***mei-1*, a Gene Required for Meiotic Spindle Formation in *Caenorhabditis elegans*, Is a Member of a Family of ATPases**

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ABSTRACT

Meiotic spindle formation in the female germline of *Caenorhabditis elegans* requires expression of the gene *mei-1*. We have cloned *mei-1* by transformation rescue and found that it resides near a hot spot for recombination, in an area of high gene density. The highest levels of *mei-1* mRNA accumulate in the female germline of adult hermaphrodites as well as in fertilized embryos. The message persists for several hours after the protein functions in embryos, implying the need for post-transcriptional regulation. Two alternatively spliced messages are made that would result in proteins that differ internally by three amino acids; the larger of the two mRNAs is preferentially enriched in the female germline. The sequence of *mei-1* shows that it is a member of a newly described family of ATPases that share a highly conserved nucleotide-binding site; four dominant-negative mutations of *mei-1* are found at or near this region. Divergent roles ascribed to this family include membrane function, proteolysis, transcription and cell cycle regulation.

FOLLOWING fertilization, zygotes of sexual eukaryotes are faced with the transition from meiosis to mitosis. These two modes of division have much in common, but there are clear differences, including the orientation and morphology of the spindles, the alignment of the chromosomes on the spindles and the presence of centrioles (there are often none during female meiosis). The time between meiosis and mitosis is often short. In the female germline of the nematode *Caenorhabditis elegans*, meiosis arrests at prophase of the first meiotic division; after fertilization, meiosis resumes and is followed within 20 min by the first mitotic cleavage (NIGON *et al.* 1960; HIRSH *et al.* 1976; STROME and WOOD 1983; ALBERTSON 1984; ALBERTSON and THOMSON 1993). Products unique to each division presumably require careful temporal or spatial regulation to restrict their activities to the appropriate spindle.

We have identified a set of interacting genes of *C. elegans* that disrupt the transition from meiosis to mitosis, (MAINS *et al.* 1990a,b; CLANDININ and MAINS 1993). This paper focuses on the molecular analysis of the gene *mei-1* (meiosis-1), which can be mutated to disrupt either the meiotic or mitotic divisions (MAINS *et al.* 1990b; CLANDININ and MAINS 1993). Genetic evidence suggests that maternal *mei-1*(+) is required for the proper organization of the meiotic spindle but this activity must be eliminated prior to mitosis. Loss-of-function (*lf*) alleles of this gene disrupt the formation of the oocyte's meiotic spindle, leading to meiotic failure and abnormal polar body formation. However, the subsequent mitotic cleavages, which include the

chromosomes of the sperm pronucleus (and occasional maternal chromosomes that join it), occur with their normal orientation and rhythm. In contrast, a dominant temperature-sensitive (*ts*), gain-of-function (*gf*) allele *mei-1(ct46)* results in the opposite phenotype, namely a normal meiosis followed by abnormal mitotic cleavages. After the maternal and paternal pronuclei fuse in *mei-1(ct46)* embryos, they usually fail to undergo their normal 90° rotation and their subsequent migration back to the center of the zygote. These abnormalities often result in a spindle with a dorsal-ventral orientation in the posterior of the embryo rather than one aligned along the anterior-posterior axis near the center. Temperature shift experiments showed that this dominant mutation can also disrupt the next several mitotic cleavages. We have identified several classes of intragenic revertants of *mei-1(ct46)* as well as extragenic suppressors and enhancers of the gene (MAINS *et al.* 1990b; CLANDININ and MAINS 1993).

We have used the correlation of the physical and genetic maps of *C. elegans* (COULSON *et al.* 1986, 1988) to clone *mei-1*. The DNA sequence shows that *mei-1* belongs to a family of proteins with diverse roles that include membrane function, proteolysis, transcription and cell cycle regulation. All members of the family share a highly-conserved ATP-binding site and several *mei-1* antimorphic alleles occur in this region.

MATERIALS AND METHODS

Strains and culture conditions: Nematode strains were maintained under standard conditions as described by MAINS *et al.* (1990a) and WOOD (1988). Nomenclature con-

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forms to that of HORVITZ *et al.* (1979). The following genes and alleles were used:

Linkage group I: *mei-1(b284, ct46, ct81, ct93, ct46sb3, ct46sb9, ct46sb17, ct46sb18, ct46sb22, ct46sb23, ct46ct82, ct46ct84, ct46ct89, ct46ct99, ct46ct100, ct46ct101, ct46ct103), dpy-5(e61), daf-8(e1393), lin-10(e1439), lin-11(n566), mei-2(ct98), unc-13(e1091), unc-29(e193 and e1072), unc-120(st364).*

Linkage group III: *glp-1(q231), unc-32(e189).*

Linkage group IV: *dpy-20(e1282), fem-1(hc17), fem-3(q20).*

Linkage group V: *dpy-11(e224).*

gaD1(I;f) is a free duplication of chromosome I that covers the genes *dpy-5, mei-2, unc-13, mei-1* and *daf-8*, but not *unc-29*.

Transformation rescue of *mei-1*: Microinjection experiments were carried out as described by FIRE (1986), as modified by MELLO *et al.* (1991), using the plasmid pRF4[*rol-6(su1006)*] as a marker of successful transformation. Progeny that inherit pRF4 display the Rol (roller) phenotype and usually contain the coinjected DNA as part of extrachromosomal arrays. Heritable lines can be established and tested for expression of the gene of interest.

The recipient for microinjection was a balanced heterozygous *mei-1* strain (BW1100) of the genotype *mei-1(ct46ct101) daf-8/unc-13 lin-11. ct46ct101* is a nonconditional, recessive maternal-effect lethal allele of *mei-1* and behaves genetically as a null allele, showing no *mei-1* activity of any kind (MAINS *et al.* 1990b). A *ts* mutation in *daf-8* was used to mark the *mei-1*-bearing chromosome. *unc-13* and *lin-11* are flanking markers, separated from each other by approximately 3 map units, and were used to balance *mei-1* and *daf-8*. At 25°, animals homozygous for *daf-8* enter an alternative third larval stage known as the dauer (the Daf phenotype), which is easily recognized. Upon downshift to 15°, they emerge from dauer and resume the life cycle. Since there is <1% recombination between *daf-8* and *mei-1*, almost all progeny of BW1100 that are homozygous for *daf-8* will also be homozygous for *mei-1*. The rare (undesired) Daf non-Mei recombinants can be recognized since they acquire *unc-13*, which will segregate in their progeny.

The DNA of interest (10–25 µg/ml) was injected with pRF4 (100 µg/ml) into BW1100 and heritable Rol lines were established. These were cultured at 25° and Rol Daf progeny were down shifted to 15° and tested for fertility. Non-Rol segregants from rescued lines were also picked and shown to be infertile, demonstrating that rescue of *mei-1* cosegregated with *rol-6* in the extrachromosomal array.

The following cosmids in the *lin-10* to *lin-28* interval (listed in left to right order, with the center of chromosome I being to the left) were tested for rescue of *mei-1*: T01G9, F54A4, C25C12, C34B7, T24H1, T19A6, F02E9, ZK254, C05D8, C48B1, C26C7, K02B12, F59D11 and C14F9.

Genetic mapping: Hermaphrodites of the genotype + *lin-10 mei-1(ct46ct101) + daf-8/unc-13 + + unc-120 +* (genes are listed in the left to right order) were selfed at 25° and Unc-13 non Unc-120 recombinants were selected. This was facilitated by the fact that the Unc-120 allele used is a larval-lethal at this temperature. *mei-1(ct46ct101)* shows a recessive nonconditional maternal-effect lethal phenotype (Mel) and the *cis*-linked *daf-8* mutation was used to indicate subsequent homozygosity for this chromosome. The recombinants (*unc-13 lin-10? mei-1? daf-8/unc-13 unc-120*) were selfed at 25° and the Daf progeny were downshifted to 15°, allowing them to emerge from dauer. Forty-two crossovers resulted in Daf progeny that were Lin Mel, indicating recombination between *unc-13* and *lin-10*. Ten crossovers occurred in the *lin-10-mei-1* region producing Daf Mel non-Lin offspring. Seventeen recombination events were distal to *mei-1* yielding Daf non-Lin non-Mel progeny. The two-factor distance

between *unc-13* and *unc-120* was determined from a subset of the animals in the above experiment where the total number of progeny (including larvae arrested due to the *unc-120* mutation) was counted. Among 1776 F₁ progeny of *lin-10 mei-1 daf-8/unc-13 unc-120* hermaphrodites, 16 were Unc-13 non Unc-120, giving a two-factor recombination value of 1.8 map units.

Molecular techniques: Standard molecular methods for restriction mapping, Southern blotting, library screening and subcloning were used throughout (SAMBROOK *et al.* 1989; AUSUBEL *et al.* 1990). All subclones were made in pBluescript SK⁺ except for pHR7 which was in pBluescript KS⁺ (Stratagene, La Jolla, California). Dideoxy sequencing (SANGER *et al.* 1977) of double-stranded DNA was performed using the Sequenase Version 2.0 (TABOR and RICHARDSON 1989; United States Biochemicals, Cleveland, Ohio). A nested set of deletions for sequencing of the DNA of interest (HENIKOFF 1984) was made using the Erase-A-Base kit (Promega, Madison, Wisconsin). With the exception of the mutants (described below), all sequences were determined for both strands.

