Metaphase to Anaphase (mat) Transition–defective Mutants in Caenorhabditis elegans

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Abstract. The metaphase to anaphase transition is a critical stage of the eukaryotic cell cycle, and, thus, it is highly regulated. Errors during this transition can lead to chromosome segregation defects and death of the organism. In genetic screens for temperature-sensitive maternal effect embryonic lethal (Mel) mutants, we have identified 32 mutants in the nematode Caenorhabditis elegans in which fertilized embryos arrest as one-cell embryos. In these mutant embryos, the oocyte chromosomes arrest in metaphase of meiosis I without transitioning to anaphase or producing polar bodies. An additional block in M phase exit is evidenced by the failure to form pronuclei and the persistence of phosphohistone H3 and MPM-2 antibody staining. Spermatocyte meiosis is also perturbed; primary spermatocytes arrest in metaphase of meiosis I and fail to produce secondary spermatocytes. Analogous mitotic defects cause M phase delays in mitotic germline proliferation. We have named this class of mutants “mat” for metaphase to anaphase transition defective. These mutants, representing six different complementation groups, all map near genes that encode subunits of the anaphase-promoting complex or cyclosome, and, here, we show that one of the genes, emb-27, encodes the C. elegans CDC16 ortholog.

Key words: meiosis • metaphase arrest • cell cycle • C. elegans • anaphase promoting complex

Introduction

Eukaryotic cell division is a highly regulated process during which cells duplicate their chromosomes, align them at a metaphase plate, and then segregate them equally to each of their two daughter cells. Accurate chromosome segregation during the metaphase to anaphase transition is essential for proper cell function, since errors in chromosome segregation can lead to cell death, sterility, birth defects, or malignant cancers. Not surprisingly, genetic studies of yeast mitosis suggest that the transition out of metaphase and through anaphase is highly regulated both in terms of the sequence of events and the existence of checkpoint regulators. Driving the process forward is a multiprotein E3–ubiquitin ligase complex known as the anaphase promoting complex or cyclosome (APC/C)1 that functions to sequentially target key cellular components for proteolytic destruction (Zachariae and Nasmyth, 1999). APC/C initiates the metaphase to anaphase transition by first targeting the destruction of the anaphase inhibitor Pds1/Cut2 (Yamamoto et al., 1996) whose absence permits the separation of sister chromatids (Giosef et al., 1998). APC/C then drives the process forward as it subsequently targets Ase1 and cyclin B for destruction (Peters, 1999). Ase1 destruction permits spindle elongation during anaphase B (Juang et al., 1997), and cyclin B destruction permits M phase exit.

Cells must avoid premature activation of APC/C and precocious exit from metaphase, because once chromosomes separate during anaphase, defects stemming from misaligned or stray chromosomes can no longer be corrected. The idea that cells might have a metaphase checkpoint (also called spindle assembly checkpoint or kinetochore attachment checkpoint) was first suggested by the observation that cells treated with microtubule inhibitors arrest in a metaphase-like state (Eigsti and Dustin, 1957). Recently, this arrest has been shown to be mediated by a set of checkpoint proteins that specifically block the activity of the APC/C in the presence of either abnormal spindles or unattached kinetochores (for review see Gardner and Burke, 2000).
A combination of genetic, cytological, and biochemical studies has elucidated many of the molecular mechanisms that underlie these critical cell cycle controls (Murray and Hunt, 1993; Nasmyth et al., 2000). These studies reveal that the same basic mechanisms drive the processes of cell division and chromosome separation in both yeast and animal cells, and that many key players are highly conserved in their molecular sequences. Nevertheless, given that many cells differ in the specific details of their cell division processes, underlying mechanistic differences are also likely to exist. For instance, animal cells disassemble their nuclear envelopes before chromosome separation, but yeasts do not. Since the mitotic proteins of yeasts and animal cells function within distinctive cellular environments, they may also be regulated differently. Likewise, the mechanisms that separate sister chromatids during mitosis and meiosis II may differ from those that separate chromosomes during meiosis I. Although the loss of sister chromatid cohesion is critical for all of these divisions, centromere cohesion is specifically protected only during meiosis I (Miyazaki and Orr-Weaver, 1994; van Heemst and Heyting, 2000). On the other hand, both the APC/C target, Pds1, and the APC/C regulator, MAD2, have been recently shown to regulate the separation of not only sister chromatids, but also homologous chromosomes (Salah and Nasmyth, 2000; Shonn et al., 2000). Another largely unexplored area of investigation is whether APC/C-related proteins and their substrates function differently during oocyte and spermatocyte meiosis, given that oocyte meiosis occurs on an acentriolar spindle, whereas sperm meiosis employs a centriole-based spindle.

Here, we present the isolation and analysis of a collection of temperature-sensitive (ts) mutants in the nematode *Caenorhabditis elegans* whose defects specifically block the metaphase to anaphase transition during the germline mitotic and meiotic divisions. Furthermore, we show that at least one of these genes encodes a subunit of the APC/C. This mutant collection provides an important addition to the analysis of the metaphase to anaphase transition for several reasons. First, because the alleles are ts, they can be used to analyze the role of essential genes during gametogenesis. Such analysis would not be possible in null mutants of genes that are required for the early mitotic divisions of either the soma or the developing germline. Second, because the germline is the only tissue in *C. elegans* adults that continues to undergo cell divisions, adult upshift experiments allow germline-specific defects to be studied in the absence of complicating somatic defects. Third, these germline cell divisions are both interesting and distinct for the following reasons. (a) A unique aspect of meiosis I is that the paired homologues are linked by chiasmata. (b) Oocyte and spermatocyte meiosis differ drastically in their associated spindle structures and cytokinesis patterns. (c) Mitotically dividing germ cell nuclei exhibit an intriguing cell cycle independence despite the fact that they are cytoplasmically linked (Hirsh et al., 1976). Finally, because the meiotic germ cells are arranged in temporal order along the distal–proximal axis of the adult gonad, sequential stages of meiosis can be easily observed. With a focus on the metaphase to anaphase transition, this collection of ts mutants provides a unique opportunity to compare the phenotypic consequences of M phase defects during three different types of cell divisions: oocyte meiosis, spermatocyte meiosis, and germline mitosis.

