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Genetic Studies of the Switch from Meiosis to Mitosis during Caenorhabditis elegans Embryogenesis

by

Thomas Robert Clandinin

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Genetic Studies of the Switch from Meiosis to Mitosis during Caenorhabditis elegans Embryogenesis" submitted by Thomas R. Clandinin in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Complex genetic circuitry directs the switch from meiosis to mitosis in the fertilised embryo of the nematode *Caenorhabditis elegans*; this work describes genetic studies of one of the pathways involved.

The *mei-1* gene encodes a maternally provided product essential for embryonic meiosis. Some dominant *mei-1* alleles retain normal activity but can function ectopically at subsequent mitotic divisions to cause aberrant cleavage. I propose that *mei-1* product may normally function as a multimer; dominant mutations cause this complex to act at mitosis; antimorphic *mei-1* mutations suppress dominant alleles by incorporating into and inactivating the multimer. Extragenic suppressors of *mei-1* dominant mutations reduce the activity of the *mei-1* genetic pathway. To extend this analysis, a method to facilitate identification of additional dominant mutations affecting embryogenesis has also been described.

We propose that *mei-1* product has an analogous but functionally non-interchangeable mitotic counterpart; a specific system may target such products to the correct spindle.

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CHAPTER I

Principles of Embryogenesis

POSITIONAL INFORMATION

In many respects, early embryogenesis represents the most complex and intricate stage in the development of a multicellular organism; the basic body plan is created and within this framework certain broad cell fates are determined. In many cases, the systems involved must generate an invariant fate map - cells in similar positions in the embryo must have similar fates from animal to animal (for example see Conklin, 1905). Conceptually, a number of mechanisms could create invariant patterns but inherent in all such models is a system specifying when and where the events of embryogenesis will occur. This "positional information system" must possess two properties. First, it must be asymmetric; for example, the posterior of the embryo must be defined as different from the anterior of the embryo. Second, it must possess some means of temporally coordinating the events of embryogenesis (Wolpert, 1969). These systems form the core of embryogenesis; early nematode development should be a useful model for their study.

Positional information has been investigated in a number of experimental systems, including *Drosophila* (for example see Carroll, 1990), *Xenopus* (Woodland, 1989) and mice (reviewed in Maro *et al.*, 1991). Recently, the small, free-living nematode *Caenorhabditis elegans* has become an alternative system

for genetic and molecular studies of early embryogenesis (reviewed in Wood, 1988). This system combines powerful genetic techniques similar to those available in *Drosophila* with a pattern of early development morphologically more similar to vertebrate embryogenesis than the embryonic syncytium of early *Drosophila* development. Furthermore, the complete cell lineage has been described, facilitating detailed examination of developmental defects (Sulston *et al.*, 1983).

Until recently, nematode development was considered an invariant, strictly cell autonomous process in which each determinative division segregated different cytoplasmic factors to each daughter blastomere resulting in each daughter having a different fate. Recent evidence (Priess and Thomson, 1987; Priess *et al.*, 1987; Schierenberg,1987; Horvitz and Sternberg, 1991; Schnabel, 1991; Goldstein, 1992) has suggested, however, that intercellular communication plays a large role in cell fate specification during many stages of *C. elegans* development and it now appears likely that a combination of cell autonomous and non-autonomous mechanisms are involved.

All models of cell autonomous determination require that different intracellular regions of a blastomere be defined as spatially distinct to allow precise localisation of the cytoplasmic factors necessary to achieve differential segregation of cell fate potential. Cell non-autonomous differentiation often has similar requirements; cell signalling components must sometimes be precisely localised to allow specific cells to interact. Therefore, both autonomous and

non-autonomous determination mechanisms require intracellular positional information systems.

While spatial control systems are well studied, temporal controls have received relatively less attention. There are three fundamental mechanisms by which a group of processes could maintain a consistent temporal order (reviewed in Murray, 1991; Hartwell and Weinert, 1989). Most simply, if each step in a multi-step pathway occurred in an invariant length of time, the relative order of events would be maintained by the completion time intrinsic to each event. However, two more sophisticated forms of temporal ordering are also conceptually possible. The first of these could be termed product-substrate dependence; the product of an upstream process is required to initiate a downstream step, enforcing a strict relative order. A yet more complex form of regulation, termed checkpoint control, requires a molecular switch which inhibits specific processes until a series of pre-conditions have been fulfilled. Most studies of temporal synchronisation have focused on the cell cycle of the budding yeast *Saccharomyces cerevisiae*; less is known about the relative importance of these controls in multicellular organisms (for a review see Glover, 1990). Given the invariant program of nematode development and the ability to observe developmental events in living embryos, *C. elegans* may allow investigation of the roles these controls play in coordinating the events of early embryogenesis.

FEATURES OF EARLY *CAENORHABDITIS ELEGANS* EMBRYOGENESIS

To date, there is only one identified cytoplasmic factor which is differentially segregated during *C. elegans* development (Strome and Wood, 1982, 1983; Wolf *et al.*, 1983). Antigens of unknown composition, the P granules, are specifically localised to the germline progenitor cell prior to cleavage of the one-cell embryo and prior to subsequent divisions. While there is no evidence that this antigen plays a role in germline specification (Schierenberg, 1988; Capowski *et al.*, 1991), the striking segregation pattern clearly demonstrates that active, asymmetric segregation of cellular components is possible and suggests that some form of positional information must exist in the early embryo. However, identification and characterisation of determinants required during *C. elegans* development and the establishment of their causal relationship to cell fate has proven difficult but may soon be possible (Bowerman *et al.*, 1992; Mello *et al.*, 1992). In this recent work, mutations disrupting cell fate specification have been shown to affect a gene whose product bears homology to a family of known transcription factors. As fate determinants must presumably regulate gene expression, this gene product may play such a role (see also Mello *et al.*, 1992). Furthermore, embryological manipulations (Schierenberg, 1985; 1987; reviewed in Schierenberg, 1989) have provided evidence that cytoplasmic determinants may also play roles in controlling cell cycle timing and cell division polarity in the early embryo. Related work (Laufer *et al.*, 1980; Cowan and Macintosh, 1985; Edgar and McGhee,

1986; 1988) supports autonomous determination mechanisms for a number of cell types.

In addition to the presumed active segregation of fate determinants, a number of cellular components move in invariant patterns during early embryogenesis (Albertson, 1984; Hyman and White, 1987; Hyman, 1989). For example, the pronuclei almost invariantly form at opposite ends of the one-cell embryo, migrate in a defined manner, and fuse in the posterior of the embryo. Of particular interest to this study are the stereotyped centrosomal rotations and migrations which occur prior to each mitotic division and are thought to be driven by interactions between microtubular elements and the embryo cortex. As the position of the centrosomes ultimately determines spindle positioning, some of the mutants described in this work may disrupt centrosome function. Positioning of the first mitotic spindle is both precise and asymmetric; the cleavage furrow therefore generates two unequally sized blastomeres. That these events occur at precise and asymmetric positions suggests they too must be directed by a positional information system. While there is no direct evidence that the same positional information system controls both the segregation of determinants and the positions of other intracellular events, it seems reasonable to suggest that similar systems might be involved in both processes. Consistent with this idea, mutations in the *par* genes and disruptions in microfilament networks (described below) affect both cell fate specification and intracellular asymmetry, implying that the two systems must share common functions. Furthermore, from a genetic

standpoint, screening for mutants affecting positioning of intracellular events might be easier than isolation of mutants disrupting determinant segregation patterns; the former would be associated with a visible morphological change in addition to a lethal phenotype while the latter would probably only appear to cause embryonic lethality until characterised in detail. Furthermore, unlike *Drosophila*, there are no obvious embryonic landmarks in *C. elegans* to facilitate scoring of terminal phenotype; examining late embryonic lineages is tedious and difficult.

A number of studies have addressed the relative roles of different cytoskeletal elements on intracellular migrations and the segregation of fate determinants (Strome and Wood, 1983; Hyman and White, 1987; Hill and Strome, 1988; 1990). Experiments in which microtubule inhibitors were applied to embryos after completion of meiosis revealed that pronuclear migration and centrosomal rotation were severely inhibited but other events, such as P granule segregation and pseudocleavage, were unaffected. Experiments in which actin microfilaments were disrupted, however, revealed that a pulse of inhibitor midway through the first cell cycle was sufficient to block certain events occurring during the pulse, such as pseudocleavage, but was also capable of disrupting later events, such as asymmetric migration of the mitotic spindle. These experiments also suggested that actin microfilaments play a crucial role in the segregation of cell fate potential and lineage specific cleavage pattern. Together this work implies that microtubules and microfilaments play distinct roles in the establishment of embryonic asymmetry and in the migration of cellular

components prior to cleavage. Given that microtubule, rather than microfilament, inhibitors can phenocopy some of the mutants discussed in this work, the genetic pathway studied here may be involved in a microtubule based process.