RNA was isolated by the method of MEYER and CASSON (1986) using standard procedures to generate staged wild-type populations (WOOD 1988). Oocytes were isolated as described by STROEHER *et al.* (1994) and were provided by B. GOSZCZYNSKI and J. MCGHEE. RNA was also isolated from hermaphrodite strains carrying *ts* mutations resulting in the lack of the female germline [*fem-3(q20)*], in a strain where it was linked to *dpy-20*], lack of the male germline [*fem-1(hc17)*], which also included an unlinked *dpy-11* mutation] or lack of both germlines [*glp-1(q231)* linked to *unc-32*]. The animals were hatched at the permissive temperature and then grown to adulthood at the nonpermissive temperature. Between 40 and 60 animals of each batch were reserved to confirm complete sterility.

PCR of reverse-transcribed mRNA (RT-PCR) was performed as described by FROHMAN *et al.* (1988). To demonstrate that the *mei-1* mRNA was trans-spliced, polyA⁺ RNA was prepared with Invitrogen Fast Track mRNA isolation kit (San Diego, California) and primed with primer 2 (see below for positions of the primers) to produce a cDNA pool. This pool was then amplified with primer 1 and a primer corresponding to the 3' end of the *trans*-splice leader SL1 (5'-GGTTTAATTAGGGAAGTTTGAG-3', KRAUSE and HIRSH 1987). The amplification products were then analyzed by Southern blots to determine which cross-hybridized with *mei-1*. The band of interest was gel purified and sequenced by asymmetric PCR (MURRAY 1989) using the Bethesda Research Laboratory (Bethesda, Maryland) thermocycle sequencing kit. To measure the relative use of the alternative splice sites in the third intron, cDNA was primed from total RNA using either oligo-dT or primer 3, and then was PCR amplified with primers 3 and 5. This product was then reamplified with primers 11 and 5. The products were electrophoresed on a polyacrylamide/urea gel and visualized by staining with ethidium bromide.

Northern blots of polyA⁺ RNA were performed as described by FOURNEY *et al.* (1988) using the hybridization buffer of AUSUBEL *et al.* (1991). RNAase protection experiments were done as described in AUSUBEL *et al.* (1990), in probe excess, using 30 µg of total RNA for each sample. Yeast tRNA was substituted for *C. elegans* RNA to determine background. The probe used for the RNAase protection experiments extended from nucleotide 1655–2067 of Figure 2. Dried gels were autoradiographed and then the bands were quantitated by cutting them out of the gel and measuring radioactivity in a scintillation counter. The total counts minus background in wild-type gravid hermaphro-

dites was 412 cpm; all other samples were normalized by dividing by this value.

DNA and protein sequence analysis used the Genetics Computer Group, Inc. (GCG, DEVEREUX *et al.* 1984) software package. Database searches used the BLAST algorithm (ALTSCHUL *et al.* 1990) and the BLAST network service of the National Center for Biotechnology Information.

Sequencing of mutant alleles: One to several mutant animals of each genotype were frozen and processed for amplification by PCR in the worm lysis buffer described by WILLIAMS *et al.* (1992). For those mutations that must be maintained as balanced heterozygotes due to their nonconditional maternal-effect lethality, individual homozygous segregants were identified by *cis*-linked morphological markers and allowed to lay eggs before freezing. Animals that produced hatching embryos, representing an undesired crossover no longer homozygous for the mutation, were discarded.

PCR reactions, using combinations of the primers described below, were carried out using the buffer described by PONCE and MICOL (1992) and the cycle times used by WILLIAMS *et al.* (1992), except an additional 0.5 units of *Taq* polymerase were often added after 30 cycles for an additional 10–15 cycles to increase the yield. The time of the extension cycle was adjusted according to the length of the expected product, assuming an extension rate of 1000 bp/min for *Taq* polymerase. The PCR products were precipitated with ammonium acetate and isopropanol and resuspended in 10 mM Tris, pH 8, 1 mM EDTA. The amplified DNAs were sequenced by asymmetric PCR as described above.

The primers used for PCR and sequencing were all 21 nucleotides in length, except for primer 11, which was 18 nucleotides. The positions in Figure 2 of the 5' base of the primers that are in the same sense as the presented sequence are as follows: primer 4, 774; primer 5, 2040. The 5' end of the primers that are complementary to the sequence in Figure 2 are as follows: primer 1, 1221; primer 2, 1252; primer 3, 2863; primer 6, 1418; primer 7, 1616; primer 8, 1807; primer 9, 1948; primer 10, 2151; primer 11, 2614.

The first group of mutations examined (*ct46sb3*, *ct46sb9*, *ct46sb17*, *ct46sb22*, *b284*, *ct81*, *ct46ct103*, *ct46ct84*, *ct46ct101* and *ct46ct82*) were sequenced in their entirety. Each had only one lesion and all except *ct46sb3* (which was induced with psoralen) were GC to AT transitions as expected from ethylmethylsulfonate (EMS) mutagenesis. Only the A and T lanes were run for the remaining mutations (all were induced by EMS) along with a standard that contained all four bases so that the exact position of the mutations could be determined. The second group of alleles were sequenced only until a mutation was found (*i.e.*, not in their entirety).

Once a mutation was identified, it was resequenced from an independently amplified PCR product to eliminate the possibility of an error caused by *Taq* polymerase (only one case of this was found). *ct46* was not directly sequenced but its change was inferred from the difference with the wild-type sequence present on all of the mutations that were induced in *cis* to *ct46* and the absence of this change from all of those alleles isolated in *trans*. The *ct46ct82* mutation was not resequenced, but it was predicted to eliminate a *BanI* restriction site, which was confirmed by showing an independent PCR product could not be cleaved by this enzyme.

RESULTS

Cloning of *mei-1* by transformation rescue: To identify cosmids that contain *mei-1*, we took advantage of the correlation of the physical and genetic maps of

C. elegans (COULSON *et al.* 1986, 1988) in combination with transformation rescue (FIRE 1986; MELLO *et al.* 1991). *mei-1* genetically maps between *lin-10* and *lin-28*, which are to the left and right of *mei-1*, respectively. *lin-10* is on the cosmid T01G9 (KIM and HORVITZ 1990), while *lin-28* is to the left of a restriction fragment length polymorphism identified by cosmid C14F9 (V. AMBROS, unpublished data). Fourteen overlapping cosmids (listed in MATERIALS AND METHODS) spanning the interval were obtained from the MRC in Cambridge.

Individual cosmids were co-microinjected into gonads of hermaphrodites with pRF4, a plasmid that includes a dominant allele of *rol-6*, which confers a Rolling (Rol) phenotype (MELLO *et al.* 1991). DNA transformed into *C. elegans* typically forms large extragenic arrays. Animals displaying the Rol phenotype, indicating the presence of pRF4, usually include the coinjected DNA as part of the array. The recipient strain used to identify *mei-1*, BW1100, was a heterozygote balanced for a null allele, *mei-1(ct46ct101)*. Heritable Rol lines were established and segregants homozygous for *mei-1* were tested for fertility (see MATERIALS AND METHODS). The overlapping cosmids T01G9 and F54A4 rescued the maternal-effect lethality of *mei-1(ct46ct101)* (Figure 1A).

The location of *mei-1* within cosmid F54A4 was determined using the method described by KIM and HORVITZ (1990). The cosmid was cut with various restriction enzymes and each total digest was injected with pRF4 into BW1100. Making the assumption that proper religation to form an active gene would be exceedingly rare, enzymes that did *not* cut in the gene were identified as those that gave rise to restriction digests that still rescued. *SalI* was one such enzyme, and a gel-purified 10.5-kb fragment was shown to contain the rescuing activity. This fragment was subcloned to make the plasmid pHR7. Further use of this technique identified a rescuing 2.5 kb *BglII* fragment that was subcloned to make plasmid pHR14 (Figure 1).