### Materials and Methods

#### Genetic Screen for Ts Embryonic Lethal Mutants

Ts embryonic lethal mutants were isolated in two separate genetic screens, both modified from Kempfes et al. (1988). All steps were carried out at 15°C, except where noted. The ts alleles were isolated as follows: L4 hermaphrodites of the genotype lin-3(e1147) IV; lin-2(e309); axIsX6 X were mutagenized with 25 mM ethylmethanesulfonate using standard procedures (Brenner, 1974). lin-2 mutant hermaphrodites lack a functional vulva, and, thus, retain most of their progeny in their uteri. axIsX6 is a chromosomally integrated transgene containing a pes-10:GFP fusion that was used in a secondary screen not described here (Wallenfang, M. and G. Seydoux, unpublished results). F2 animals, synchronized by hypophyseal treatment of F1 gravid adults (Emmons et al., 1979), were shifted as L4 larvae to 25°C for 20 h and back down to 15°C for an additional 20 h. F2 animals surviving dead embryos were transferred individually to new plates at 15°C. As a control for viable F3 progeny, indicating an embryonic lethal mutant was rescued by the shift to the permissive temperature. From approximately 1,000,000 mutagenized genomes, 1,197 ts embryonic lethal mutants were isolated. With the protocol used, most, but perhaps not all, of these alleles are independent. The terminal phenotypes of these mutants was determined in a secondary screen by shifting L4 hermaphrodites to 25°C for 12–16 h and viewing the accumulated in utero embryos under DIC (differential interference contrast) optics on a Zeiss Axioplan II compound microscope (ZEISS). From 1,197 ts embryonic lethal mutants, we recovered 30 mutants that arrest at the one-cell stage. Five of these one-cell arrested mutants have cytokinesis defects and will not be described here. The remaining 25 ts alleles are the subject of this report.

The or alleles described here were isolated in a similar screen, except for the following differences: the starting lin-3 strain lacked lin-1 mutations and integrated transgenes. ethyl methanesulfonate was used at 20 mM, and F2 animals were upshifted as L4 larvae for 25–30 h. For screening, F2 animals were suspended in M9 buffer, and bloated animals containing dead embryos were transferred individually to new plates at 15°C. From approximately 1,000,000 mutagenized genomes, ~300 to embryonic lethal mutants were isolated. Of these, 14 mutants arrested at the one-cell stage. Seven of these are described here, and the remaining seven have unrelated cytokinesis defects (Encalada et al., 2000).

#### Strains and Alleles

The Bristol strain N2 was used as the standard wild-type strain. The marker mutations, deficiencies, and balancer chromosomes used are listed from Kemphues et al. (1988). All steps were carried out at 15°C, except where noted. The Bristol strain N2 was used as the standard wild-type strain. The marker mutations, deficiencies, and balancer chromosomes used are listed from Kemphues et al. (1988). All steps were carried out at 15°C, except where noted.

### Genetic Analysis

 Newly isolated mutations were outcrossed three to five times, and complementation was tested to one another until map positions were established. Linkage analysis, deficiency complementation, two factor mapping, and transgene mapping were used to map the three factor mapping were used to map the three loci and the new alleles to LGI between the left breakpoint of mat-1 or the right breakpoint of mat-1, but not under either map data was downloaded previously (Cassada et al., 1981; Sigurdson et al., 1984; Furuta et al., 2000). Our mapping crosses place mat-1 on LGI close to, or left of, unc-63. Deficiency crosses place mat-2 on LGI between the left breakpoint of mndD(87) and the right breakpoint of mndD90, but not under either mndD86 or mndD87. mat-3 maps left of dpy-1 and daf-7 on the far left arm of LGIII (see Fig. 2). Data from these crosses are available from the *C. elegans* database WormBase (http://www.wormbase.org). Because mat-1(ax72) and mat-1(ax520) homozygotes were difficult to maintain at 15°C,
these alleles were maintained as heterozygotes over an unc-38(x20) dpy-5(67u1) chromosome. Likewise, emb-27(ax189) was maintained over the ncrC1 balancer chromosome. Because mat-1(ax212) is leaky at 25°C, it was characterized at 26°C. To facilitate complementation tests and the analysis of male phenotypes, genetic doubles of various mat alleles with either him-5(V) or him-8(IV) were constructed; all male and spermatogenesis phenotypes were secondarily confirmed in him+/+ strains.

**Growth Conditions**

Mutant stocks were maintained at either 15 or 16°C. For the two-cell embryo shift-up experiments, adult hermaphrodites were raised at 15°C, and then dissected in a drop of egg salts (Edgar, 1995), using 27.5 gauge needles. Isolated two-cell embryos were transferred to prewarmed 25.5°C plates for further development. In L1 shift experiments, third- and fourth-stage embryos were picked to fresh plates at 15°C and shifted to 25.5°C after hatching. Alternatively, L1s were directly picked from 15 to 25.5°C. For the analysis of spermatocyte meiosis defects, him-8 doubles of mat mutants were grown at 16°C until the late L3 larval stage. Males were then picked to fresh plates and grown at 25°C until they reached early adulthood. For the analysis of embryos and gonads within somatically unaffected adults, young adult hermaphrodites with few, if any, embryos in their uteri were shifted up to 25.5°C for 6–7 h. Embryos and gonads were then isolated for analysis, as described below. For many alleles, it proved essential to maintain the incubators at 25.5 ± 0.5°C to attain fully penetrant mutant phenotypes.