RECESSIVE MUTATIONS DISRUPTING EMBRYOGENESIS

Initial genetic screens for mutations disrupting embryogenesis focused on conditional, recessive, loss-of-function mutations causing embryonic lethality (Hirsh and Vanderslice, 1976; Cassada *et al.*, 1981; Isnenghi *et al.*, 1983; reviewed in Wood, 1988). However, this type of screen is complicated by the fact that embryogenesis represents one of the most metabolically active stages of the worm life cycle. As a result, a large number of loci appear capable of mutating to an embryonic lethal phenotype. Since most of these loci also function at other stages in the life cycle and many are involved in basic metabolic functions, many mutations isolated in this type of screen will not be informative with regard to developmental processes *per se* (Kemphues *et al.*, 1988b). Recent efforts, in which the primary screen for embryonic lethals is supplemented by an intensive secondary screen examining lineages of individual embryos, have identified three loci that probably play important roles in cell fate specification (Priess *et al.*, 1987; Schnabel and Schnabel, 1990a; b). Similar screens have recently identified recessive, maternal-effect lethal mutations defining several additional loci which may also be informative (Bowerman *et al.*, 1992; Mello *et al.*, 1992).

An alternative approach was based on the idea that non-conditional null mutations could be isolated in genes that are only required maternally; that is, their product is only essential for viability when supplied in the oocyte. While this type of screen has not yet been performed exhaustively, apparently few loci can mutate to this phenotype (Kemphues *et al.*, 1988b). Five of these loci, designated the *par* (for *par*titioning defective) genes, appear to disrupt many of the cytoplasmic rearrangements that take place in the one cell embryo, including P granule segregation (Kemphues *et al.*, 1988a; Kirby *et al.*, 1990; Morton *et al.*, 1992). Overall, these mutants lose many of the asymmetric features of the early embryo. It appears, therefore, that these loci play an important role in controlling the spacial arrangement of certain events during early embryogenesis and may represent components of a positional information system.

DOMINANT MUTATIONS DISRUPTING EMBRYOGENESIS

As has been summarised above, all previous genetic screens for disruptions in early embryogenesis have focused on recessive loss- or reduction-of-function mutations. Such screens are biased towards the isolation of mutations in non-redundant loci. However, it has been estimated that at least half of all genes in *C. elegans* may have wild-type loss-of-function phenotypes (Park and Horvitz, 1986; Sulston *et al.*, 1992; Waterston *et al.*, 1992); similar estimates have been obtained for other organisms (Goebl and Petes, 1986; Dove, 1987; Oliver

et al., 1992). As a result, such screens would probably not detect many developmentally important loci. Recently a screen for dominant, temperature-sensitive, maternal-effect mutations has been undertaken in a preliminary effort to identify such genes (Mains *et al.*, 1990a). This type of screen should identify genes belonging to functionally redundant multigene families, loci in which the multimeric gene product can be disrupted by a defect in one subunit of the complex, and haplo-insufficient loci in which two functional copies of the locus are required for viability. Finally, screens for dominant mutations should also identify lesions that cause ectopic expression of gene activity; subsequent chapters describe a dominant mutation of this type. Furthermore, unlike screens for loss-of-function mutations, screens for dominant mutations are not biased against functionally redundant genes, allowing a more representative assessment of the genetic functions of the genome.

This initial screen identified dominant, gain-of-function mutations at two linked loci, designated *mei-1* and *mel-26,* which result in defects in the mitotic spindle of the one-cell embryo. Normally, the mitotic spindle forms along the anterior-posterior axis of the embryo; cleavage produces a larger, anterior AB cell and a smaller, posterior P_1 cell. In embryos from *mei-1(ct46)* or *mel-26(ct61)* parents, the spindle is typically shifted posteriorly and oriented perpendicularly to spindles in wild-type embryos. The first cleavage in mutant embryos, therefore, proceeded along the anterior-posterior axis; the resultant blastomeres often failed to cleave completely, forming poorly defined cell boundaries and, often,

multinucleate cells (Mains *et al.*, 1990a). Recessive, loss-of-function alleles at a third locus, *zyg-9*, produced a similar phenotype (Kemphues *et al.*, 1986). Mutations at all of these loci were capable of phenotypic enhancement; double mutant combinations displayed phenotypic severities consistent with synergistic effects. Genetic studies of these three loci suggest that they may respond to, or be involved in, embryonic positional information. A detailed analysis of one of these loci, *mei-1*, presented below, suggests mechanisms by which events of early embryogenesis may be coordinated.

CHAPTER II

Genetic Studies of *mei-1* Alleles

INTRODUCTION

Completion of meiosis and initiation of mitosis in *Caenorhabditis elegans* requires extensive reorganisations of the embryonic cytoplasm (Strome and Wood, 1983; Albertson, 1984; Hill and Strome, 1988, 1990). Meiotic and mitotic spindles are morphologically different but presumably contain components that perform similar functions. Certain meiotic spindle components might, therefore, be analogous to but functionally non-interchangeable with mitosis-specific structures. Mechanisms must, therefore, ensure that meiotic and mitotic components are used only in the appropriate spindle. We are interested in the genetic pathways that coordinate these two forms of nuclear division.

Prior to fertilisation, the oocyte nucleus is arrested at diakinesis of meiosis I. Upon fertilisation, it completes both meiotic divisions, migrates to and fuses with the sperm pronucleus, and the first mitotic spindle is assembled (Nigon *et al.*, 1960; Hirsh *et al.*, 1976; Strome and Wood, 1983; Albertson, 1984). In *C. elegans*, these processes take place in a spatially distinct and temporally restricted manner. For example, the meiotic spindle is positioned near the anterior pole of the embryo, pronuclear fusion occurs in the posterior region and the mitotic spindle assembles along the anterior-posterior axis, slightly posterior of centre. At 25^0 these steps are complete approximately 35 minutes after

fertilisation, implying strict temporal control of the activities involved.

Previous work (Mains *et al.*, 1990b) identified the *mei-1* locus as essential for meiosis. Embryos lacking maternal *mei-1* activity fail to form proper meiotic spindles, leading to defective polar body formation and variable incorporation of maternal chromosomes into the zygote nucleus; subsequent mitotic cleavages are morphologically normal. However, a dominant, temperature-sensitive (*ts*), gain-of-function (*gf*) allele at the locus, *mei-1(ct46)*, caused mitotic rather than meiotic defects. While the mitotic defects suggest that the *gf* allele is neomorphic (displaying an activity not seen in wild type), gene dosage studies revealed that wild-type activity competed with mutated product, making precise genetic classification of this allele problematic.

The recessive, loss-of-function (*lf*), meiosis-defective mutations mentioned above were isolated as suppressors of the dominant *mei-1(ct46)* allele in a screen which identified two classes of intragenic pseudo-revertants. The expected group of alleles were capable of suppressing only in *cis*; these were interpreted to be null alleles (designated class 1) that had converted the dominant, *gf* product into a recessive, *lf* allele. The second class of intragenic mutations arose at a high frequency, lacked functional *mei-1* activity, and were both *cis* and *trans* dominant suppressors of the *gf* allele. It was proposed that *mei-1(+)* gene product is involved in meiosis and is normally inactivated prior to the first mitotic cleavage. The dominant mutation caused *mei-1* gene product to retain normal activity during meiosis but become resistant to post-meiotic inactivation.

Trans-suppression was thought to require a non-specifically defective product which could complex with the *gf* product and promote, perhaps in an antimorphic (dominant negative) manner, its inactivation. However, one might expect that *trans*-suppressors could also complex with wild-type or *ct46* product, cause a reduction in *mei-1(+)* activity during meiosis, and create meiotic defects. As no meiotic defects caused by *trans*-suppressors were observed, one must postulate that either the inactivation of meiotic activity by *trans*-suppressors must specifically occur after meiosis or that a threshold of *mei-1* activity exists; enough *mei-1* activity is eliminated by *trans*-suppressing product to correct the mitotic defects but sufficient activity remains to support meiosis. In this study we have extended the genetic analysis of this locus and have distinguished between these two models, finding the latter to be more accurate.

We have undertaken an alternate genetic screen for suppressors of the dominant maternal-effect lethal defect of *mei-1(ct46)* and have identified a third type of intragenic suppressor arising at high frequency. Characterisation of these alleles in *trans*-heterozygous combinations with previously identified *mei-1* alleles has provided evidence for a threshold of *mei-1* activity; a certain level of activity is required for meiosis but must be eliminated prior to mitosis. Analysis of these strains also demonstrated that *trans*-suppressors do indeed act in an antimorphic manner, reducing *mei-1* activity at both meiosis and mitosis. Finally, we have obtained genetic evidence suggesting that *mei-1* gene product may function as a multimeric complex.