The gene density in the vicinity of *mei-1* is high. Several genes previously assigned to the physical map are on the same cosmids as *mei-1*. Southern blots of the cosmids established the positions, shown in Figure 1, of *lin-10* (KIM and HORVITZ 1990), *kin-10* (HU and RUBIN 1991) and *kup-2* (SPIETH *et al.* 1993). In addition, a cDNA (in pHR30) isolated using pHR7 as a probe (see below) also mapped to the region. The high gene density near *mei-1* may not be unusual in *C. elegans*; reports from the *C. elegans* sequencing project found an apparent density of one gene for every 3–4 kb (SULSTON *et al.* 1992; WATERSTON *et al.* 1992). This gene density is many times higher than predicted from genetic analysis, perhaps indicating a high degree of genetic redundancy in *C. elegans*.

mei-1(+) activity provided by extragenic arrays was

FIGURE 1.—Restriction maps of the region containing *mei-1*. Heavy lines indicate genomic DNA and restriction enzyme sites and scales bars are as shown. Dotted lines indicate that the ends of the clones extend beyond the range of the figure or that their ends are not known with certainty within a restriction fragment. The center of chromosome I is to the left. The cosmid and plasmid clones are shown above each map and the positions of known genes and their direction of transcription are indicated below. The direction of transcription of *mei-1* and pHR30 were inferred from polyA tracks in cDNAs, while those for *kin-10* and *kup-2* (which are cotranscribed) were determined by use of a *kin-10* probe 3' to the *Sal I* site. (A) Restriction map of cosmid T01G9. pHR7 resulted from a *Sal I* partial digest, and contains 4 kb of the cosmid vector attached to the left end of the clone. (B) Restriction map of the genomic *Sal I* fragment of pHR7.

apparently limiting; 14 independent pHR14 transgenic lines showed cold-sensitive (*cs*) rescue of null *mei-1* alleles at 15°. In addition, pHR14 could not rescue a weak antimorphic allele (*ct46ct100*) at higher temperatures (25°). Although transgenic lines of the larger *mei-1* clones were viable at 15°, most grew better at 25°. The cold-sensitivity did not stem from overexpression or inappropriate expression of the *mei-1* transgene since +/*mei-1*(null) transgenic lines were not *cs*. In addition, the observed *cs* embryonic phenotypes were the same as *mei-1* null alleles (data not

shown). These results may indicate that expression of *mei-1* by extragenic arrays is inefficient. Consistent with this, we observed no germline expression of *lacZ* constructs (FIRE *et al.* 1990) fused to 2 kb of *mei-1* 5' flanking sequence (data not shown).

There is a recombination hotspot near *mei-1*: The recombination frequency is 1000–1500 kb/map unit near the central cluster of the autosomes of *C. elegans* (GREENWALD *et al.* 1987; PRASAD and BAILLIE 1989; STARR *et al.* 1989; SULSTON *et al.* 1992; ALBERTSON 1993). *mei-1* is on the right side of the cluster of linkage group I. Therefore, we did not expect to find *mei-1* on the same cosmid as *lin-10* since the previous estimate of the genetic distances between the genes was 0.3 map units (MAINS *et al.* 1990a), which should correspond to a physical distance much greater than one cosmid. To investigate the relationship between physical and genetic distances near *mei-1*, we quantitated the two- and three-factor map distance between *lin-10*, *mei-1* and the flanking genes *unc-13* and *unc-120*, whose positions on the physical map are known. As shown in Table 1, recombination frequency per kb between *mei-1* and *lin-10* is 10–40 times higher than previous estimates for recombination frequency in the autosomal clusters. (The position of the mutation within *lin-10* used for the mapping experiments is not known and so the physical distance between the *lin-10* and *mei-1* alleles could be anywhere from 6–25 kb, leading to a large range of recombination per map unit). Recombination frequency drops to nearer the previously reported values on either side of the *lin-10* to *mei-1* interval. This recombination hotspot may correspond to a region affecting recombination that MCKIM *et al.* (1993) recently identified by deficiency mapping in same vicinity. The nonuniformity of recombination over relatively short physical distances has been observed in several other regions in the *C. elegans* genome (STARR *et al.* 1989; HODGKIN 1993; PILGRIM 1993).

Genomic and cDNA sequence of *mei-1*: The genomic clones in pHR14 and pHR18 (the *Bgl II*-*Eco RI* fragment immediately to the left of pHR14, Figure 1B) were sequenced (Figure 2).

cDNA clones of *mei-1* were obtained using the 10.5-kb *Sal I* fragment of pHR7 to probe a mixed-stage *C. elegans* cDNA library (BARSTEAD and WATERSTON 1989). Twelve clones were isolated; most appeared by restriction digest analysis to represent a nested family originating from the 3' end of the gene (data not shown). The sequence of the longest cDNA, in pHR8, is collinear with the genomic clones, except for three introns (Figure 2). However, the cDNA and genomic sequences diverge 5' of the G at position 910 of Figure 2, which precedes an ATG start codon. The seven bases in pHR8 5' to this (GTTTGAG) are the same as the end of the *trans*-splice leader (SL1), which is spliced onto the beginning of many *C. elegans* mRNAs

TABLE 1
Correlation of the physical and genetic distances in the *mei-1* region

Distance	Genetic markers			
	<i>unc-13</i>	<i>lin-10</i>	<i>mei-1</i>	<i>unc-120</i>
Physical distance (kb) ^a	975	6–25	210	
Genetic distance (map units) ^b	1.10	0.26	0.44	
kb/map unit	885	25–100	420	

^a The physical distance was estimated from Figure 1 or by counting the number of cosmids on the physical map between the markers and assuming an average insert length of 40 kb per cosmid.

^b See MATERIALS AND METHODS for details.

(KRAUSE and HIRSH 1987). 5' to this, pHR8 contains 19 consecutive A residues preceded by a sequence that did not match the genomic sequence in Figure 2. Thus, pHR8 probably contains a near full length *mei-1* cDNA with a second, unrelated cDNA ligated in a tail to head fashion to its 5' end.

To confirm the interpretation that *mei-1* is trans-spliced and that pHR8 includes a near full-length cDNA, we performed RT-PCR (FROHMAN *et al.* 1988) on mRNA isolated from gravid hermaphrodites. The 5' primer, corresponding to the end of SL1, was used in conjunction with two nested primers several hundred base pairs 3' of the putative initiator ATG. A band of the appropriate molecular weight was readily amplified. This DNA fragment was subjected to asymmetric PCR sequencing (MURRAY 1989), which showed that it exactly matched the genomic sequence (data not shown).

Analysis of additional cDNAs indicated that there is an alternative splice acceptor site at the end of the third intron. Sequencing this region in nine cDNAs revealed that five used a 3' acceptor site nine nucleotides upstream of that used in pHR8 (Figure 2); these would encode a protein with three additional amino acids. (There is some ambiguity in the actual donor and acceptor sites for the longer transcript since the donor and acceptor sites can be shifted three nucleotides upstream of those shown in Figure 2; the same sequence, AAG, is found at both locations. However, only the downstream alternative would include a consensus GT at the splice donor). The acceptor site used in pHR8 (as well as those used for the first two introns) exactly matches the consensus used for *C. elegans*, namely TTTCAG, while the other acceptor site does not (FIELDS 1990). The *in vivo* use of the two splice sites will be discussed later. We isolated another cDNA corresponding to an RNA from which the third exon had not been removed. However, we detected no unspliced *mei-1* messages, either by Northern blots or by RT-PCR.

Two cDNA clones, represented by pHR30 and pHR28, identified a gene adjacent to *mei-1* that is included in the 10.5-kb *SalI* fragment used to screen the cDNA library. The ends of these cDNAs were sequenced and the location of their polyA tails indi-

cated that the gene is convergently transcribed with *mei-1* and that the 3' ends of *mei-1* and the pHR30/pHR28 mRNAs are only 10 bp apart (Figures 1 and 2). The 5' ends of pHR30/pHR28 extended beyond the 3' end of the genomic sequence shown in Figure 2. Northern blots using pHR30 as a probe showed that similar levels of this mRNA accumulated at each stage of the life cycle (data not shown). Searches of databases with the limited sequence of the 3' and 5' ends of pHR30 and pHR28 revealed no significant similarities to known genes.

Analysis of the *mei-1* sequence: The inferred translation products of *mei-1* are 472 and 475 amino acids, corresponding to molecular masses of 51.7 and 52.1 kDa, respectively. BLAST searches (ALTSCHUL *et al.* 1990) of databases revealed that *mei-1* is a member of a recently described family of putative ATPases. The proteins share two conserved motifs implicated in the binding of ATP, which are underlined in Figures 2 and 3. Several diverse functions have been ascribed to members of this family, including cell cycle regulation, transcription regulation, membrane function and proteolysis. These will be described in more detail in the DISCUSSION. Similarities between *mei-1* and other members of the family extend well beyond the confines of the ATP-binding consensus and range from 50–60% (identities 30–40%) over several hundred amino acids (Figure 4). *mei-1* is no more closely related to any single protein in the group than any other, although subsets of the family do show high similarity throughout their entire lengths (shown by the boxes in Figure 4). Similarity across the entire family is limited to a region of about 200 amino acids, which is presented in Figure 3 (the sequence of pig VCP is not included in the alignment since it differs from mouse VCP by only 2/806 amino acids). The alignment was made using the shorter *mei-1* protein; alignments made with the longer protein were not significantly different. The extra three amino acids in the longer *mei-1* isoform are beyond the region of highest similarity shown in Figure 3.