**Immunohistochemistry and Other Phenotypic Analysis**

For the analysis of early embryos and hermaphrodite gonads, upshifted adult hermaphrodites were transferred to a 5-μl drop of egg buffer (Edgar, 1995) on a slide subbed with poly-L-lysine (Sigma-Aldrich). Embryos and gonads were released using 27.5 gauge needles, freeze-cracked, and processed for antibody staining (Miller and Shakes, 1995). For tubulin immunostaining, sperm spreads were generated for Hoechst/DIC analysis by placing a coverslip over the isolated gonad and gentle pressure was applied to generate a monolayer of spermatocytes and spermatids. The spreads were immediately analyzed using Nomarski/DIC optics while simultaneously illuminating the specimen with UV epifluorescence to visualize the DNA. For DAPI/tubulin immunostaining, sperm spreads were generated on ColorFrost Plus slides (Fisher Scientific) in buffer without Hoechst. The samples were freeze-cracked in liquid nitrogen before a 12–20 h incubation in –20°C methanol. Tubulin immunostaining was carried out as above.

**Physical Mapping of emb-27**

emb-27 lies within the gene subcluster of L2I (Cassada et al., 1981). Fine-scale genetic mapping studies using a large set of overlapping deficiencies placed emb-27 to the right of unc-105 (Sigurdson et al., 1984) and to the left of mig-5 (Guo, 1995). The cloning and physical mapping of unc-105 and mig-5 to the cosmid C41C4 (Liu et al., 1996) and TOSC512 (Guo, 1995), respectively, positioned emb-27 to a region of three overlapping cosmids, C41C4, F10B5, and TOSC512. Information from the C. elegans Sequencing Center was used to identify candidate genes from this region.

**RNA-mediated Interference (RNAi)**

An expressed sequence tag clone of F10B5.6 (CEESZ14) was used as the template for CeCdc16 cDNA synthesis. CEESZ14 is a truncated cDNA that contains the last 20% of the predicted F10B5.6 open reading frame and the 3’ untranslated region. M13 forward and reverse primers were used to amplify this cDNA insert. Sense and antisense RNAs were synthesized using a T3 and T7 in vitro transcription kit (Ambion). The RNA synthesis reactions were mixed and incubated at 70°C to denature the RNAs and then slow cooled at room temperature to anneal the complementary strands. Young wild-type adult hermaphrodites were microinjected by standard protocols (Mello et al., 1991) with dsRNA, and they were allowed to recover 12 h before analysis by DIC microscopy or fixation for DNA staining. Similarly, methods were used to analyze the RNAi phenotypes of other emb-27 gene candidates.

**cDNA Synthesis and DNA Sequencing**

Total RNA was prepared from wild-type and emb-27(g48) hermaphrodites by standard RNA isolation methods. First strand cDNA was prepared with a reverse transcription–PCR kit according to the manufacturer’s instructions (Gene Amp Reverse Transcription-PCR; PerkinElmer). F10B5.6-specific cDNAs were PCR amplified with oligonucleotide primers corresponding to either the SL1 trans-spliced leader RNA or the predicted start codon coupled with a primer that contained the predicted translation stop codon. The PCR products were gel purified using Wizard columns (Promega) and cloned into a pBluescript vector (Stratagene). Automated DNA sequencing of the F10B5.6 cDNA was performed using standard methods. A contiguous consensus contig was generated by the CAP Sequence Assembly program (Huang, 1992) accessed via the Baylor College of Medicine Search Launcher. Two independent PCR reactions were sequenced for both wild-type and g48, and they were used to determine the consensus sequence of both the wild-type and g48 F10B5.6 gene sequence.

**Results**

emb-27(g48) Mothers Produce Meiotic One-Cell Arrested Embryos

Our initial interest in mutants that arrest at the metaphase to anaphase transition of meiosis I arose from our analysis of the maternal effect embryonic lethal (Mel) mutant emb-27(g48). The emb-27(g48) mutant was originally isolated in a genetic screen for ts Mel mutants (Cassada et al., 1981). emb-27(g48) was reported to produce embryos that arrested at the one-cell stage and failed to form polar bodies, the discarded karyocytes of oocyte meiotic divisions (Denich et al., 1984). Although the terminal phenotype of the mutant emb-27(g48) embryos has been reported to be somewhat variable, growth under strict temperature conditions (see Materials and Methods) resulted in a highly penetrant and consistent meiotic one-cell arrest.

Whereas wild-type hermaphrodites continue rapidly cleaving embryos of many different ages, emb-27(g48) mutant hermaphrodites contain only one-cell embryos (Fig. 1, A and B). Typically, wild-type hermaphrodites have only a single newly fertilized embryo that is at the same meiotic one-cell stage as the entire clutch of emb-27 embryos. A similar early arrest phenotype has been observed for embryos in which the expression of three C. elegans cdc-25 genes has been disrupted by RNAi; both emb-27(g48) and cdc-25 (RNAi) embryos arrest at the one-cell stage without forming pronuclei (Ashcroft, N., and A. Golden, manuscript in preparation). This phenotype is distinct from cleavage-defective mutants such as air-2 and cys-1,
which arrest at the one-cell stage but nevertheless complete meiosis, exit M phase, and produce pronuclei (Schumacher et al., 1998; Swan et al., 1998).