MATERIALS AND METHODS

Culture Conditions: *Caenorhabditis elegans* (var. Bristol) was grown as described by Brenner (1974). All stocks were maintained at 15° and all crosses between strains bearing *ts* mutations were performed at this temperature; other crosses were performed at 20°. Embryonic viability was determined by scoring embryos laid by 2 to 12 self-fertilised hermaphrodites (1 or 2 animals per plate) over 48 hours at 25° (this usually represented a complete brood). Animals were transferred to fresh plates after 24 hours and hatched embryos were scored 12 hours after removal of the parent. Arrested larvae (animals much smaller than their sibs) and males were noted the following day. For calculation purposes, arrested larvae are not considered to be viable progeny. The same shelf of the same incubator was used to minimize variation between experiments.

Genes, alleles and rearrangements: The nomenclature of Horvitz *et al.* (1979) is followed. Briefly, mutations are designated as gene name (allele number); in this work the original dominant mutation was designated *mei-1(ct46)*; suppressors of this mutation were assigned the prefix *sb* and numbered appropriately. The recessive marker mutations used in this work can be described by the following. Dpy animals are unusually short, Lin animals are egg-laying-defective (Egl), Unc animals display defects in movement and Lon animals are unusually long. The following genes and alleles were used.

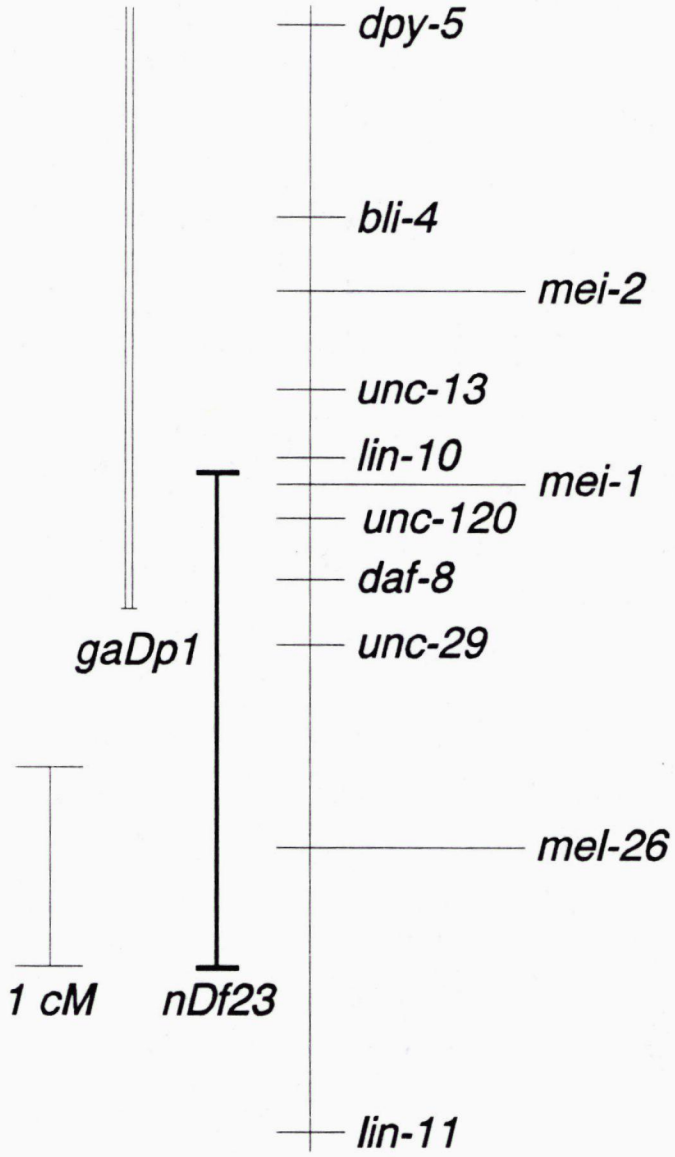
LG I: *dpy-5(e61), lin-10(e1438), lin-11(n566), mei-1(b284, ct46ts, ct46ct82, ct46ct99, ct46ct100, ct46ct103, ct46sb8ts, ct46sb9ts, ct46sb10ts, ct46sb16ts, ct46sb17ts, ct46sb18ts, ct46sb19ts, ct46sb21ts, ct46sb22ts, ct46sb23ts, and ct46sb24ts), mei-2(ct102), unc-13(e1091), unc-29(e193 and e1072), and unc-120(st364ts)*. The deficiency *nDf23* and the duplication *gaDp1 (I;f)* were also used (Figure 1).

LG X: *him-8(e1489), lon-2(e678)*.

Isolation and characterisation of suppressors of *mei-1(ct46)*: *mei-1(ct46)* is a dominant, *ts*, maternal-effect lethal mutation (Mains *et al.*, 1990a). A previous screen for dominant suppressors of *ct46* employed strains in which *ct46* was heterozygous with wild-type and primarily identified intragenic suppressors displaying recessive maternal-effect lethality as described above (Mains *et al.*, 1990b). We therefore designed a different screen to eliminate this 'background' of intragenic suppressors (Figure 2 and below) which we anticipated would be biased towards extragenic events.

mei-1(ct46ct99) unc-29/unc-13 mei-1(ct46) lin-11; lon-2 heterozygotes were mutagenised with ethylmethanesulfonate (EMS, Sigma, St. Louis, MO) under standard conditions (Brenner, 1974). Two mutagenised animals were placed on each plate and allowed to produce self progeny at 15^0 for 2 days. 623 plates representing a total of approximately 45,500 F_1 animals were shifted to 25^0; those bearing relatively large numbers of F_2 animals were saved. A total of 14

Figure 1. Genetic map of the central region of LG I. The extent of the deficiency *nDf23* and the duplication *gaDp1* are displayed below.


dpy-5
bli-4
mei-2
unc-13
lin-10
mei-1
unc-120
daf-8
unc-29
gaDp1
mel-26
1 cM
nDf23
lin-11

independent lines were retained, 13 of which were outcrossed at least five times to remove extraneous mutations and maintained as homozygous Unc-13 stocks at 15°. The remaining line (designated *ct46sb24*) was inviable as an Unc-13 stock due to a spurious larval lethal mutation and was maintained as an *ct46ct99 unc-29/unc-13 ct46sb24 lin-11; lon-2* strain.

Mapping *mei-1* mutations by their suppression of *ct46*: If the *cis*-linked suppressors of *mei-1(c46)* are extragenic, then they may be separable from *ct46* by recombination. At 25° *ct46/+* animals fail to produce significant numbers of viable progeny whereas an intermediate percentage of embryos produced by *ct46sup/+* animals were found to be viable (see below). Two mutations, *lin-10(e1438)* and *unc-120(st364)*, flank a 0.7 centimorgan (cM) region defining approximately 200kb of genomic DNA known to contain *mei-1* (Clark-Maguire and Mains, personal communication). For each of three suppressors (*ct46sb17*, *ct46sb18* and *ct46sb21*) 7 to 9 Lin-non-Unc-120 recombinants were isolated from an *unc-13 ct46 sup/lin-10 unc-120* background at 25° (*unc-120* is a *ts* larval lethal, facilitating selection of these recombinants). Embryos raised at 25° were excised from gravid Lin-non-Unc hermaphrodites into embryo buffer (Hawkins and McGhee, personal communication), transferred to fresh plates at 25°, and scored for hatching. Given that recombinants were identified at 25°, animals which had lost the suppressor but retained *ct46* would not be detected as these animals

would produce only inviable eggs. In each case, recombinants either displayed a level of embryonic lethality consistent with the presence of both *ct46* and the suppressor (for example, for *ct46sb17*, approximately 66% of the excised embryos hatched) or they displayed no maternal-effect lethality [*mei-1(+)sup(+)*] (for example, for *ct46sb17*, approximately 99% of the excised embryos hatched). This observation is consistent with two explanations. Either both *ct46* and the suppressor map within the *lin-10 unc-120* interval and are tightly linked (approximately $p<0.09$cM) or the suppressor maps distal to *ct46* and possesses no apparent phenotype in the absence of *ct46*. To exclude the second possibility, two independent Lin-non-Unc recombinants (one each from *ct46sb22* and *ct46sb24*) displaying no embryonic lethality were tested for their ability to suppress *mei-1(ct46)* in *trans*; such suppression was not observed.