Sequence analysis of *mei-1* mutations: We previously described 26 alleles of *mei-1* that fall into four categories based upon their phenotypic properties (MAINS *et al.* 1990b; CLANDININ and MAINS 1993). To

1 gaattccgctaaattcgaaaattgaaagtgttcaaatgcaagcgattgtgcatcagacgtgacagtgctctgggtgtattgctccgacattttaactgacga
106 cacttgatcttttcgcatactccggagctccagctccgagcgctgagcaattatcttttactttttatgaaaagcttctatagatatcttttaagaagtta
211 cactataattgtgcaaatcaaaactggctccggacacacaaatttcgtctatacttttatgatctttttttgttaacaagtgaacaattatttccttttcaa
316 actgctctgtttctctcttttataatcaattttttttttgtttgtgtaaggaaattgttgcgaggatgagctaatctgaggttgaccagcagaaaa
421 tctgttttctgaaaaatcaataactcgccttaattttgtttttatcaagtgatagcaattagaaggttctaatcatttatatctcgtgaaagatctcaga
526 tttcaagccttttctgaaggatttaattcctaaacttttttgacatcatctttttgtgtgatctaccgctgaaataactgtgtttttgaggctaaactctt
631 tcaatgtttccaacaagttagccaatatcaagtaaaaaaagaaaaatcgttttctattcaaccattttattctgtaataataataaattcatcttcacggtaca
736 atcttcttctcccatctaataaagtcacgcacactccgttccgctgtttccctattcgttatcattcatcatcttgccattttcttctccgcaaatccattg
1 M N G D V Q S V I R G Y
841 tcttatactaaatttcacctctcgtctgtagaagtgtatattattgaaaaattaaagtatatcttcagGATGAATGGGATGTGCAGTCAGTCATTCGGGATA
13 L E R A Q V A K T M S D A G R W N E A G D L L R Q L M T D V K S C K I
946 TCTGGAGCGAGCCCAAGTCGCGAAAACATGTCCGATGCCGACGATGGAATGAGGCTGGTGATCTTCTTCTCGAGCTCATGACAGATGTCAAAGCTGTAAT
48 S A S N R D E H D A R N T F L R A L
1051 TTCGGAAGCAACAGAGACGAGCATGACGCAAGAAATgtacgtaataataatcttttaaaagcttgttgaattatttgaatttcagACATTCCTACGACCCCTA
66 E A N L K L V Q Q N V R D E D D L H E A M T R Q S G S P E P P A D P D
1156 GAGGCTAATTGAAAGCTTGTCCAACAAATGTGCGCATGAAGATGACCTACACGAGCGATGACGCGACAAAGTGAAGTCCAGAGCCACCTGCAGACCCAGAT
101 V W S K P S P P L P S S S K F G A T K K G V G A A G P R P R E I S K S
1261 GTTGGTCCAAACCATCGCCACCGTTACCATCCTCATCGAAATTTGGAGCAACAAAGAAAGAGTTGGAGCAGCAGGTCCAGTCCAGAGAAATATCAAAGTCA
136 T S S M S T N P A D V K P A N P T Q G I L P Q N S A G D S F D A S A Y
1366 ACGTCGTCGATGAGCAGCAATCCGGCTGATGTGAAGCTGCGAATCCAACCAAGAACTACTGCTCAAATAGTCTGGAGATTTCATTCGATTCGATTCGCTTAT
171 D A Y I V Q A V R G T M A T N T E N T M
1471 GATGCGtaagttattatgttttccagaaacaaaaatcagaatcttttcagTACATTGTTCAAGCGGTGCGTGGTACAATGGCTACAAATACGAAATACAAT
191 S L D D I I G M H D V K Q V L H E A V T L P L L V P E F F Q G L R S P
1576 GTCTTTAGATGATATAATCGGAATGCACGAGTGAAGCAAGTTCTGCACGAAGCGTCACTCTTCACTCTTGGTCCAGAATTCTTCAGGGTCTCCGTTACC
226 W K A M V L A G P P G T G K T L I A R A I A S E S S S T F F T V S S T
1681 ATGGAAGCAATGGTTCTCGCTGGACCACTGGAAGTGAAGACTCTTATCGCAGCTGCGATTGCTTCAGAGTCTAGTTCAACTTTTTTACC GTTCTCGAC
261 D L S S K W R G D S E K I V R L L F E L A R F Y A P S I I F I D E I D
1786 TGATCTGTCAGCAAGTGGCGTGGTATTCCGAGAAGATTGTCGTCTGTATTGCAACTTGACGGTCTATGCTCCATCCATAATCTTCATCGATGAGATTGA
296 T L G G Q R G N S G E H E A S R R V K S E F L V Q M D G S G Q N K F D S
1891 CACACTTGGTGACACCTGGAAATCTGGAGAACACGAAAGCGATCGCGCTGTTAAATCCGAGTTCTGGTACAAATGACGGGTACAGAAATAAATTCGATTC
331 R R V F V L A A T N I P W E L D E A L R R R F E K R I F I P L P D I D
1996 GCGACGTGATTCTGCTGCGGCCCAAAATATTCATGGAGCTCGATGAGGCACTCCGAAGACGTTTCGAAAAGCGAATCTTCATTCCACTGCCAGATATAGA
366 A R K K L I E K S M E G T P K S D E I N Y D D L A A R T E G F S G A D
2101 TGCAAGAAAGAGCTTATCGAAAATCAATGGAAGGAAGTCCGAATCCGATGAAATTAATATGATGACTTGGCGGCAAGGACTGAAGGATTCTCAGGAGCTGA
401 V V S L C R T A A I N V L R R
2206 TGTGTGTCTCTATGCAGAACTGCTGTATCAATGTGTGAGAGtaggtagcaggaatgtgcagaaaatcttttggaaaacgtgcttgtttcaataagataa
2301 tccaatatttcgaatcaaaactggatatagaatggcaactttcatgcactatttaattgttttaacgctttagacaaaattaaataacattttaagatgaatga
2416 accgtttttataaatttcgatatacaatttaagattgaaaagtggtattatcataataaaaaaagccaattttgtacgtttgctgaaaaattttgctaactgc
416 Y F R Y D T K S L R G G E L T A M E S
2521 gatgttgcaacacttttttcaagaatcgacactaagaatccttaagATATTTCAGATACGACACCAATCAATTGCGAGCGGGGAAGTGCAGCTGCAATGGAAT
436 L K A E L V R N I D F E A A L Q A V S P S A G P D T M L K C K E W C D
2625 CACTGAAAGCGGAAGTCTGAGGAACATTGACTTTGAGCAGCTCTTCAAGCAGTTTCAACAGTCTGGTCCAGATACAATGCTCAAATGCAAGAATGGTGTG
471 S F G A M
2731 ACTCTTTTGGTGCATGTAATCAACTATTTATTGTGATCTCTTTTAGTTTAAATATTGTGGCCTAGCTTTGGTTTTGAAACTCTGAAACATTGTACAGATA
2836 AAATTTTCAAGGCCACCTTCATCCAAACATCTCTCCCGATTCACTCTTCTCTGTAATAACTATTGGTTAAATTTGTATGTAATAATGAATCATTTTGCCACTC
2941 ACTggattatttggaaatatataaaagttcatttgtgaagaaatataagaaaagaagacagtgaaaaaaaacaatacaggaagaaaaataaacatcgattag
3046 aaaagaaactgaaataaaaaaccgccctttagaaggaatcatattgtggataagtggttaggaatttcatgtgtgaattttaattgtctaaagggttaacatt
3151 ttggaagaagaagatcaaaaaaaagaagcaattttgtggaacacgattctcttacgggggatacaaacggaattatgacggtggactgaaggaataat
3256 tgagaagatct

FIGURE 2.—Genomic sequence of the *mei-1* region. The sequence of the genomic inserts of plasmids pHR18 (5') and pHR14 (3') are shown, the joint being at the *Bgl*II site at position 496 (this joint was confirmed by sequencing an overlapping subclone). The center of linkage group I is in the 5' (left) direction. The sequence corresponding to the mature mRNA is indicate by upper case letters and the inferred translation is indicated by the single amino acid code, centered above the first base of each codon. The nine shaded nucleotides and corresponding three amino acids result from alternate splicing of the third intron. The putative nucleotide binding sites are indicated by a single underline beneath the appropriate amino acids. The amino acids and nucleotides changed by the *mei-1* mutations listed in Table 2 are indicated by double underlines. The single underline beginning at nucleotide 2953 represents the last 10 bp of the convergently transcribed gene represented by the cDNA contained in pHR30. The Genbank accession number of the *mei-1* sequence is L25423.

see how these different types of mutations might affect gene function, DNA was sequenced from PCR products amplified from 15 different mutants. The alterations caused by each mutation are listed in Table 2 and their positions within the sequence are indicated in Figures 2 and 3.

ct46, is a dominant, temperature-sensitive, gain-of-function "poison" that retains the normal meiotic activity but then appears to persist into mitosis, where it disrupts that division. *ct46* results in a change from P to L at amino acid 99.

