCP, is a ubiquitous clathrin-binding protein. Nature (Lond.). 365:459-462.

Roof, D. M., P. B. Meluh, and M. D. Rose. 1992. Kinesin-related proteins required for assembly of the mitotic spindle. J. Cell Biol. 118:95-108.

Rose, M. D., S. Biggins, and L. L. Satterwhite. 1993. Unravelling the tangled web at the microtubule-organizing center. Curr. Opin. Cell Biol. 5:105-115.

Rothman, J. E., and L. Orci. 1992. Molecular dissection of the secretory pathway. Nature (Lond.). 355:409-415.

Saunders, W. S., and M. A. Hoyt. 1992. Kinesin-related proteins required for structural integrity of the mitotic spindle. Cell. 70:451-458.

Sawada, T., and G. Schatten. 1988. Microtubules in ascidian eggs during meiosis, fertilization, and mitosis. Cell Motil. Cytoskeleton. 9:219-230.

Sawin, K. E., and S. A. Endow. 1993. Meiosis, mitosis and microtubule motors. Bioessays. 15:339-407.

Sawin, K. E., K. LeGuellec, M. Philippe, and T. J. Mitchinson. 1992a. Mitotic spindle organization by a plus-end-directed microtubule motor. Nature (Lond.). 359:540-543.

Sawin, K. E., T. J. Mitchinson, and L. G. Wordeman. 1992b. Evidence for kinesin-related proteins in the mitotic apparatus using peptide antibodies. J. Cell Sci. 101:303-313.

Schatten, G., C. Simmerly, and H. Schatten. 1985. Microtubule configurations during fertilization, mitosis, and early development in the mouse and the requirement for egg microtubule-mediated motility during mammalian fertilization. Proc. Nat. Acad. Sci. USA. 82:4152-4156.

Smith, D. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione-S-transferase. Gene (Amst.). 67:31-40.

Stearns, T., L. Evans, and M. Kirschner. 1991. γ-Tubulin is a highly conserved component of the centrosome. Cell. 65:825-836.

Strome, S., and W. B. Wood. 1983. Generation of asymmetry and segregation of germ-line granules in early C. elegans embryos. Cell. 35:15-25.

Steuer, E. R., L. Edgar, and J. M. Scholey. 1993. Roles of kinesin and cytoplasmic dynein in the meiotic spindle and kinetochores. J. Cell Biol. 123:681-689.

Strobe, S. 1993. Determination of cleavage planes. Cell. 72:3-6.

Studer, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff. 1990. Use of T7 RNA polymerase to directly express of cloned genes. Methods Enzymol. 180:65-89.

Theurkauf, W. E., and R. S. Hawley. 1992. Meiotic spindle assembly in Drosophila females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. J. Cell Biol. 116:1167-1180.

Vaisberg, E. A., M. P. Koonce, and J. R. McIntosh. 1993. Cytoplasmic dynein plays a role in mammalian mitotic spindle formation. J. Cell Biol. 123:849-858.

Verde, F., J.-M. Berrez, C. Antony, and E. Karsenti. 1991. Taxol-induced microtubule asters in mitotic extracts of Xenopus eggs: requirement for phosphorylated factors and cytoplasmic dynein. J. Cell Biol. 112:1177-1187.

Wood, W. B., R. Hecht, S. Carr, R. Vanderslice, N. Wolf, and D. Hirsh. 1980. Parental effects and phenotypic characterization of mutations that affect early development in Caenorhabditis elegans. Dev. Biol. 74:446-469.

Wood, W. B. 1988. The Nematode Caenorhabditis elegans. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 667 pp.

Wright, B. D., M. Terasaki, and J. M. Scholey. 1993. Roles of kinesin and kinesin-like proteins in sea urchin embryonic cell division: evaluation using antibody microinjection. J. Cell Biol. 123:681-689.

Yang, C. H., and M. Synder. 1992. The nuclear-mitotic apparatus protein is important in the establishment and maintenance of the bipolar mitotic spindle apparatus. Mol. Biol. Cell. 3:1259-1267.

Zhang, B., B. A. Knowles, L. S. B. Goldstein, and R. S. Hawley. 1990. A kinesin-like protein required for distributive chromosome segregation in Drosophila. Cell. 62:1053-1062.

Zheng, Y., M. K. Jung, and B. R. Oakley. 1991. γ-Tubulin is present in Drosophila melanogaster and Homo sapiens and is associated with the centrosome. Cell. 65:817-823.