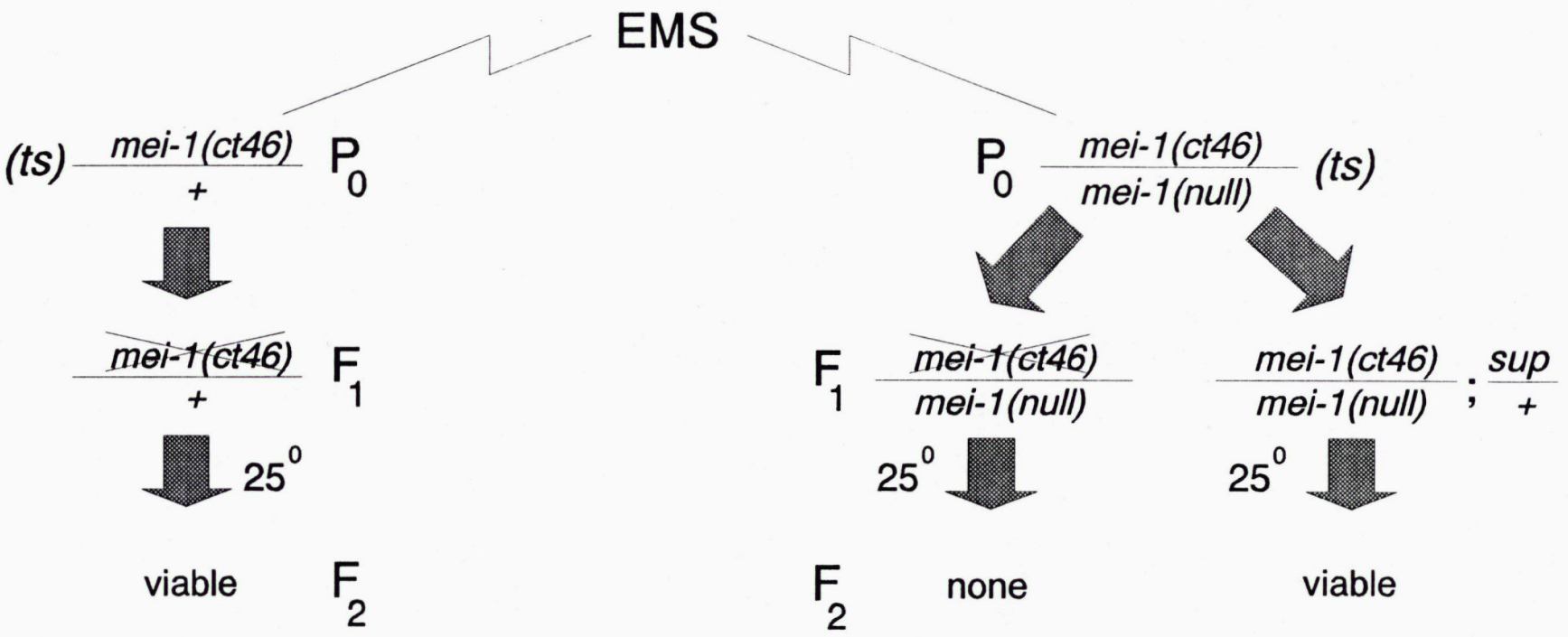
Microscopy: Gravid hermaphrodites were dissected in embryo buffer and living embryos were observed on agar pads using a Zeiss Axioplan microscope equipped with Nomarski optics. The embryos were time-lapse videotaped with a Panasonic WV1800 camera and a Panasonic AG6750 videorecorder or flash-photographed with Kodak (Rochester, NY) Tech Pan film developed at ASA 100. In all figures, embryos are oriented anterior-left, dorsal-up.

RESULTS

Isolation of suppressors of *mei-1(ct46)*: The dominant, *ts*, maternal-effect lethal mutation *mei-1(ct46)* appears to express an abnormal mitotic 'poison' while retaining its normal meiotic function. Previous screens for suppressors of *mei-1(ct46)* isolated two classes of intragenic suppressor; both types of alleles arose at high frequency (Figure 2, left-hand column). Of the 12 intragenic mutations, only one retained functional *mei-1* activity. Only two additional suppressors isolated in this screen were extragenic; both defined *lf* mutations at a linked locus, *mei-2*. We therefore designed a genetic screen biased against intragenic *ct46* suppressors lacking all functional *mei-1* activity by utilising a strain in which *mei-1(ct46)* provided the only functional *mei-1* product (see **MATERIALS AND METHODS** and Figure 2, right-hand columns). Animals bearing *mei-1(ct46)* in *trans* to a *mei-1* null allele, *ct46ct99*, should acquire recessive maternal-effect lethality if additional mutational events eliminate the activity of the *ct46* gene copy (as frequently occurred in the previous screen). Such mutational events would not, therefore, be detected, biasing the screen toward other types of suppressors. Approximately 45,500 F_1 progeny of EMS-mutagenised *ct46ct99 unc-29/unc-13 ct46 lin-11; lon-2* parents were screened for suppression of the dominant maternal effect lethality and a total of 14 independent lines were isolated.

We anticipated that this screen would be biased toward extragenic sup-

Figure 2. Schematic illustration of the two alternative genetic screens used to identify suppressors of *mei-1(ct46)*. The method outlined on the left was previously used to isolate class 1 and class 2 suppressors, the right-hand columns describe the procedure used in this work to isolate class 3 mutations and extragenic suppressors.



isolates primarily intragenic recessive maternal effect lethal suppressors

isolates extragenic and weak dominant maternal effect lethal suppressors

suppressors, but, surprisingly, 11 of 14 suppressor mutations were linked to *unc-13* (which marked the *ct46*-bearing chromosome). Ten of these 11 mutations display *ts*, dominant, maternal-effect lethality and, as described below, are probably allelic to *ct46*. The remaining allele in this group displays no apparent phenotype, may represent a revertant to wild-type, and was not investigated further. The remaining three suppressors were unlinked to *unc-13* and may identify a single locus (Clandinin, personal communication).

Intragenic *ct46* suppressors define a new class of dominant mutation: A number of observations suggest that the 10 suppressors represent *mei-1* alleles producing reduced levels of both the normal meiotic and the abnormal mitotic activities of the *ct46* mutation. First, as described below, complementation analysis of these suppressors with previously identified alleles of *mei-1* resulted in a pattern consistent with these mutations representing new *mei-1* alleles. Second, three of these alleles were mapped to the same interval as *ct46*, between *lin-10* and *unc-120*, the two known loci that flank *mei-1* most closely (a 0.7cM region approximately 200kb in size). Gene dosage studies (to be discussed in detail below) demonstrate that these alleles display properties distinct from all previously identified *mei-1* mutations (Table 1 lines 1-4, compare with lines 5-7). Therefore, extending the previous nomenclature (Mains *et al.*, 1990b), these alleles have been designated class 3 suppressors of *mei-1(ct46)* (null mutations defining class 1; alleles with *trans*-suppressing activity representing class 2). In

Table 1. Allele dosage effects on viability of class 3 embryos at 25°

Mutation[a]	*M*/+[b]	*M*/*M*[c]	*M*/*ct46ct99*[b]	*M*/*Df*[d]	*M*/*ct46*[e]
1. *ct46sb22*	5.3	14	41	22	0
2. *ct46sb17*	13	26	62	49	0.10
3. *ct46sb23*	24	86	77	67	0.48
4. *ct46sb18*	46	86	92	76	4.3
5. *ct46*[f]	1[g]	0.2[g]	0.3	0.3	0
6. *ct46ct99*[f]	97	0	0	0	0.3
7. *ct46ct82*[f]	98	0.2	0	0	93

[a]Headings indicate maternal genotypes. All values are percent of self-progeny that were viable. Between 350 and 1050 embryos of each genotype were scored.

[b]All hermaphrodites were morphologically wild type and coisogenic for the heterozygous recessive markers *unc-29* and *unc-13*.

[c]These hermaphrodites were morphologically Unc-13.

[d]These hermaphrodites were morphologically wild type and coisogenic for the heterozygous recessive marker *unc-13*. *nDf23* was used in these experiments (values were corrected for the 25% zygotic lethality caused by the deficiency).

[e]These hermaphrodites were morphologically wild type and coisogenic for the heterozygous recessive markers *him-8* and *lon-2*.