Intragenic revertants of the *ct46* mutation fall into

	190						247
NSF	feKmgIGGLd	KefsdIfrra	fasrvfpPEi	veqMGcKhvK	GILLYGPPGc	GKTLARqIg	
SEC18	fedIgvGGLd	KefstkIfrra	fasrIfpPsv	iekLGishvK	GILLYGPPGT	GKTLiARkIg	
Mouse VCP	vgYdDVGGr	KQlaql.kEm	VELPLrHPaL	FkaIGVKPPr	GILLYGPPGT	GKTLiARAVA	
p97	vgYdDIGGr	KQlaql.kEm	VELPLrHPaL	FkaIGVKPPr	GILLYGPPGT	GKTLiARAVA	
CDC48	etYaDIGGLd	KQmaql.rEm	VELPLrHPaL	FkaIGIKPPr	GVLMYGPPGT	GKTLMARAVA	
4S Protease	etYaDIGGLd	nQiqei.kEs	VELPLrHPaL	YeeMGIKPPK	GVLMYGPPGT	GKTLAKAVA	
TBP-1	eqYsDIGGLd	KQiqei.vEa	IvLPMnHkEk	FenLGiqPPK	GVLMYGPPGT	GKTLARAcA	
SUG1	stYdmVGLt	KQikeI.kEv	IELPvkHPEL	FesLGiaqPK	GVLMYGPPGT	GKTLARAVA	
MSS1	vtYsDVGgck	eQiekI.rEv	VEtPLIHPEr	FvnLGiePPK	GVLFLGPPGT	GKTLcARAVA	
ftsH	ttFaDVaGcd	eakeev.aEl	VEy.LrePsr	FqkLGgKiPK	GVLmVgPPGT	GKTLAKAIA	
PAS1	ikWgDIGaLa	nakdvI.lEt	lEwPtKyepi	FvncpIrlrs	GILLYGyPGc	GKTLAsAVA	
mei-1	msLDIIGMH	.dvkvVlH Ea	VtLPLlvPEF	FqgLr.sPwK	amvLaGPPGT	GKTLiARAIa	
Consensus	--Y-DIGGL-	KQ---I--E-	VELPL-HPeL	F--LGIKPPK	GVLLYGPPGT	GKTLARAVA	
		*		*	*		
	248						298
NSF	kmInArepkv	VnGPEiLnKY	VGESeAnIRk	LFAdAEeeqr	rlgansglhI	IIfDEIDAic	
SEC18	tmInAkefki	VnGPEiLsKY	VGSESeNIRn	LFkdAEaeyr	akgeesslhI	IIfDEIDSVf	
Mouse VCP	neTgAFFF1	InGPEiMsKL	aGESEsNIRk	aFEaAEknAP	a.....I	IIfDEIDAIA	
p97	neTgAFFF1	InGPEiMsKL	aGESEsNIRk	aFEaAEknAP	a.....I	IIfDEIDAIA	
CDC48	neTgAFFF1	InGPEVmsKm	aGESEsNIRk	aFEaAEknAP	a.....I	IIfDEIDSIa	
4S Protease	nqTsAtFLr	VvGsEliqKY	IGDgpkLVRE	LFRvAEehAP	s.....I	IFIDEIDAIG	
TBP-1	aqTKAtFLk	laGpQlvqMF	IGDgkLVRE	aFaLAkeAP	s.....I	IFIDEIDAIG	
SUG1	hhTdckFir	VsGaEiVqKY	IGEGsrMVRE	LFvmArehAP	s.....I	IFmDEIDSIg	
MSS1	nrTdAcFir	IsGsEiVqKY	VGEGarmVRE	LFEmArtkka	c.....I	IFIDEIDAIG	
ftsH	geakvPFft	IsGsDfvmEY	VGvgasrVRE	MFEqAkkAP	c.....I	IFIDEIDAIG	
PAS1	qqcglnFis	VkGPEiLnKF	IGaSeqNIRE	LFERaQsvkP	c.....I	lFfDEfDsIa	
mei-1	sessstFFt	VsstdIssKW	rGDESEKIVR1	LFELArfyAP	s.....I	IFIDEIDtIG	
Consensus	---T-A-FF--	V-GPE---KY	VGESe-NVRE	LFE-AE--AP	-----I	IFIDEIDAIG	
		*		*	*		
	299						355
NSF	kqRg.smaGs	tgvhdtVvNq	LLskiDGVeq	lnn...lIVI	gmTNRPDliD	eALLRPGRIE	
SEC18	kqRg.srGdG	tgvgdnVvNq	LLakMD.vDq	lnn...lIVI	gmTNRkDliD	sALLRPGRIE	
Mouse VCP	pKRkethGev	Er...RIVsQ	LLtIMDGLkq	rah...ViVm	AATNRPNsID	PALrRfGRFD	
p97	pKRkethGev	Er...RIVsQ	LLtIMDGLkq	rah...ViVm	AATNRPNsID	PALrRfGRFD	
CDC48	pKRdktGev	Er...RVVsQ	LLtIMDGMKA	rsn...VvVI	AATNRPNsID	PALrRfGRFD	
4S Protease	tKRydsnsGG	EreiqRtmle	LLnQLDGFds	rgd...VkvI	MAATNRiEtID	PALiRPGRIID	
TBP-1	tKRfidskaG	DrevqrTmle	LLnQLDGFp	ntq...VkvI	MAATNRvDliD	PALLRsGRID	
SUG1	stRvegsGGG	DsevgRtmle	LLnQLDGFET	skn...IKIi	MAATNRDliD	PALLRPGRIID	
MSS1	garfddgaGG	DnevgRtmle	LinQLDGFDP	rgn...IKVl	MAATNRPDtID	PALMRPGRIID	
ftsH	qRfagagIGG	hdereqtlInQ	MLveMDGFE	neg...IiVI	MAATNRPDvID	PALLRPGRIID	
PAS1	pKRghdstGv	t...dRVVnQ	LLtQMDGaEg	ldg...VyIl	AATSRPDliD	sALLRPGRIID	
mei-1	qGR...gnsGe	heasrRVKse	FLvQMDGsqn	kfdsrVfVl	AATNiPwleD	eALLR..RFE	
Consensus	-GR---GGG	E----RVV-Q	LL-QMDGFE-	-----V-VI	AATNRPD--D	PALLRPGRIID	
		*		*	*		
	356						402
NSF	vkmEiGLPDe	kGRLqILh..	IHT	arMrghqLLS	aDVDikeLAV	eTknFSGAEI	
SEC18	vqVEiHLPDe	kGRLqIFd..	IqT	kkMrennmMs	DDVnLaeLaa	lTknFSGAEI	
Mouse VCP	ReVDIgiPDa	tGRLeILq..	IHT	knMk.....La	DDVDLEqVAn	eThGhVGAD1	
p97	ReVDIgiPDa	tGRLeILq..	IHT	knMk.....Ls	DDVDLEqVAn	eThGhVGAD1	
CDC48	ReVDIgiPDa	tGRLeVLr..	IHT	knMk.....Ls	DDVDLEaLaa	eThGhVGAD1	
4S Protease	RkIEfPLPDe	ktkkrIFq..	IHT	srMt.....La	DDVtLdDliM	akddLSGADI	
TBP-1	RkIEfPLPDe	eaRarIMq..	IHT	rkMn.....vs	pDvNiYeELAr	cTddFNGAqc	
SUG1	RkIEfppPsv	aaRaeILr..	IHT	rkMn.....Lt	rgInLrkVae	knnGcSGADV	
MSS1	RkIEfPLPD1	eGRthIFk..	IHT	rsMs.....ve	rDirFEILAr	lcpnstGAEl	
ftsH	RqVvVgLPDv	eGRthILK..	VHM	rrvp.....La	pDIDaaiiAr	gTpgFSGAD1	
PAS1	ksVlcnIPte	seRLdILqai	vnskdkdtqg	kkfa.....Le	knaDLkIiAe	kTaGfSGAD1	
mei-1	krIfIPLPD1	daRkklIie..	ksmeptks	DEInYdDLAA	rTeGfSGADV		
Consensus	R-VEI-LPD-	-GRL-IL---	-----IHT	--M-----L-	DDVDLE-LA-	-T-GFSGADI	
		*		*	*		

FIGURE 3.—Alignment of *mei-1* related sequences. The single amino acid code is used and upper case indicates agreement with the consensus sequence or conservative substitutions. Dots indicate gaps inserted to produce the alignment. Underlined residues represent the putative ATP-binding site and asterisks indicate the position of the *mei-1* mutations listed in Table 2. The amino acid numbering is that for *mei-1*. Sequences were initially aligned using the GCG Pileup program. The sequences that have two copies of the ATP-binding site were truncated so that only the region including the one that had the greatest homology to *mei-1* was used. Most of the homology across the 13 *mei-1* related sequences was found to be limited to approximately a 200 amino acids portion of each protein. The position of this region of highest similarity was noted for each protein and the alignment presented here resulted from rerunning the Pileup program using these limits to maximize the alignment in this region. The GCG Pretty program calculated the consensus sequence using a minimum plurality of six. The following region of each of the sequences were used in the alignments: NSF, 223–440; SEC18, 236–462; mouse vcp, 201–412; p97, 201–412; CDC48, 210–421; 4S protease, 182–396; TBP-1, 148–362; SUG1, 145–359; MSS1, 172–386; ftsH, 149–362; PAS1, 694–914; *mei-1*, 190–402. The sequence of pig VCP differs from that of mouse VCP by only 2/806 amino acids, and is not included in the alignment.

three general classes. Mutations of the first class convert the dominant *gf* mutation into recessive null alleles showing defects in meiosis rather than mitosis. One of these, *ct46ct101*, is a nonsense mutation.