Before fertilization, emb-27 oocytes are indistinguishable from their wild-type counterparts. When stained with the DNA dye DAPI, diakinetic emb-27 oocytes were found to contain a normal complement of six bivalents, indicating that the early meiotic events of homologue pairing, homologous recombination, and chromosome condensation occurred normally. As in wild-type animals, mature emb-27 oocytes undergo nuclear envelope breakdown just before ovulation. After fertilization, they exhibit many, but not all, of the normal events of egg activation. For instance, the fertilized emb-27 (g48) oocytes assume an oval-shaped morphology and initiate eggshell deposition (Fig. 1 B). However, none of these mutant embryos progress beyond the normal meiotic one-cell stage (n > 200) (Fig. 1, B and D). DAPI staining of the mutant g48 embryos revealed that both the sperm chromosomes and oocyte chromosomes remain condensed and positioned near the cortex. This persistent pattern, a cluster of tightly arrayed oocyte chromosomes and a single sperm chromatin mass (Fig. 1 D), is identical to what occurs during the normally transient, meiotic one-cell stage of wild-type embryogenesis (Fig. 1 C). Likewise, the eggshells of emb-27 embryos remain osmotically sensitive, another
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Identification and Isolation of Additional emb-27–like Mutants

Because Cdc16 is only 1 of the 8–12 APC/C subunits, it seemed likely that genetic screens for similar one-cell arrest mutants would identify mutants in additional APC/C and APC/C-related genes. Using the emb-27(g48) phenotype as a guide, we screened preexisting collections of ts Mel mutants for additional members of this class. From several different mutant collections (Miwa et al., 1980; Wood et al., 1980; Cassada et al., 1981), we identified two such genes: emb-1(hc57, hc62) and emb-30(g53) (Fig. 2). Although emb-1 has not yet been cloned, emb-30 encodes an ortholog of APC4 (Furuta et al., 2000).

We also initiated a large scale screen for new one-cell arrested ts mutants. Our goal was to isolate additional alleles of emb-1, emb-27, and emb-30, as well as novel members of this phenotypic class. To facilitate the isolation of Mel mutants, screens were carried out in a genetic background of an egg laying–defective mutant (lin-2) (Kemphues et al., 1988). The mutant phenotypes were scored in hermaphrodites that had been upshifted to the restrictive temperature of 25°C as L4 larvae. Of ~1.9 million haploid genomes screened, >1,400 ts Mel mutants were isolated. Of these, 44 mutants produced one-cell embryos when homozygous L4s were shifted to 25°C, and 32 of these exhibited meiotic progression defects similar to those of emb-27(g48). Mapping crosses and complementation tests revealed that seven of these mutants are new alleles of emb-27 (6 alleles) and emb-30 (1 allele), whereas the remaining 25 alleles define three new complementation groups: mat-1 (6 alleles), mat-2 (7 alleles), and mat-3 (12 alleles). The genetic map positions of these genes are shown in Fig. 2.

Meiotic One-Cell Mutant Embryos Are Blocked in Metaphase of Meiosis I

Between fertilization and the first mitotic division, the one-cell stage of C. elegans embryogenesis encompasses the meiotic divisions of the oocyte, a round of DNA synthesis, the conjunction of the pronuclei, and anterior–posterior polarization of the embryo (Albertson, 1984; Albertson and Thomson, 1993; Kemphues and Strome, 1997;...
Sadler and Shakes, 2000). Fig. 3 shows DNA and tubulin images of the events that lead up to the first mitosis in wild-type embryos. Before fertilization, the primary oocytes undergo a prolonged pause in diakinesis of meiosis I. At this stage, they contain six highly condensed bivalents within a nuclear envelope and a uniform meshwork of cytoplasmic tubulin (Fig. 3, A and B) (Albertson, 1984). Immediately before fertilization, they undergo meiotic maturation, which includes nuclear envelope breakdown (McCarter et al., 1999). After fertilization, the oocyte-derived bivalents align and congress to form a metaphase plate with the five autosomal bivalents surrounding the sex chromosome bivalents in an axial orientation. This arrangement appears as either a “pentagonal array” or, in side view, as a cluster of two or three axially aligned bivalents (Fig. 3 C). The associated meiotic spindle is a barrel-shaped structure that lies adjacent and parallel to the cortex (Fig. 3 D) (Albertson and Thomson, 1993). During this time, the quiescent sperm chromatin remains as a single mass of condensed DNA near its site of entry (Sadler and Shakes, 2000). During anaphase, the spindle rotates to its telophase position, perpendicular to the cortex (Fig. 3, E and F), before extruding one set of oocyte chromosomes into the polar body (Fig. 3 G). Meiosis II proceeds in a similar manner (Fig. 3, G and H) (Albertson, 1984). After these meiotic divisions, the maternal and paternal chromosomes decondense, are enclosed within nuclear envelopes, and undergo DNA synthesis (Sadler and Shakes, 2000). During this same period, the sperm centrioles duplicate and begin nucleating microtubules (Fig. 3 J) (Albertson, 1984). Subsequently, the zygote enters a period of transitional prophase during which the maternal pronucleus migrates posteriorly to meet the paternal pronucleus (Fig. 3, I and J). The joined pronuclei then move centrally before initiating mitosis (Fig. 3 L).

To further investigate the mutant defects, oocytes and embryos of adult-upshift mutant mothers were examined. No prefertilization defects were detected; DAPI staining revealed the expected six bivalents per oocyte and maturation defects were undetectable by DIC optics. To analyze defects after fertilization, wild-type and mutant embryos were costained with DAPI and anti-tubulin antibodies. Embryos within the uteri of wild-type mothers ranged in age from the 1–50-cell stage (Fig. 4, A and B), and, because the 1-cell stage lasts <1 h at 25°C (Ward and Carrel, 1979; McCarter et al., 1999), only one or two of these embryos were at the one-cell stage of development. In contrast, each of the mutant mothers was filled with 1-cell embryos, all of which were arrested at the same stage of development (Fig. 4, C–N, compare with Fig. 3, C and D). Within the mutant embryos, the oocyte chromosomes had properly condensed and remained hypercondensed, and the sperm centrosomes remained quiescent. As assessed by DAPI/tubulin staining, all 32 alleles exhibited an identical oocyte meiosis defect; the embryos arrest with what appears to be a normal meiotic spindle (compare Fig. 3, C and D, with Fig. 4, C–N). Because these meiotic one-cell mutants share a common metaphase-arrest defect, we call them “mat” for metaphase to anaphase transition defective.