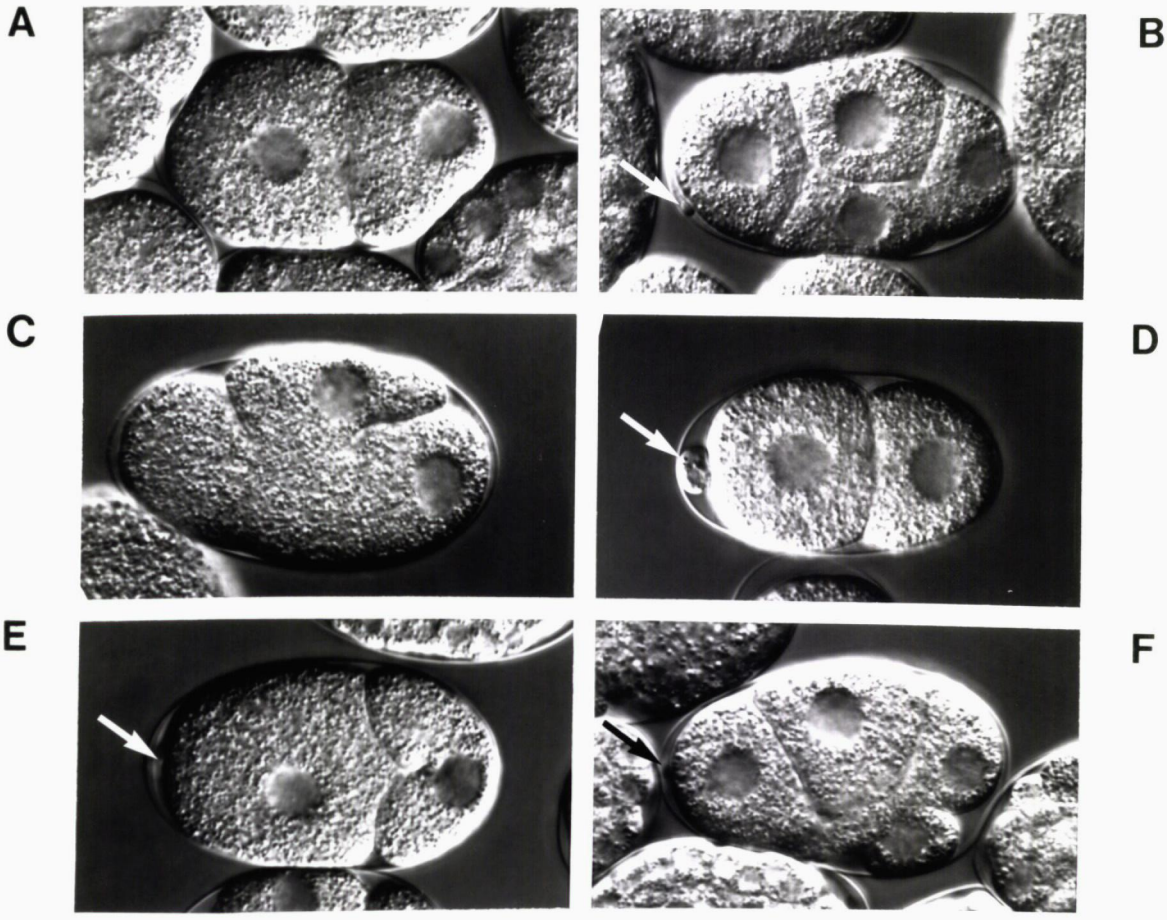
[f]These values were obtained previously (Mains *et al.*, 1990b). *ct46ct99* and *ct46ct82* are class 1 and class 2 alleles respectively.

[g]The corresponding values at 20° are 33% and 3% for *ct46*/+ and *ct46*/*ct46* respectively (Mains *et al.*, 1990b).

this paper, "class 3 embryos" or "mutant embryos" refer to embryos produced by class 3 mutant hermaphrodites.

Class 3 *mei-1* alleles display weak mitotic defects: Defective embryos produced by class 3 mutant hermaphrodites display mitotic spindle defects similar to, but not as severe as, defects produced by the original *ct46* mutation (Figure 3). Maternal meiosis normally occurs near the anterior pole of the embryo and produces two small polar bodies. The subsequent mitotic spindle assembles along the anterior-posterior axis of the embryo; cleavage produces two unequally sized blastomeres, AB (left) and P_1 (Figure 3A, a wild-type four cell embryo is displayed in 3B). In embryos produced by *ct46* hermaphrodites, meiosis appears to occur normally but the first mitotic spindle is often oriented perpendicular to the anterior-posterior axis of the embryo; the first cleavage usually occurs improperly and often incompletely (Figure 3C; compare with the null phenotype displayed in 3D). [The temperature-sensitive period for this mutation extends for the first several cleavages, suggesting that the mutant product can disrupt subsequent mitotic cleavages as well.] In class 3 embryos, the first mitotic spindle is usually oriented correctly but division is often defective and results in abnormal distributions of cytoplasm between the AB and P_1 blastomeres (Figure 3E). In some embryos the first cleavage is apparently normal but cleavages to the 4 cell stage occur improperly, producing unusual blastomere

Figure 3. Nomarski photomicrographs showing representative phenotypes in self-progeny embryos produced by *mei-1(lf)*, *mei-1(gf)* and wild-type hermaphrodites. (A) wild-type, two-cell. (B) wild-type, four-cell. Note the small polar body (arrow). (C) *mei-1(ct46)*, two-cell. Note the incomplete mitotic cleavage and the aberrant plane of division. (D) *mei-1(ct46ct99)*, two-cell. Note the large polar body (arrow) and the normal mitotic cleavage. (E) *mei-1(ct46sb18)*/+, two-cell. Compare the plane of mitotic division with that seen in (C) and compare the size of the polar body (arrow) with the polar body (arrow) seen in (D). (F) *mei-1(ct46sb18)*/+, four-cell. Compare relative sizes of blastomeres in this embryo with those in (B) and note the small polar body (arrow).


A
B
C
D
E
F

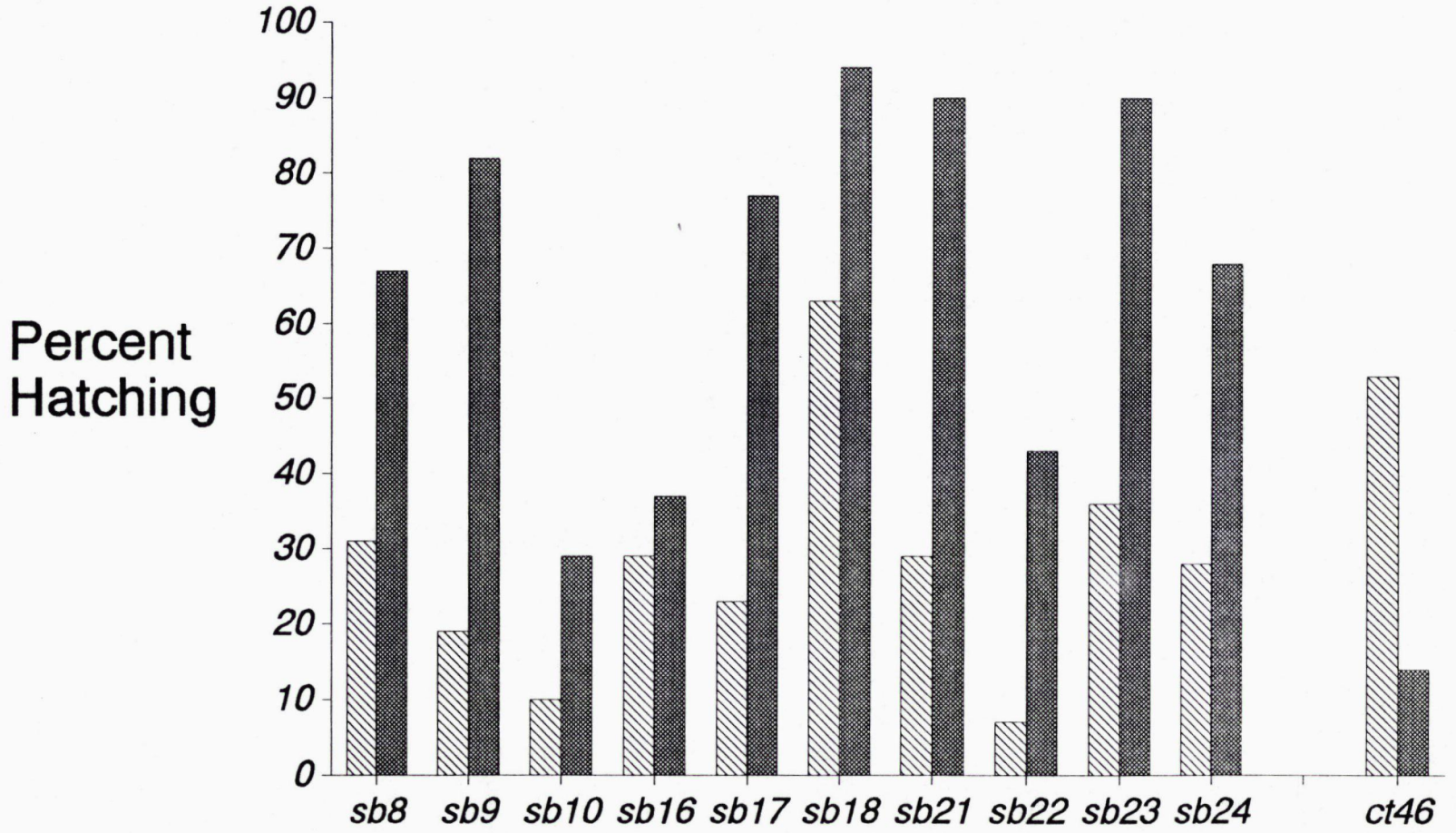
sizes and positions (Figure 3F). Weaker (i.e. more *ts*) class 3 suppressors display mitotic defects very similar to those produced by *mei-1(ct46)* (data not shown).

Class 3 suppressors are phenotypically enhanced by wild-type activity and can cause normal product to function inappropriately at mitosis: Surprisingly, the phenotype of many class 3 mutations was more severe in *trans* to wild type than when homozygous or when in *trans* to the null allele *ct46ct99*; embryonic hatching percentage was reduced by as much as six fold (Table 1, lines 1-4; Figure 4). This result was unusual because *ct46* did not show an analogous enhancement (Table 1, line 7; Figure 4).