Members of the second class of intragenic revertants of *ct46* show the same recessive meiotic defects as the nulls but suppress the mitotic defects caused by *ct46* in *trans* in an antimorphic (dominant-negative) manner. These mutations are found throughout the last 60% of the protein. Three mutations, *ct93*, *b284* and *ct81*, are within or adjacent to the nucleotide binding site consensus.

Mutations of the third class of intragenic revertants of *mei-1(ct46)* behave as weakened dominants. These alleles retain the ability of their *ct46* parent to persist into mitosis, but the overall amount of *mei-1(+)* activity is decreased (but not eliminated) at both meiosis and mitosis (CLANDININ and MAINS 1993). Four of the five sequenced mutations are in the first one-quarter of the protein. Two independent mutations, *ct46sb9* and *ct46sb17* are exactly the same.

ct46ct103 is an unusual allele having properties common to both the dominant negatives (it can suppress *ct46* in *trans*) and weakened dominants (*mei-1* activity is decreased but not eliminated at both meiosis and mitosis). The *ct46ct103* mutation is at the same position of the ATP-binding consensus as the dominant-negative mutation *ct93*, but the two alleles result in different amino acid changes.

Of our mutations, perhaps the most interesting are those at or near one of the putative ATP-binding motifs. In previous work, we showed that the two mutations adjacent to the site, *b284* and *ct81*, behaved as antimorphic mutations (CLANDININ and MAINS 1993). We used gene dosage experiments to confirm that the ATP-binding site mutation *ct93* behaved in a similar manner. Antimorphic *mei-1* activity leads to meiotic nondisjunction, resulting in inviable embryos as well as a high proportion of XO males among the self-progeny of XX hermaphrodites. In wild-type broods, spontaneous X chromosome nondisjunction

	<i>SEC18</i>	mouse VCP	pig VCP	p97	<i>CDC48</i>	Protease 4S	<i>TBP-1</i>	<i>SUG1</i>	<i>MSS1</i>	<i>ftsH</i>	<i>PAS1</i>	<i>mei-1</i>
NSF	70 (82)	42 (61)	43 (61)	43 (62)	42 (61)	39 (60)	37 (59)	38 (58)	38 (56)	37 (59)	41 (55)	30 (52)
<i>SEC18</i>		41 (61)	40 (61)	41 (61)	42 (61)	35 (57)	35 (57)	35 (57)	38 (56)	36 (60)	37 (55)	32 (53)
mouse VCP			100	98 (99)	87 (95)	47 (67)	42 (64)	42 (63)	42 (65)	45 (63)	43 (58)	37 (59)
pig VCP				99 (99)	87 (95)	47 (67)	42 (64)	42 (63)	42 (65)	45 (63)	43 (58)	38 (59)
p97					86 (94)	47 (66)	43 (65)	42 (63)	42 (65)	44 (63)	43 (58)	38 (60)
<i>CDC48</i>						48 (66)	43 (62)	44 (65)	44 (65)	45 (63)	42 (58)	39 (59)
Protease 4S							60 (78)	58 (76)	57 (75)	42 (66)	33 (56)	37 (60)
<i>TBP-1</i>								57 (73)	55 (72)	44 (66)	34 (53)	38 (60)
<i>SUG1</i>									59 (73)	43 (64)	38 (56)	36 (58)
<i>MSS1</i>										46 (76)	35 (55)	34 (54)
<i>ftsH</i>											36 (61)	30 (53)
<i>PAS1</i>												29 (51)

FIGURE 4.—Pairwise percent identity and similarity scores of the *mei-1* related sequences. Pairwise percent identity (and similarity in brackets) scores are for the regions shown in the sequence alignment presented in Figure 3. Sequences that fall into subfamilies showing higher similarity scores are boxed. Scores were calculated using the GCG Distances program, summing pairwise identities (or similarities, using the program's default values for conservative substitutions) and dividing by the length of the shorter sequence, without gaps. VCP from mouse and pig are not identical, but differ by only 2/806 amino acids.

TABLE 2
Mutations of *mei-1*

Allele	Nucleotide ^a	Amino acid ^b	Type of mutation ^c
<i>ct46ct99</i>	CGT → TGT (1016)	Arg → Cys (36)	Null
<i>ct46sb18</i>	GAG → AAG (1156)	Glu → Lys (66)	Weakened dominant
<i>ct46</i>	CCA → CTA (1256)	Pro → Leu (99)	Dominant
<i>ct46sb9</i>	GGT → AGT (1336)	Gly → Ser (126)	Weakened dominant
<i>ct46sb17</i>			
<i>ct46sb22</i>	CGT → TGT (1342)	Arg → Cys (128)	Weakened dominant
<i>ct46sb3</i>	ATA → AAA (1590)	Ile → Lys (195)	Dominant negative
<i>b284</i>	CCA → CTA (1680)	Pro → Leu (225)	Dominant negative
<i>ct81</i>	CTC → TTC (1697)	Leu → Phe (231)	Dominant negative
<i>ct46ct103</i>	CCT → TCT (1709)	Pro → Ser (235)	Weakened dominant/dominant negative
<i>ct93</i>	CCT → CTT (1710)	Pro → Leu (235)	Dominant negative
<i>ct46ct84</i>	GAA → AAA (1928)	Glu → Lys (308)	Dominant negative
<i>ct46ct101</i>	TGG → TGA (2035)	Trp → Stop (343)	Null
<i>ct46sb23</i>	CCA → TCA (2084)	Pro → Ser (360)	Weakened dominant
<i>ct46ct89</i>	AGA → AAA (2247)	Arg → Lys (414)	Dominant negative
<i>ct46ct82</i>	GGT → GAT (2740)	Gly → Asp (473)	Dominant negative

^a The wild-type and mutant codons are shown, with the mutated nucleotide in bold. The position of the mutated base (in parentheses) corresponds to the numbering system of Figure 2, where they are indicated by a double underline.

^b The wild-type and mutated amino acids are shown, and the positions (in parentheses) of the mutated amino acids correspond to the numbering system of Figure 2, where they are indicated by a double underline. Mutant amino acids between positions 195 to 360 are indicated by asterisks in the multiple sequence alignment in Figure 3.

^c See text.

leads to 0.2% male progeny (HODGKIN *et al.* 1979). As shown in Table 3, *ct93* did indeed behave as an antimorph. There were fewer viable progeny and the proportion of males among the survivors rose as the dose of *ct93* was increased.

The antimorphic behavior of *ct93* was more pronounced at lower temperatures. To determine if the *cs* antimorphic activity was specific to the allele *ct93*, we compared the affect of gene dosage and temperature for several other *mei-1* alleles that *trans*-suppress the dominant mutation *mei-1(ct46)*. As shown in Table 3, *b284* also exhibited stronger antimorphic effects at a lower temperatures. The temperature effects were

less pronounced for *ct46ct82*. The gene dose of the complex allele *ct46ct103* had little effect at either temperature. Perhaps the assay is not sensitive enough to detect weak dominant-negative properties of *ct46ct103*, which is the least potent of the *mei-1(ct46)* *trans*-suppressors in Table 3. Alternatively, *ct46ct103* might suppress *mei-1(ct46)* by specific interaction to correct the *ct46* defect rather than doing so in an antimorphic fashion.

The *cs* properties of multiple antimorphic alleles could indicate that *mei-1* participates in an inherently *cs* process, which is revealed under conditions of low *mei-1* activity. This is consistent with the *cs* rescue or

TABLE 3
Gene dosage of *mei-1* mutations

<i>mei-1</i> maternal genotype ^a	25°		20°		15°	
	Viable progeny (%) ^b	Male (%)	Viable progeny (%) ^b	Male (%)	Viable progeny (%) ^b	Male (%)
+/+	82	0.2	81	0	81	0.1
<i>ct93</i> /+	93	2.5	ND ^c	ND	87	3.6
<i>ct93/ct93</i> /+	54	4.8	4.3	32	0.4	— ^d
<i>b284</i> /+	94	0.4	ND	ND	92	0
<i>b284/b284</i> /+	67	1.8	ND	ND	21	8.8
<i>ct46ct82</i> /+	98	0.4	ND	ND	92	0.6
<i>ct46ct82/ct46ct82</i> /+	11	26	ND	ND	6.7	24
<i>ct46ct103</i> /+	87	0	ND	ND	94	0
<i>ct46ct103/ct46ct103</i> /+	65	0.4	ND	ND	77	0

^a Complete maternal genotypes are as follows: *gaDp1*; *dpy-5 unc-29*, *unc-13 mei-1(ct93) daf-8/unc-29*, *gaDp1*; *unc-13 mei-1(ct93) daf-8*, *dpy-5 mei-1(b284) unc-29/unc-13 lin-11*, *gaDp1*; *dpy-5 mei-1(b284) unc-29*, *mei-1(ct46ct82) unc-29/unc-13 lin-11*, *gaDp1*; *mei-1(ct46ct82) unc-29*, *mei-1(ct46ct103) unc-29/+*, *lon-2/+*, *gaDp1*; *dpy-5 mei-1(ct46ct103) unc-29*.