**mat Mutant Embryos Remain in a Prolonged M phase–like State**

Because cell cycle dynamics are ultimately regulated by the activation and inactivation of cyclin-dependent kinases (CDKs) (Gitig and Koff, 2000), the phosphorylation state...
of direct and indirect CDK targets can also be used to determine the stage of cell cycle arrest. For instance, histone H3 is phosphorylated during M phase entry and subsequently dephosphorylated before M phase exit (Su et al., 1998; Wei et al., 1998, 1999). Therefore, to test whether the mutant embryos remain in a prolonged M phase state, embryos were analyzed using an antibody against phosphorylated histone H3 (phospho-H3). In both wild-type and mutant embryos, anti–phospho-H3 antibodies intensely stained oocyte chromosomes in their metaphase configuration (Fig. 5). In the mutants, the oocyte chromosomes continued to stain even in the oldest in utero embryos, suggesting that the mutant embryos enter, but never leave, M phase. Note that this antibody does not recognize the highly condensed sperm chromatin mass in either wild-type or mutant embryos (Fig. 5).

Mutant embryos were also analyzed using the MPM-2 monoclonal antibody that recognizes various mitotic phosphoproteins (Davis et al., 1983). The MPM-2 epitope is widely conserved, as this antibody stains kinetochores, centrosomes, and microtubules during early, but not late, M phase (Vandre et al., 1983; Hecht et al., 1987; Vandre and Borisy, 1989; Hirano and Mitchison, 1991; Renzi et al., 1997). As shown in Fig. 6, MPM-2 stains a “halo” of material around or, in some case, between the congressed oocyte meiotic chromosomes in both wild-type (Kitagawa and Rose, 1999) and mutant embryos. MPM-2 also stains the sperm chromatin mass (Fig. 6). In the mutants, MPM-2 continued to stain the chromosomes in older embryos, a further indication that these aging embryos remain blocked in M phase.

Terminal Phenotype

Under stringent conditions, every allele from this ts mutant collection exhibits a block in both the meiosis I metaphase to anaphase transition and in M phase exit. Nevertheless, after 3–4 h at 25.5°C, the oocyte and sperm
DNA within the aging mutant embryos leave their position near the cortex and move centrally, and the metaphase chromosomes begin to unravel. Importantly, this DNA unraveling is distinct from DNA decondensation, since the unraveled DNA continues to stain with the phospho-H3 antibody (Fig. 5 F, bottom embryo), and it never become encased within a nuclear envelope (data not shown).

When mutant mothers were grown under suboptimal conditions (e.g., growth at 20–24°C or after brief upshifts), their mutant embryos were sometimes observed to exit M phase and reform nuclear envelopes in the absence of chromosome separation. Other embryos even underwent a few rounds of mitotic cell divisions. The particular conditions required to produce these hypomorphic phenotypes are allele specific, and the hypomorphic defects themselves are consistent with the previously reported phenotypes of both \textit{emb-27(g48)} and \textit{emb-30(g53)} (Cassada et al., 1981), as well as \textit{emb-1(hc62} and \textit{hc57}) (Miwa et al., 1980).

\section*{Mutant Spermatocytes Are Also Blocked in Metaphase of Meiosis I}

In \textit{C. elegans}, the first meiotic division of the oocyte takes place on an acentriolar barrel-shaped meiotic spindle and culminates in a highly unequal division in which one set of homologues are discarded into a tiny polar body. In contrast, the first meiotic division of a primary spermatocyte takes place on a centriole-based, mitotic-like spindle, and, though cytokinesis is often incomplete, the division culminates in the symmetric division of the primary spermatoocyte into two secondary spermatocytes (Ward et al., 1981) (Fig. 7 A). Unlike oocytes, in which the first meiotic division only occurs after fertilization, spermatocytes initiate their first meiotic division autonomously as soon as they mature and separate from the syncytial gonad. This first meiotic division is followed by a round of centriole duplication and the assembly of a bipolar meiosis II spindle, orthogonal to the first division axis. The separation of sister chromatids during meiosis II is accompanied by an unusual asymmetric division (spermatid budding) during

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\textbf{Figure 5.} Phosphohistone H3 and DAPI localization in one-cell arrested \textit{mat} embryos. Embryos were dissected from mothers that had been upshifitd to 25°C for 6–7 h (A and B). The three wild-type, one-cell stage embryos are in meiosis I metaphase (left), meiosis II metaphase (middle), and S phase (right), based on their DNA staining patterns (B). In the wild-type panels, phosphohistone H3 stains the pentagonal array of the oocyte metaphase meiosis I chromosomes (arrow), but it does not stain the sperm chromatin mass on the other side of the embryo. In the middle embryo, the sperm chromatin mass is out of focus. Phosphohistone H3 brightly stains both the oocyte meiosis II chromosomes (arrowhead) and the chromatin within the adjacent polar body. In the S phase embryo, phosphohistone H3 stains the two polar bodies and the oocyte pronucleus, but not the male pronucleus, which, in this particular embryo, lies next to the oocyte pronucleus. All three \textit{mat-2(ax102)} embryos are in metaphase of meiosis I (D), and their congressed chromosomes (D, arrows) stain brightly (C). Congressed chromosomes also stain in \textit{mat-1(ax144)} (E) and \textit{mat-3(ax68)} (F). The bottom \textit{mat-3} embryo is older and displays the terminal phenotype: the chromosomes continue to stain with phospho-H3 antibody, but have moved centrally and lost their condensed metaphase chromosome morphology. The out of focus DAPI-stained body in the top embryo in D is outside of the embryo.

\textbf{Figure 6.} MPM-2 and DAPI localization in one-cell arrested \textit{mat} embryos. Embryos were dissected from mothers that had been shifted to 25°C for 6–7 h. These embryos were stained with the MPM-2 monoclonal antibody (A, B, E, and F) and DAPI (C, D, G, and H) to identify congressed M phase–like chromosomes (A, B, E, and F). Wild-type embryos in metaphase of meiosis I (A and C) are shown compared with arrested \textit{mat-1} (B and D), \textit{mat-2} (E and G), and \textit{mat-3} (F and H) embryos. Note, anterior is to the left. In most images, the hypercondensed sperm chromatin mass is visible at the right end of each embryo. A magnified view of the oocyte chromosomes are shown in each panel.