One trivial explanation for this observation is that perhaps the null allele used, *mei-1(ct46ct99)*, possesses weak *trans*-suppressing activity. Phenotypic enhancement by wild-type activity would then be an artifact of the 'null' allele used. However, since a deficiency known to uncover *mei-1* also increases embryonic viability of class 3 *trans*-heterozygotes relative to wild type (Table 1, lines 1-4), this model seems unlikely. [It should be noted, however, that the deficiency failed to increase viability to the same level as that achieved by *ct46ct99*. The deficiency used also uncovers *mel-26*, a locus that interacts with *mei-1* (Mains *et al.*, 1990a); this may account for the difference].

The earlier suppressor screen (Mains *et al.*, 1990b) had identified one homozygous viable *mei-1* allele, *mei-1(ct46ct103)*, which caused weak, *ts* meiotic defects as a homozygote (Mains *et al.*, 1990b) and was interpreted as producing

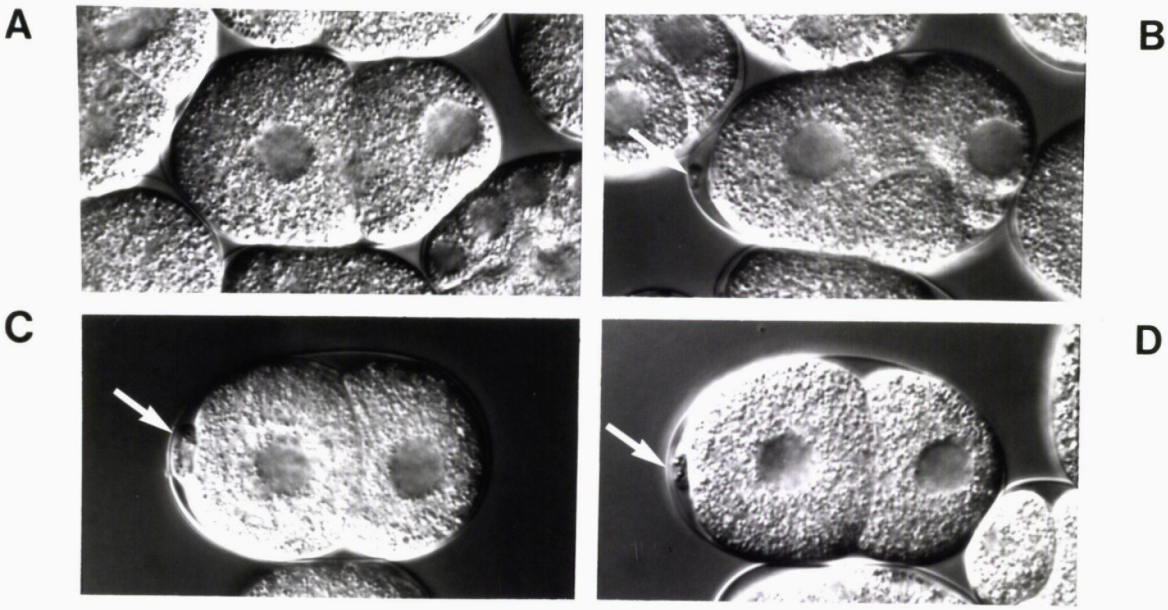
Figure 4. Bar graph plot of the percentage of hatched self-progeny embryos produced by all known class 3 alleles as heterozygotes to either wild-type (hatched bars) or *mei-1(ct46ct99)* (dark bars). The rightmost pair of bars represents analogous values for *mei-1(ct46)*.


Percent Hatching
100
90
80
70
60
50
40
30
20
10
0
sb8
sb9
sb10
sb16
sb17
sb18
sb21
sb22
sb23
sb24
ct46

reduced *mei-1* activity (Figure 5C). Although homozygous class 3 embryos display mitotic defects, we postulated that *ct46ct103* was similar to class 3 alleles isolated in the present screen. As described below, *ct46ct103* and other class 3 alleles display similar interactions when tested in *trans* to previously identified *mei-1* alleles. Furthermore, like other class 3 mutations, *ct46ct103* displays a weak dominant defect with low expressivity; approximately 75% of the embryos produced by *ct46ct103*/+ hermaphrodites hatch and defective embryos display mitotic defects (Figure 5B). Since *ct46ct103* homozygotes produce embryos with meiotic defects (Figure 5C; the embryonic phenotype of *ct46ct103/ct46ct99* is displayed in 5D), *ct46ct103* must enable wild-type activity provided by the homolog in heterozygotes to function aberrantly at mitosis. This conclusion is supported by the observation that mitotic defects caused by other class 3 alleles are quantitatively and qualitatively enhanced by wild-type activity (above and data not shown). Therefore, wild-type activity potentiates the mitotic defects and eliminates the meiotic defects caused by class 3 alleles (discussed further below).

We propose the following model. *mei-1(ct46)* product can function normally at meiosis but its activity can also act ectopically at mitosis. Class 3 suppressors encode products which can act ectopically like *ct46* but which have reduced activity; as a result, ectopic expression of these products results in mitotic defects that are less severe. *ct46ct103* has so little meiotic activity that it can neither support normal meiosis nor cause visible mitotic defects. That all class 3 products (including *ct46ct103*) can still function ectopically is supported

Figure 5. Nomarski photomicrographs of self-progeny embryos produced by *mei-1(ct46ct103)* and wild-type hermaphrodites. (A) wild-type, two-cell. (B) *ct46ct103*/+, two-cell. (C) *ct46ct103/ct46ct103*, two-cell. (D) *ct46ct103/ct46ct99*, two-cell. Compare the sizes of the polar bodies produced in (A) and (B) with those produced in (C) and (D) (indicated by arrows). Compare the mitotic division in (B) with the remaining plates.


A
B
C
D

by their interaction with wild type; they act in *trans* to allow wild-type activity provided by the homolog in heterozygotes to also act ectopically at mitosis. Implicit in this model is a balance between a minimum level of *mei-1* activity necessary for meiosis and a maximum level of ectopic activity tolerated at mitosis. Hypothesising that class 2 *trans*-suppressing alleles of *mei-1* act as antimorphs to reduce *mei-1* activity at both meiosis and mitosis (demonstrated below), we used these alleles to vary the amount of *mei-1* activity present in different class 3 suppressor backgrounds. If our model is correct and class 3 alleles produce reduced levels of normal *mei-1* activity in meiosis which can function ectopically at mitosis, then a weak *trans*-suppressor might ameliorate the mitotic defects caused by a class 3 allele but allow enough residual *mei-1* activity to support normal meiosis. Stronger *trans*-suppressors would not be expected to further improve mitosis but would reduce *mei-1* activity present at meiosis and cause meiotic abnormalities.

We will first demonstrate that *mei-1 trans*-suppressors can act as antimorphs to reduce the level of wild-type *mei-1* activity present. We will then examine the interactions between these mutations and the class 3 suppressors.

***trans*-suppressors of *mei-1(ct46)* are antimorphic:** These experiments rely on an assay specific for defects in meiosis. Autosomal meiotic non-disjunction leads to embryonic lethality or larval arrest in *C. elegans*. However, since males are genotypically XO while hermaphrodites are XX, meiotic non-disjunction of the X

chromosome in hermaphrodites can produce viable male embryos. In wild-type hermaphrodite populations, occasional X chromosome non-disjunction results in a spontaneous male frequency of approximately 1 in 500 (Hodgkin *et al.*, 1979). An increase in this frequency (causing a high incidence of males, or Him phenotype) should be indicative of inaccurate chromosome segregation and, possibly, meiotic spindle defects.

If *trans*-suppressors inactivate *gf* products, do they also inhibit *mei-1(+)* activity during meiosis? Using the strongest *trans*-suppressor available, *ct46ct82*, we were unable to detect consistent effects in *ct46ct82*/+ animals (Table 2, line 4). However, a slight increase in the relative proportion of *ct46ct82* product to wild-type activity (using *ct46ct82/ct46ct82*/+ animals and comparing with *ct46ct82*/+ animals) dramatically reduced embryonic viability (Table 2, line 1; compare with lines 2-5). Consistent with this lethality being caused by *mei-1(+)* activity being reduced by a dominant-negative effect, this strain produces large numbers of spontaneous males (Table 2, line 1). Given that a small difference in gene dosage has severe phenotypic effects, *ct46ct82*/+ animals probably produce just enough *mei-1(+)* activity to support normal meiosis and demonstrates that *trans*-suppressors act during, not after, meiosis.