^b Excludes unhatched embryos and arrested larvae. Between 500 and 2000 progeny were scored for each genotype.

^c Not determined.

^d Of three viable progeny, there was one dauer, one hermaphrodite and one male.

FIGURE 5.—Northern blot analysis of *mei-1* mRNA. Approximately equal amounts of polyA⁺ mRNA were loaded in each lane. A fragment of pHR8, from the *EcoRV* site at 942 in Figure 2 to the end of the coding sequence, was used as the probe.

mei-1 in transgenic lines. However, the *cs* antimorphic behavior may not be universal and so the conclusion must remain tentative.

Expression of *mei-1* mRNA: Genetic analysis indicates that *mei-1* is essential only during female meiosis (MAINS *et al.* 1990b). We used Northern blots and RNAase protection assays to determine how the temporal and spatial pattern of *mei-1* mRNA accumulation compares to the genetic analysis. A single message of 1.7 kb was seen on Northern blots of polyA⁺ RNA (Figure 5) at all stages of the life cycle. The steady state message levels were quantified by RNAase protection assays (Figure 6A). The highest levels of *mei-1* mRNA were seen in gravid adults as well as oocytes and embryos. By L3, the amount of accumulated *mei-1* message decreased to less than 10% of that found in gravid hermaphrodites; message levels then began to increase by L4.

High levels of *mei-1* mRNA in wild-type embryos were unexpected since meiotic cells lack eggshells and would have been destroyed by the alkaline hypochlorite procedure used to isolate them. Therefore, either the message persists for at least several hours after the protein functions or a small percentage of early em-

FIGURE 6.—Quantitation of *mei-1* mRNA levels. (A) RNAase protection to quantitate total *mei-1* message. Equal amounts of total RNA were treated as described in MATERIALS AND METHODS. The total counts as a percentage of the signal of adult wild-type hermaphrodites ("Gravid") are shown below each lane. Yeast tRNA was substituted for *C. elegans* RNA in the left-most lane, which represents background. The *mei-2(ct98)* lane was done in a separate experiment and was normalized to a sample of gravid wild-type hermaphrodites done in parallel. The times after harvesting (not post-fertilization) are indicated for the staged embryos. (B) Relative use of alternate *mei-1* splice sites. RT-PCR was performed on RNA from the same samples as used in (A) and was visualized with ethidium bromide. The PCR products from cDNAs corresponding to the shorter (pHR8) and longer (pHR24) forms of the *mei-1* mRNA were mixed and included as standards in the right-most lane; the upper and lower bands are 261 and 252 nucleotides in length, respectively. The relative amount of RT-PCR product between lanes is of no significance; lanes were loaded to give roughly equal amounts of the lower band. Several higher molecular weight PCR products were also detected. These did not correspond to any of the cDNAs and there are no other open reading frames in the third intron. These products were not investigated further.

bryos in the preparation (21% were less than 2 hr post-fertilization) contain high levels of *mei-1* mRNA.

To distinguish between these possibilities, embryos were harvested as before and the sample was split; one portion was immediately frozen (microscopic inspection showed it had the same age distribution as the previous preparation) while the rest were allowed to incubate for 3 or 7 hr at 25°. Only 1–2% of the embryos in these latter preparations had not passed the 2 hr stage, and these were probably dead. As shown in Figure 6A, unfertilized oocytes and freshly harvested embryos had high levels of *mei-1* mRNA and this level persisted for at least 3 hr. Therefore, the *mei-1* signal in freshly harvested embryos was not restricted to the youngest embryos and so the message persists long after its essential function.

To determine which adult tissues express *mei-1* mRNA, we used mutations that eliminate either the male or the female germline or both germlines. During the last larval stage (L4) of the *C. elegans* hermaphrodite, the germline produces 300–400 sperm, which are stored for later self-fertilization. When the animal matures, the germline switches to oocyte production. The *gf* mutation *fem-3(q20)* prevents the sperm to oocyte transition and so mutant hermaphrodites contain large numbers of sperm but no oocytes (BARTON *et al.* 1987). Hermaphrodites carrying *fem-1(hc17)* do not undergo the transient period of spermatogenesis and so adults contain oocytes but no sperm (KIMBLE *et al.* 1984). The mutation *glp-1(q231)* essentially lacks both germlines; about 15 germ cells (about 1% the normal number) are produced and all differentiate into sperm (AUSTIN and KIMBLE 1987). None of these mutations result in fertilized embryos. RNAase protection assays (Figure 6A) of adult hermaphrodites carrying these mutations showed that *mei-1* mRNA accumulation was almost entirely restricted to oogenesis: only about 10% of the wild-type level was seen in animals that had either no germline (*glp-1*) or had sperm but no oocytes [*fem-3(gf)*], while animals having oocytes but no sperm (*fem-1*) contained twice the wild-type level of *mei-1* message (increased levels may result because the animals bloat with unfertilized oocytes). However, *mei-1* mRNA was clearly present in tissues other than the female germline. The low levels of *mei-1* mRNA detected in *glp-1* and *fem-3* were above background and were unlikely due to contamination of nonsterile worms.

RT-PCR was used to determine the stage-dependent relative use of the alternatively spliced *mei-1* messages. Since the mRNAs differ by only nine internal nucleotides, their relative efficiencies of reverse transcription and PCR amplification should be similar when flanking primers are used. Therefore, within a sample, the relative levels of product should be proportional to the *in vivo* levels, even though the PCR reactions were not done in a quantitative fashion. Expression of the longer form was almost exclusively restricted to the female germline; it was barely de-

tectable in other tissues and stages (Figure 6B).

Mutations of the gene *mei-2* were identified as dominant extragenic suppressors of *mei-1(ct46)* and show recessive meiotic defects similar to *mei-1(lf)* (MAINS *et al.* 1990b). To determine if *mei-2* regulates *mei-1* expression at the level of transcription, *mei-1* mRNA accumulation was measured for the viable allele *mei-2(ct98)*, because it could be grown as a pure (homozygous) population. Although 80% of the embryos from *mei-2(ct98)* hermaphrodites hatch at 25°, two-thirds show abnormal polar body formation, indicating that the level of *mei-2* activity is barely adequate. Nevertheless, *mei-1* mRNA accumulated to near normal levels in *mei-2(ct98)* hermaphrodites (Figure 6A).

DISCUSSION

Genetic evidence indicates that the *mei-1* gene of *C. elegans* is required only for meiotic spindle function in the female germline (MAINS *et al.* 1990b; CLANDININ and MAINS 1993). The oocyte's meiotic and the zygote's mitotic spindles differ in many respects, including their orientation, morphology and alignment of the chromosomes on the spindle (NIGON *et al.* 1960; HIRSH *et al.* 1976; STROME and WOOD 1983; ALBERTSON 1984; ALBERTSON and THOMSON 1993). In addition, centrioles are present in the zygote, where they are contributed by the sperm, but are absent during female meiosis. *mei-1* activity is a candidate for some aspect of the spindle that differs between the two types of division. In its absence, meiotic spindle formation is defective while its presumed ectopic activity during embryogenesis can disrupt the early zygotic cleavages.

We have cloned *mei-1* using transformation rescue. The gene resides near a hotspot for recombination, in an area of high gene density (Table 1 and Figure 1). *mei-1* mRNA is most highly expressed in post-mitotic oocytes, and high levels remain in mid- to late-stage embryos (Figure 6). Thus, *mei-1* mRNA persists well beyond the time the *mei-1* product functions. Since temperature-shift experiments with the dominant allele *mei-1(ct46)* showed that post-meiotic *mei-1* expression could be lethal for at least 2 hr post-fertilization (MAINS *et al.* 1990a), there must be some form of post-transcriptional inactivation of *mei-1* after its meiotic function. Possible candidates for this regulation include the genes *mel-26* and *zyg-9*, mutants of which show mitotic phenotypes similar to *mei-1(ct46)* and act as genetic enhancers of that mutation. However, *mei-1(null)* is not epistatic to *zyg-9*, making it unlikely that *zyg-9* is the inactivator of *mei-1* (MAINS *et al.* 1990b). In contrast, *mei-1(null)* is epistatic to *mel-26* (P. E. MAINS, unpublished data), so *mel-26* is a candidate for the post-meiotic inactivator of *mei-1(+)*.

An alternative explanation for the persistence of *mei-1* mRNA is that although *mei-1(ct46)* may assemble into the mitotic spindle, *mei-1(+)* cannot, and so its

post-meiotic expression is irrelevant (CLANDININ and MAINS 1993). Levels of *mei-1* mRNA that are clearly above background persist, albeit at low levels, throughout the larval stages and are found in tissues other than the germline (Figure 6). The genetic analysis shows that this mRNA fulfills no essential function and it is possible that this expression is also irrelevant.