DNA within the aging mutant embryos leave their position near the cortex and move centrally, and the metaphase chromosomes begin to unravel. Importantly, this DNA unraveling is distinct from DNA decondensation, since the unraveled DNA continues to stain with the phospho-H3 antibody (Fig. 5 F, bottom embryo), and it never become encased within a nuclear envelope (data not shown).

When mutant mothers were grown under suboptimal conditions (e.g., growth at 20–24°C or after brief upshifts), their mutant embryos were sometimes observed to exit M phase and reform nuclear envelopes in the absence of...
Figure 7. Tubulin and DAPI localization in wild-type and mutant spermatocytes. Shown in A is a depiction of wild-type spermatogenesis. After nuclear envelope breakdown, the chromosomes of primary (1°) spermatocytes congress to form a meiosis I metaphase plate. The first meiotic division yields two secondary (2°) spermatocytes, which can either be separated or remain attached through a cytoplasmic connection due to incomplete cytokinesis. Secondary spermatocytes subsequently undergo a polarized budding division during which two haploid sperm separate from a central residual body. (B) A wild-type male germline squashed in the presence of Hoechst dye 33342 and observed by DIC and UV epifluorescence. All meiotic stages can be seen. Primaries (1°) and secondaries (2°) can be distinguished by size (B). A budding figure (BF) is visible in the lower right corner. Haploid sperm (S) and residual bodies (RB) are also indicated. (C) A sperm spread from a mat-3(ax82) male lacks 2° spermatocytes, but contains many anucleate sperm. Abnormal budding figures are also present in which all the chromosomes remain in the center of the developing residual body. Staining with anti-α-tubulin antibody (E, G, I, and K) and DAPI (D, F, H, and L) reveals the underlying spindle structures in these sperm spreads (E, G, I, and K). In wild-type sperm spreads, distinctive meiotic spindles are apparent in 1° and 2° spermatocytes and in budding figures (D and E). A magnified view of a wild-type meiotic spindle is shown in (I) with its corresponding DAPI image (H). In the mat mutants, normal meiosis I–like spindles form, but anaphase figures are never observed (F, G, J, and K). The diameter of a primary spermatocyte equals 12 μm.
which the two haploid sperm bud from a large residual cytoplasm (Fig. 7 A).

Given these striking differences between oocyte and spermatocyte meiosis, we investigated whether the mat genes functioned in both processes. Testes from young adult mutant males, which had been upshifted to 25.5°C as L3 larvae, were isolated and processed for DIC/Hoechst analysis. Such “sperm spreads” from wild-type males contain cells in all stages of spermatogenesis, including primary spermatocytes, secondary spermatocytes, budding figures, and haploid sperm (Fig. 7 B). In contrast, mat mutant spreads exhibit a striking abundance of primary spermatocytes and sperm (Fig. 7 C). Notably, secondary spermatocytes are completely absent, and the DNA staining patterns suggest that meiosis in the mutant primary spermatocytes fails to progress past meiosis I. Despite this apparent meiosis I arrest, cells that resemble haploid sperm still form, but they lack DNA (which we refer to as anucleate). Similar sperm spreads were seen for most mat alleles (data not shown) under L3 shift-up conditions, with the striking exceptions that mat-3 (or172, or180, and or187) and emb-1 (hc62 and hc57) males produce morphologically normal secondary spermatocytes and haploid sperm and can sire viable offspring when mated to feminized hermaphrodites (data not shown) (Sadler, P., D. Fox, A. Pletcher, and D. Shakes, unpublished observations). As previously reported for emb-27 and emb-30 males (Sadler and Shakes, 2000), affected mat-1, mat-2, and mat-3 males are unable to sire viable offspring, however, their anucleate sperm are surprisingly functional in that they can activate, crawl, and even fertilize oocytes.

To further understand the meiotic defects, we analyzed stage-specific DNA and tubulin structures in wild-type and mutant sperm spreads. In wild-type animals (Fig. 7, D and E), sperm spreads reveal a diversity of microtubule structures ranging from the large, closely opposed asters of the metaphase primary spermatocytes to the highly dynamic spindles of the secondary spermatocytes and budding figures. In mat mutants, including mat-2(ax143) (Fig. 7, F and G), sperm spreads are dominated by large spermatocytes, each of which contains a metaphase array of chromosomes and a bipolar, astral, meiosis I metaphase spindle. In enlarged views (Fig. 7, H–K), these spindles are closely associated with properly congressed and aligned metaphase chromosomes. Some of the mutant spindles eventually elongate, but homologue separation and other anaphase events never occur. Thus, with the possible exception of emb-1, whose two alleles lack spermatocyte defects, these data indicate that every mat gene is required for the meiosis I metaphase to anaphase transition in both oocytes and spermatocytes.

The mat Genes Are Also Required for Germline Mitosis

Our analysis of oocyte and spermatocyte meiosis in the mutants suggested that the mat gene products function in a region of germlines from isolated mutant gonads. DAPI staining also reveals fewer mitotic figures in the wild-type (E) than mat-2(ax76) (F) or mat-2(ax143) (G) gonads. Some phenotypes were more obvious at an intermediate temperature of 20°C (G).

Figure 8. Phospho-H3 staining of adult upshifted wild-type (A), mat-1(ax144) (B), mat-2(ax102) (C), and mat-3(or180) (D) hermaphrodites reveals an excess of mitotic plates in the mitotic re-
shared step of these two structurally dissimilar meiotic processes. To address whether these mat genes function specifically to separate axially aligned homologues during meiosis I or whether they also function to separate sister chromatids during mitosis, we examined the mitotic divisions of germline nuclei within adults. Because germline mitotic divisions continue at a time when the animal’s somatic cells have ceased to divide (Beanan and Strome, 1992), they can be studied in adult upshifted mutants in the absence of potential complications from somatic defects. To test for mitotic defects, young adult hermaphrodites were upshifted to 25°C for 6 h and then were dissected to release their gonads. Gonads were fixed and stained with the phospho-H3 antibody. Shown here is quantitation of the number of phospho-H3 positive germ cell nuclei per gonadal arm.