***mei-1* protein activity must be eliminated after meiosis to allow normal mitosis:** We examined *trans*-heterozygotes between class 3 alleles and a representative series of other *mei-1* mutations which showed increasing ability to

Table 2. *mei-1 trans*-suppressors antagonise *mei-1(+)* activity at 25°

Maternal Genotype[a]	Viable Progeny (percent)	Male (percent)
1. *ct46ct82/ct46ct82/+*[b]	9	32
2. *ct46ct99/ct46ct99/+*[b]	80	0.3
3. +/+/+[b]	83	0.36
4. *ct46ct82/+*[c]	96	0.24
5. *ct46ct99/+*[c]	98	0.07

[a]Between 650 and 3100 embryos of each genotype were scored. The duplication used was *gaDp1(l;f)*.

[b]These hermaphrodites were morphologically Unc and homozygous for the recessive marker *dpy-5* (covered by the duplication). Hermaphrodites in line 1 were also heterozygous for the recessive marker *lon-2*.

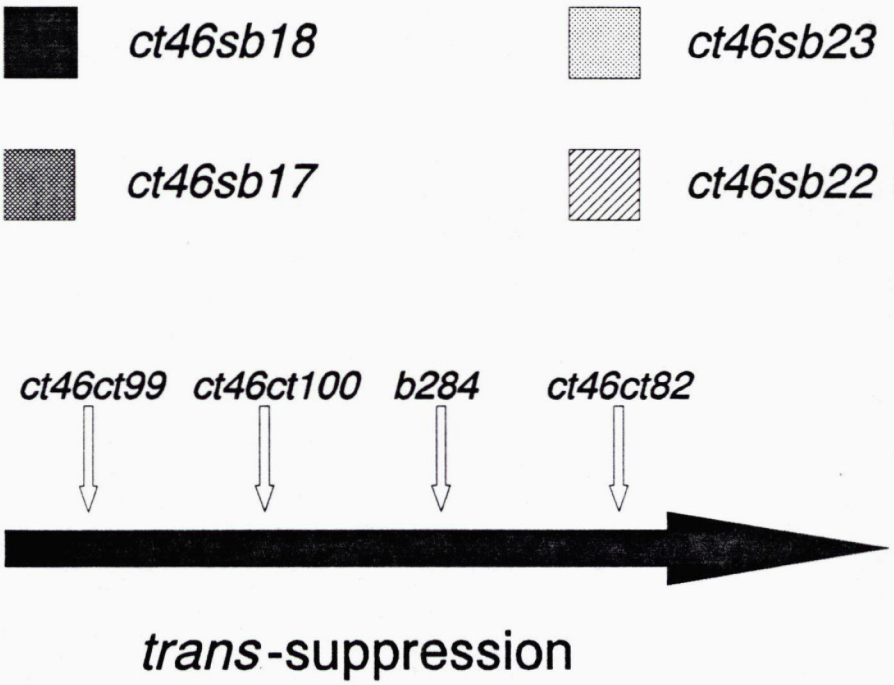
[c]These hermaphrodites were morphologically wild type and coisogenic for the recessive markers *unc-29*, *unc-13* and *lin-11*.

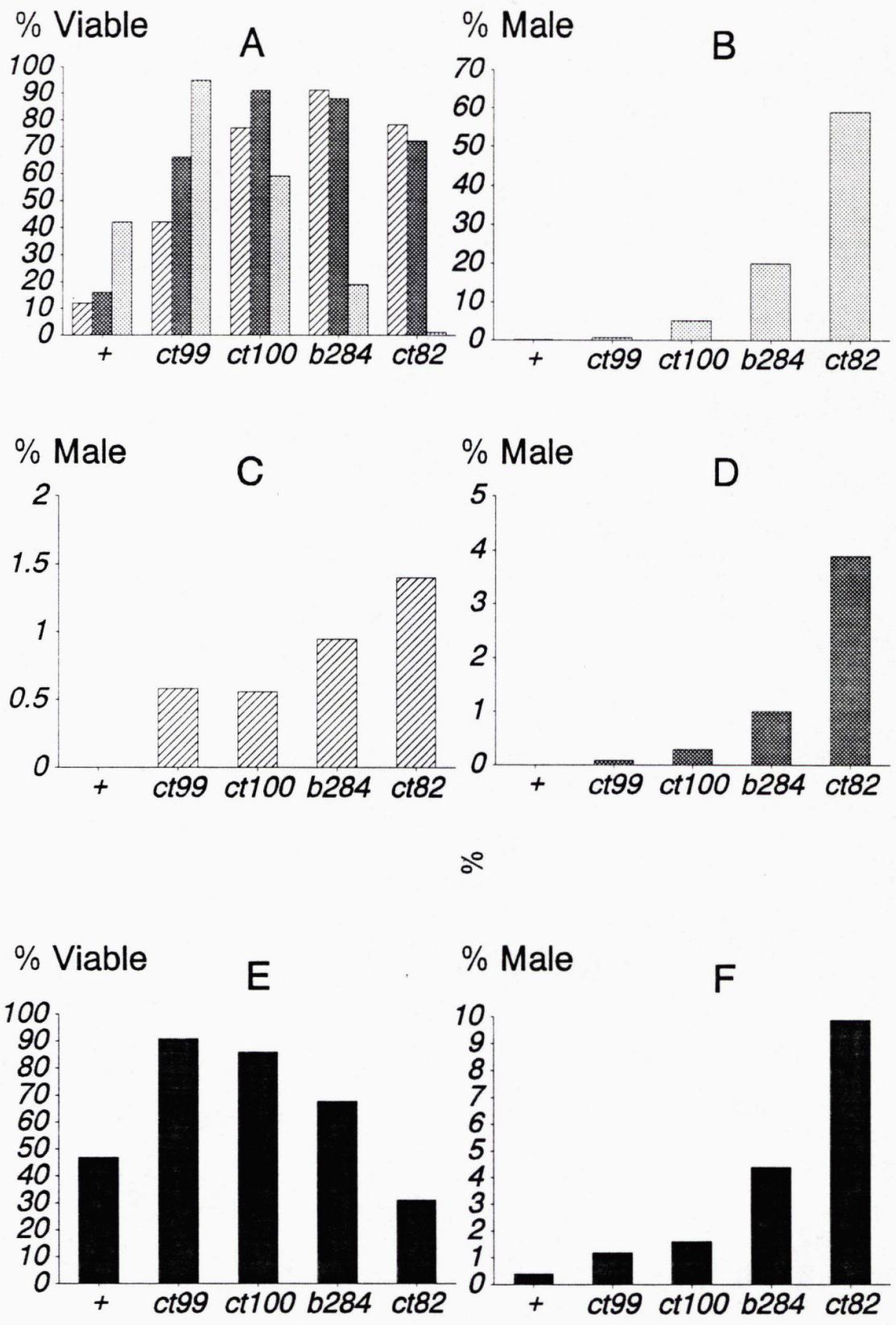
trans-suppress *ct46* (and had greater antimorphic activity) in the order *ct46ct99*, *ct46ct100*, *b284* and *ct46ct82* (Figure 6 and data not shown). In these experiments, the percentage of viable embryos is a measure of maternal defects which, as we will demonstrate, represent either meiotic or mitotic abnormalities, depending on genetic background. As discussed above, meiotic defects can be specifically assayed by noting the frequency of spontaneous males in

hermaphrodite populations. Strikingly, all class 3 alleles displayed similar patterns of embryonic viability and spontaneous male frequency. As mentioned earlier, embryonic viability increased relative to the wild-type heterozygote when the wild-type copy was replaced by the null allele *ct46ct99*. Testing against a battery of *trans*-suppressors resulted in variable changes in viability, depending on the class 3 allele used. Most notable, however, was that class 3 alleles (for example *ct46sb23*) that displayed good viability in *trans* to wild type displayed poor viability in *trans* to the strongest *trans*-suppressor known, *ct46ct82*. Conversely, class 3 alleles (for example *ct46sb22*) that hatched poorly in *trans* to wild type displayed high viability in *trans* to strong *trans*-suppressors. 7/11 class 3 alleles produced viabilities intermediate to those displayed by *ct46sb22* and *ct46sb23* in all *trans*-heterozygous combinations tested (for example, see *ct46sb17* in Figure 6A and data not shown). Two class 3 alleles, *ct46sb18* and *ct46ct103*, were anomalous, displaying relatively good viability in *trans* to both wild type and strong *trans*-suppressors (Figure 6E and data not shown). The significance of this observation is unclear; perhaps the suppressing mutations in these two alleles affect a second functional property of the *mei-1* protein in addition to reducing its activity. For example, perhaps these mutants interact inefficiently with *trans*-suppressing products, reducing the antimorphic effect.