Two potential *mei-1* protein products result from alternate use of splice acceptor sites in the fourth exon (Figure 2). It is not obvious how the two forms might differ in function; the addition of the three amino acids specific to the longer form, Y-F-R, result in no dramatic differences in predicted protein motif or secondary structure based on computer analysis (using the GCG software package, version 7.2). It may be significant that a dominant negative mutation, *ct46ct89*, is two amino acids immediately amino terminal to the site of the alternative sequences. The alteration caused by *ct46ct89*, R to K, is a conservative substitution and yet it has a pronounced affect on protein function. This probably indicates the importance of this region to the protein. It is also interesting that the expression of the mRNA encoding the longer form is almost exclusively restricted to the female germline. It may be significant that the acceptor splice site for the longer message, CTTAAG, differs from the consensus used in *C. elegans*, TTTACG (FIELDS 1990). In contrast, the shorter *mei-1* message as well as the acceptor sites in the first two introns exactly match the *C. elegans* consensus. The germline-specific expression of the longer form could reflect germline specific splicing.

mei-1 is related to a group of putative ATPases with rather disparate functions, including transcription, proteolysis, membrane function and cell cycle regulation (Figures 3 and 4). Although *mei-1* shows the same degree of divergence from all of the genes, most of the proteins fall into groups sharing higher sequence similarity. Several members of one subfamily have been implicated in cell cycle control. *cdc48* mutations of *Saccharomyces cerevisiae* (FRÖHLICH *et al.* 1991) arrest cell division prior to spindle pole body separation, a defect that is perhaps analogous to the failure of meiotic spindle formation in *mei-1(lf)*. The mouse valosin containing protein (VCP, EGERTON *et al.* 1992) has also been implicated in control of cell division since it is among the first proteins to be tyrosine phosphorylated upon T lymphocyte activation. However, a subset of cellular VCP is associated with clathrin and it has been suggested that VCP might have a chaperone-like activity (PLEASURE *et al.* 1993). VCP has been found in a number of mammalian species (KOLLER and BROWNSTEIN 1987; PLEASURE *et al.* 1993). The related *Xenopus* protein p97 (PETERS *et al.* 1990, 1992), whose function is unknown, shows *in vitro* ATPase and GTPase activities.

The genes *SEC18* of *S. cerevisiae* (EAKLE *et al.* 1988)

and NSF of hamsters (WILSON *et al.* 1989) are involved in the vesicle fusion pathway. Other genes, while not closely related to *SEC18* and NSF, are also involved in membrane function. The *S. cerevisiae* gene, *PAS1* is required for peroxisome assembly (ERDMANN *et al.* 1991) and the *Escherichia coli* gene *ftsH* is an integral membrane protein needed for cell septation (TOMOYASU *et al.* 1993).

Another subfamily of *mei-1* related genes are thought to affect transcription. These include the human proteins *TBP-1* (NELBOCK *et al.* 1990) and *MSS1* (SHIBUYA *et al.* 1992) and *SUG1* of *S. cerevisiae* (SWAFFIELD *et al.* 1992; GOYER *et al.* 1992). However, another protein closely related to these three is the 4S subunit of the human 26S ATP-dependent protease, which degrades ubiquitinated proteins (DUBIEL *et al.* 1992). As pointed out by DUBIEL *et al.* (1992), the effect of the other gene products on transcription could be an indirect effect of proteolysis. *TBP-1*, *MSS1*, *SUG1* and the 4S protease subunit include only one copy of the conserved region, which in all others sequences (excepting *mei-1*), is found twice.

It is hard to relate the divergent activities of the *mei-1*-related proteins by a common theme and so it seems likely that the sequence similarities do not reflect the conservation of a specific activity. Perhaps the proteins share a more general type of function, for example, ATP-mediated protein-protein interactions as suggested for the VCP family (PLEASURE *et al.* 1993)]. It is not clear if *mei-1* is the true *C. elegans* homolog of any of these genes. For example, the cDNA cm20a12 is much more closely related to the *CDC48* subfamily of genes than is *mei-1*. This cDNA was reported by WATERSTON *et al.* (1992) among their collection of partial sequences of 1500 random *C. elegans* cDNA sequences. We have completed the sequence of cm20a12 (S. C. MAGUIRE and L. STEBLECKI, unpublished data) and found that it codes for a protein that is 69% identical to the *CDC48*. This cDNA is even more similar to the VCPs, showing 80% identity. As an extension of another compilation of partial sequences of *C. elegans* cDNAs (MCCOMBIE *et al.* 1992), a gene more closely related to *TBP-1* (showing 85% identity) than is *mei-1* has been found (R. MCCOMBIE, unpublished data).

One structural feature shared by the *mei-1* related proteins is a likely nucleotide binding site. The "Walker motif A" or "P-loop" (phosphate-binding loop, first of the two underlined regions in Figures 2 and 3) is common to both ATP and GTP-binding proteins. The proposed consensus sequence is G-X₄-G-K-S/T, where X is any residue, but G and P are common (WALKER *et al.* 1982; DEVER *et al.* 1987; CHIN *et al.* 1988; GORBALENYA and KOONIN 1989; SARASTE *et al.* 1990; STORY and STEITZ 1992). This region shows almost complete conservation among the *mei-1* related genes, even though the consensus

sequence includes a large degree of flexibility. ATP-binding proteins have an additional conserved region, the "Walker motif B," which may be involved in Mg^{2+} binding (the second underlined region in Figures 2 and 3, FRY *et al.* 1986; YOHDA *et al.* 1988; BLACK and HRUBY 1992; THOMAS *et al.* 1992). A consensus sequence for this site is R/K/H-X₅₋₈- ϕ ₄-D/E, where ϕ is any hydrophobic amino acid. Again, the *mei-1* related proteins show remarkable conservation of this region; most include the sequence I-I-F-I-D. (The VCP-related sequences require an X₄ rather than X₅₋₈ to meet the consensus).

Four mutations of *mei-1* with dominant-negative properties occur within an 11 amino acid region at or near the first ATP-binding motif. Two mutations, *ct46ct103* and *ct93* are in the Walker motif A, showing that this region is indeed important for *mei-1* function. Two additional mutations, *b284* and *ct81*, are found adjacent to it. Genetic analysis of this motif demonstrated that it is critical to the activity of a number of proteins, including members of the *ras* gene family (TODA *et al.* 1985; BARBACID 1987; WAGNER *et al.* 1987; BEITEL *et al.* 1990), *UvrA* (MYLES *et al.* 1991), thymidine kinase (BLACK and HRUBY 1990), the cystic fibrosis transmembrane conductance regulator (TSUI 1992), ATP synthase (FUTAI *et al.* 1992; THOMAS *et al.* 1992), adenylate kinase (REINSTEIN *et al.* 1988), *RAD3* (SUNG *et al.* 1988) and *EF-Tu* (JACQUET and PARMEGGIANI 1988). *ct46ct103* and *ct93* change the second X of the Walker motif A consensus from a P to an S or an L, respectively. This position corresponds to the critical G₁₂ residue of *ras*, which if changed to any residue but P (the wild-type amino acid in *mei-1*), results in oncogenic transformation (SEEBURG *et al.* 1984; COLBY *et al.* 1986; TRAHEY *et al.* 1987). One might suspect that mutations in this residue would result in a simple null phenotype, but both *ct46ct103* and *ct93* have dominant-negative properties. *ct46ct103*, which results in the more conservative of the two amino acid changes, is homozygous viable.

Dominant-negative mutations have been described in the Walker A motif of other proteins, although not at the corresponding position as *ct46ct103* and *ct93*. Included are mutations of *ras* and *ras*-like genes from mammals (FARNSWORTH and FEIG 1991), *C. elegans* (HAN and STERNBERG 1991) and yeast (POWERS *et al.* 1989; RUGGIERI *et al.* 1992), kinesin-like genes of *Drosophila* (RASOOLY *et al.* 1991) and yeast (MELUH and ROSE 1990) and the *C. elegans* myosin heavy chain (BEJSOVEC and ANDERSON 1990). Dominant-negative mutations (MULLER 1932) could act by competing with wild type for the same site or a limiting factor, as has been proposed for the kinesin- and *ras*-like genes, or by the coassembly of mutant and wild-type subunits resulting in defective multimers (HERSKOWITZ 1987). We favor an assembly mechanism for the

mei-1 dominant-negative mutations since genetic evidence suggests that *mei-1* forms multimers (CLANDININ and MAINS 1993). Indeed NSF, CDC48 and p97 have been shown to form multimers (BLOCK *et al.* 1988; FRÖHLICH *et al.* 1991; PETERS *et al.* 1992). The ATPase activity might be necessary for proper assembly, as has been proposed for antimorphic myosin alleles (BEJSOVEC and ANDERSON 1990). The exact role of the nucleotide-binding motif and its relationship to *mei-1* function awaits further molecular analysis.

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