**Table I. Quantitation of Phospho-H3–Positive Nuclei in the Distal Germelines of mat Mutants**

<table>
<thead>
<tr>
<th>Gene (allele)</th>
<th>Nuclei</th>
<th>n</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2 (wild-type)</td>
<td>5.0 ± 2.2</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>mat-1 (ax161)</td>
<td>5.0 ± 3.0</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>mat-1 (ax144)</td>
<td>9.8 ± 5.7</td>
<td>32</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>mat-2 (ax76)</td>
<td>5.4 ± 2.5</td>
<td>51</td>
<td>NS</td>
</tr>
<tr>
<td>mat-2 (ax102)</td>
<td>8.3 ± 2.6</td>
<td>38</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>emb-27 (ax148)</td>
<td>3.8 ± 2.5</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>emb-27 (ax162)</td>
<td>7.1 ± 3.8</td>
<td>35</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>mat-3 (or180)</td>
<td>8.6 ± 3.5</td>
<td>55</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>mat-3 (ax68)</td>
<td>9.8 ± 4.3</td>
<td>32</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>emb-1 (hc57)</td>
<td>7.2 ± 3.8</td>
<td>22</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>emb-1 (hc62)</td>
<td>7.1 ± 3.9</td>
<td>24</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Statistical comparison to N2 using a one-tailed t-test. NS = not significant.

**In Embryos and Larvae, the mat Genes Are Also Essential for Somatic Cell Divisions**

In summary, we have isolated and characterized a large set of ts mutant alleles that define five complementation groups and share a common defect: mutant mothers shifted to the nonpermissive temperature of 25°C produce embryos that arrest at the meiotic one-cell stage. Our premise, that these affected one-cell embryos reach a normal Meiosis I metaphase state, but then fail to progress to anaphase, is supported by the following observations: (a) the oocytes undergo normal maturation events and, after fertilization, their chromosomes align and congress in a

ble II and data not shown). In the most severe cases, L1 upshifts resulted in sterile (Ste) adults with fewer than 50 germ cells and no apparent gametes. Similar defects in mitotic germline proliferation have been reported for null alleles of *emb-30* (Furuta et al., 2000).

**Discussion**

In summary, we have isolated and characterized a large set of ts mutant alleles that define five complementation groups and share a common defect: mutant mothers shifted to the nonpermissive temperature of 25°C produce embryos that arrest at the meiotic one-cell stage. Our premise, that these affected one-cell embryos reach a normal meiosis I metaphase state, but then fail to progress to anaphase, is supported by the following observations: (a) the oocytes undergo normal maturation events and, after fertilization, their chromosomes align and congress in a
metaphase pentagonal array on a morphologically normal meiotic spindle, (b) in such embryos, the sperm chromatin remains condensed and the sperm microtubule organizing center remains quiescent, and (c) neither meiotic anaphase figures nor polar bodies are ever observed. In addition, the stable presence of phospho-histone H3 and MPM-2 epitopes indicates that the mutant embryos are permanently arrested in a M phase–like state. These results suggest that functional meiotic CDKs drive the mutant embryos into metaphase of meiosis I, but because these CDKs are not subsequently deactivated, the mutant embryos are unable to transition out of a metaphase state. Thus, we have chosen the name “mat” (defined above) as an appropriate designation for this general mutant class.

Importantly, the metaphase to anaphase transition defects in these mutants are not restricted to oocyte meiosis I. Analogous defects are observed during spermatogenesis: primary spermatocytes undergo normal budding from the gonadal syncytium, undergo nuclear envelope breakdown, but then arrest in a normal meiosis I metaphase state and never form secondary spermatocytes. Thus, the same fundamental defect is seen in both spermatocyte and oocyte meiosis, despite the fact that spermatocytes employ astral, rather than anastral spindles. On the other hand, the developmental consequences of this common meiosis I block are strikingly dissimilar. The mutant oocytes appear to be blocked from further development as their sperm chromatin remains condensed and their sperm-contributed microtubule organizing centers remain quiescent. In contrast, differentiation of the mutant spermatocytes proceeds with the eventual formation of motile, albeit anucleate, spermatozoa.

These mutants also display metaphase to anaphase transition defects during mitosis. Most dramatically affected are the mitotic divisions of proliferating germ cells; upshifted adults accumulate an excessive number of phospho-H3–staining germ cell nuclei, and upshifted larvae exhibit moderate to severe defects in germ cell proliferation. However, unlike the complete metaphase blocks observed during meiosis, the metaphase block during germ cell mitosis is frequently incomplete and results in an M phase delay. The identification of defects in somatic tissues, such as the male tail and the hermaphrodite vulva, suggests that the mat genes are also required for mitotic divisions of the soma.

Each of the mat genes, except for emb-1, is represented by an allelic series that encompasses the full range of described metaphase to anaphase transition defects. Because identical mutant defects are observed in multiple alleles of multiple genes, they are likely to reflect important functions of this gene class. Thus, our data implies that a common mechanism governs the transition from metaphase to anaphase, regardless of whether this transition (a) involves the separation of homologues or sister chromatids, (b) is mediated by anastral or astral spindles, (c) occurs within syncytiot or nonsyncytial cells, or (d) involves germline or somatic cells. On the other hand, whereas all of the mutant alleles have defects in oocyte meiosis and most have defects in spermatocyte meiosis, many fewer exhibit defects in the mitotic divisions of either the germline or soma. Furthermore, we observed strong metaphase blocks during meiosis I, but primarily M phase delays during germ line mitosis. Thus, whereas the mat genes are required for all of these various cell divisions, mitotic divisions may be particularly sensitive to low levels of gene product. Alternatively, the design of our mutant screens may have favored the isolation of mutants with lesions in mitotic-specific or germline-specific domains. Sequence analysis of the mutant lesions may preferentially support one of these two explanations, and, in the latter case, identify specific functional subdomains within each protein.

What is the molecular nature of these genes that are required for the metaphase to anaphase transition during various types of cell divisions? Evidence from the cell cycle literature suggests that mitotic metaphase blocks can stem either from defects in the machinery that drives the metaphase to anaphase transition or from spindle and/or kinetochore attachment defects that secondarily trigger the metaphase checkpoint system. Recent studies suggest that this checkpoint is functioning within one-cell C. elegans embryos (Kitagawa and Rose, 1999), but, because the mat mutants assemble morphologically normal metaphase plates and spindles, we suspect the mat genes encode com-

<table>
<thead>
<tr>
<th>Allele</th>
<th>L4 shift-up</th>
<th>L1 shift-up</th>
<th>Two-cell shift-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>mat-1 (LG I): ax161, ax227 Mel</td>
<td>Mel</td>
<td>Mel</td>
<td>Mel</td>
</tr>
<tr>
<td>mat-2 (LG II): ax144, ax212 Mel</td>
<td>Mel</td>
<td>Mel</td>
<td>Mel</td>
</tr>
<tr>
<td>mat-3 (LG III): ax182, ax136, or192 Mel</td>
<td>Mel</td>
<td>Mel</td>
<td>Mel</td>
</tr>
<tr>
<td>emb-27: ax348, g48 Mel</td>
<td>Mel</td>
<td>Mel</td>
<td>Mel</td>
</tr>
<tr>
<td>emb-30: ax80, ax81, ax162, or279 Mel</td>
<td>Mel</td>
<td>Ste</td>
<td>Ste</td>
</tr>
<tr>
<td>emb1: he57, he62 Mel</td>
<td>Mel</td>
<td>Mel</td>
<td>Mel</td>
</tr>
</tbody>
</table>

shown here are the 32 alleles recovered in the screen for the mat phenotype. Also included are the previously existing alleles of emb-27 (g48), emb-30 (g53), and emb-1 (he57 and he62) that belong in this class of Mat mutants. All ax and or alleles were identified based on L4 shift-up experiments in which L4s produce entire broods of one-cell arrested embryos at 25°C (and hence are Mel). Shift-up experiments were also carried out with L1 larvae. Most L1 larvae shifted to 25°C developed to the adult stage and produced broods of one-cell embryos (Mel) or were Ste. Shift-up experiments with two-cell embryos were also carried out: most hatched and developed to the adult stage where they were either Mel or Ste. The phenotypes shown for the two-cell shift-ups represent the phenotypes of those embryos that hatched. For some alleles, a fraction of the embryos routinely die before hatching, regardless of the temperature conditions. In this table, the Ste category is broad, ranging from hermaphrodites with little or no germline to hermaphrodites that produce one or two embryos. The three alleles listed at the bottom were isolated in the ts screen, but proved to be nonconditional upon outcrossing.
ponents of the cell cycle machinery per se. Consistent with this hypothesis, we report that *emb-27* encodes an ortholog of the APC/C subunit, Cdc16. Molecular analysis of the other *mat* genes is ongoing, but we have recently discovered that *mat-1* encodes an ortholog of another APC/C gene, *cde-2* (Schumacher, J., D. Shakes, and A. Golden, manuscript in preparation), and Furuta et al. (2000) report that *emb-30* encodes APC4. Thus, the Mat phenotype appears to be an excellent predictor of APC/C and APC/C-related genes, and it is not surprising that *emb-1*, *mat-2*, and *mat-3* all map to regions in which APC/C subunits have been identified.

It is somewhat surprising that this large mutant screen, which is at or near saturation, yielded multiple alleles of only five genes (*emb-1* was not reisolated), all of which are either known or likely to encode conserved APC/C components. First, this screen was expected to identify several meiosis-specific APC “accessory proteins” or regulators that enable the APC/C to separate homologues during meiosis I without disrupting the centromeric sister chromatid cohesions that are APC/C’s primary, but indirect target, during mitosis and meiosis II. Although our results do not rule out the existence of such modifiers, they do indicate that such modifiers do not easily mutate to temperature sensitivity. This result suggests that APC/C functions largely in the same capacity in distinct types of cell divisions. Second, even if all six *mat* genes prove to encode APC/C subunits, there are at least nine APC/C subunits encoded within the *C. elegans* genome (King et al., 1995, Yu et al., 1998; Zachariae et al., 1998; Furuta et al., 2000; Wille, L., and D. Shakes, manuscript in preparation). It is not clear why additional genes were not hit, but similar ts screens in yeast likewise identified only a subset of APC/C genes. Thus, something about either the function or molecular structure of the individual subunits must make them more or less likely to be isolated in such ts screens. This possibility is supported by studies in *S. cerevisiae* in which only a small number of essential genes could be mutated to be ts (Kabaek et al., 1984; Harris and Pringle, 1991).

The phenotypic analysis of this large set of ts *mat* mutants and the molecular identification of *emb-27* as an ortholog of Cdc16 have enabled us to define an essential role for APC/C in a variety of specialized cell divisions, including the first meiotic division of both oocytes and spermatocytes. Importantly, our analysis reveals that all of these metaphase to anaphase transitions are driven by a common cellular mechanism, and that many or all of the individual APC/C subunits are essential for M phase progression in these various cell types. Ts mutants proved critical to our analysis, since meiotic functions could not have been analyzed in nonconditional mutants with defects in germine proliferation. In continuing studies, analysis of the molecular lesions associated with each allele should reveal which protein domains are important for general and/or cell-type-specific functions. Additionally, screens for genetic suppressors may identify additional components that act in each of these pathways.

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References