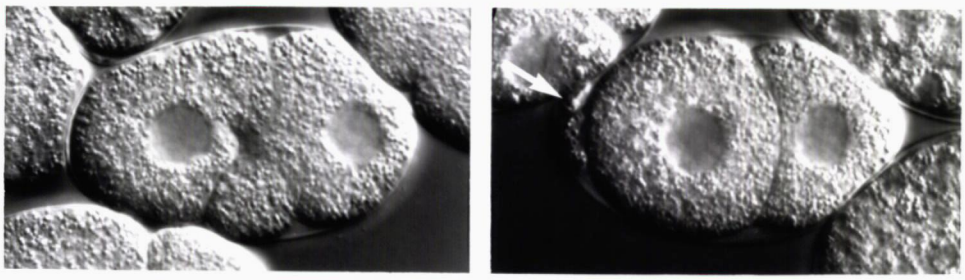
When class 3 alleles were in *trans* to wild type, no increase in the frequency of spontaneous males was observed. However, when class 3 alleles were in *trans* to strong *trans*-suppressors, large increases in male frequency were

Figure 6. Bar graph plots and nomarski photomicrographs of a series of class 3 *trans*-heterozygotes at 25°. (A) embryonic viabilities of certain *ct46sb22*, *ct46sb17* and *ct46sb23* *trans*-heterozygotes. (B-D) frequency of spontaneous males produced by the same *trans*-heterozygotes. (B) *ct46sb23*. (C) *ct46sb22*. (D) *ct46sb17*. Note the difference in scales. Note the correlation between low embryonic viability and high frequencies of males in strains bearing strong *trans*-suppressors. (E, F) embryonic viabilities and male frequencies respectively for *ct46sb18* *trans*-heterozygotes. Note how embryonic viabilities in (E) are much higher than the corresponding values for *ct46sb23* in (A). (G) 2-cell embryo produced by a *ct46sb23*/+ parent. (H) 2-cell embryo produced by a *ct46sb23*/*ct46ct82* animal. Compare the mitotic defects in (G) with the aberrant polar body (arrow) in (H).




% Viable
A
100
90
80
70
60
50
40
30
20
10
0
+
ct99
ct100
b284
ct82
% Male
B
70
60
50
40
30
20
10
0
+
ct99
ct100
b284
ct82
% Male
C
2
1.5
1
0.5
0
+
ct99
ct100
b284
ct82
% Male
D
5
4
3
2
1
0
+
ct99
ct100
b284
ct82
%
% Viable
E
100
90
80
70
60
50
40
30
20
10
0
+
ct99
ct100
b284
ct82
% Male
F
10
9
8
7
6
5
4
3
2
1
0
+
ct99
ct100
b284
ct82

G



H

observed (Figure 6, B-D and F). (While the spontaneous male percentage was not investigated for *ct46ct103*, it seems likely that *ct46ct103* behaves like other class 3 alleles).

Taken together, these results suggest a phenotypic transition: in *trans* to wild type, class 3 embryos display mitotic defects; *trans*-suppressors of intermediate antimorphic strength alleviate these defects, strong *trans*-suppressors reduce activity such that meiotic defects become apparent. To confirm this hypothesis, maternal-effect defects in the progeny of the *trans*-heterozygous animals were examined directly. Embryos produced by animals bearing class 3 alleles in *trans* to wild-type display the mitotic defects noted previously (Figure 6G). Embryos produced by animals bearing class 3 alleles in *trans* to strong *trans*-suppressors (where large numbers of males were observed) instead display meiotic defects characterised by large polar bodies and abnormal incorporation of maternal chromosomes into the zygote nucleus (Figure 6H). Embryos lacking maternal *mei-1* activity (produced by hermaphrodites homozygous for class 1 or class 2 alleles) display a similar range of defects.

***mei-1* and *mei-2* interact during normal embryogenesis:** Given the dominant negative properties of *mei-1(ct46ct82)*, we tested for interactions between this mutant and a *lf* mutation, *mei-2(ct102)*, in the same genetic pathway. Previous work (Mains *et al.*, 1990b) demonstrated that mutations at *mei-2* could *trans*-suppress *mei-1(ct46)*. Like *mei-1 trans*-suppressors, these *mei-2* alleles appear

to require the presence of defective product (Mains, unpublished observations). On this evidence alone, it is formally possible that *mei-2(lf)* alleles interact with *mei-1(gf)* product but *mei-2(+)* gene product does not normally interact with *mei-1(+)* activity. We examined the viability of embryos produced by + *mei-1(ct46ct82)/mei-2(ct102)* + animals (Table 3, line 1) and although only weak synthetic maternal-effect lethality was observed, the frequency of spontaneous males was high, suggesting meiotic defects. Heterozygotes of each mutation alone display wild-type levels of hatching and produce few spontaneous males; a null allele of *mei-1* did not interact with *ct102* (Table 3, lines 2-5). This implies that *mei-1* and *mei-2* normally interact and supports the idea that *ct46ct82*/+ animals produce barely enough *mei-1(+)* activity to carry out meiosis; *lf* mutations in the same genetic pathway reduce this activity below a critical level.

DISCUSSION

Meiotic and mitotic spindles are structurally distinct but must fulfil somewhat similar functions. It seems reasonable to suggest, therefore, that meiotic and mitotic spindles share certain analogous but functionally non-interchangeable components. If this is the case, some system must ensure that division-specific components are targeted to the correct spindle. Since meiosis and mitosis occur at different times, perhaps the simplest such system would ensure that analogous components are never present at the same time. Perhaps

Table 3. Synergistic interactions between *mei-1* and *mei-2* at 25°

Maternal Genotype[a]	Viable Progeny (percent)	Male (percent)
1. *ct102+/+ct46ct82*	60	4.7
2. *ct102+/+ct46ct99*	97	0.19
3. *ct102/+*	97	0.08
4. *ct46ct82/+*	96	0.24
5. *ct46ct99/+*	98	0.07

[a]Hermaphrodites in lines 1 and 2 were morphologically Unc-29 and coisogenic for the recessive marker *lon-2*, those in 3 were morphologically Lon, those in 4 and 5 were wild type and coisogenic for the heterozygous recessive markers *unc-29*, *unc-13*, and *lin-11*. Between 1500 and 3500 embryos of each genotype were scored.

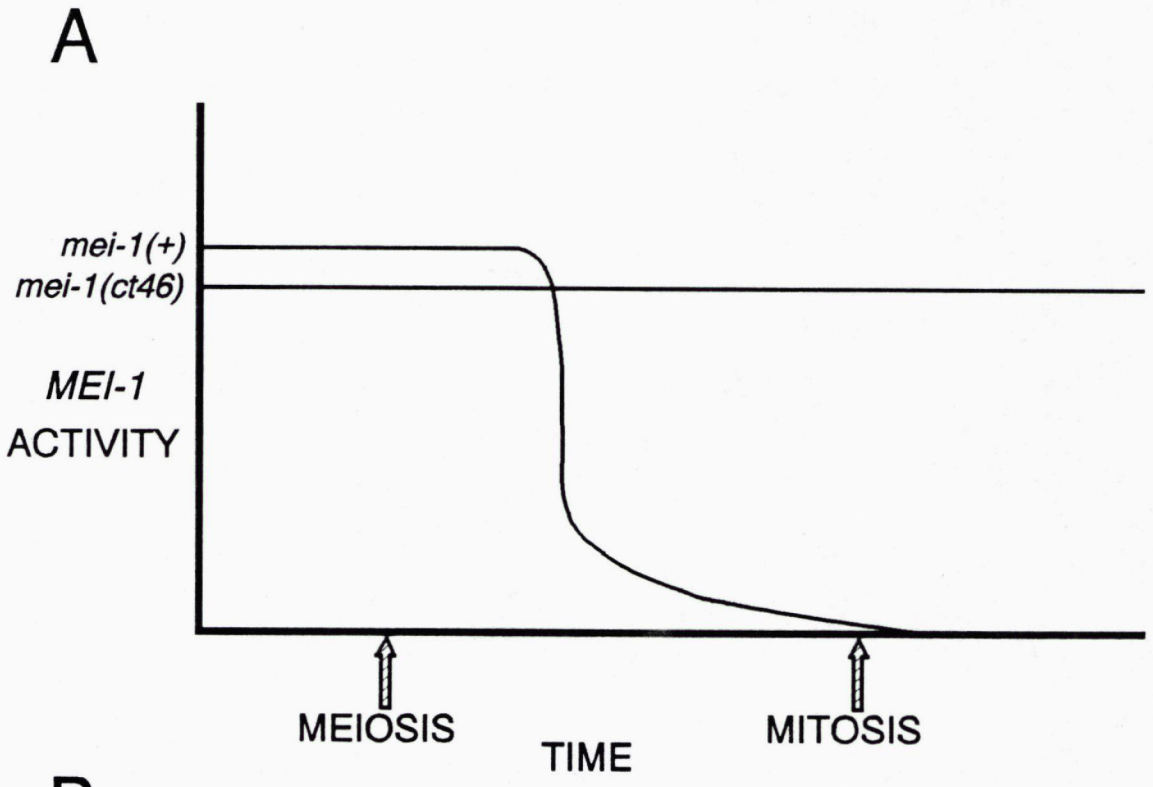
after meiosis, all meiosis specific structures are inactivated or eliminated; the only active components present at the time of mitosis are then mitosis specific.

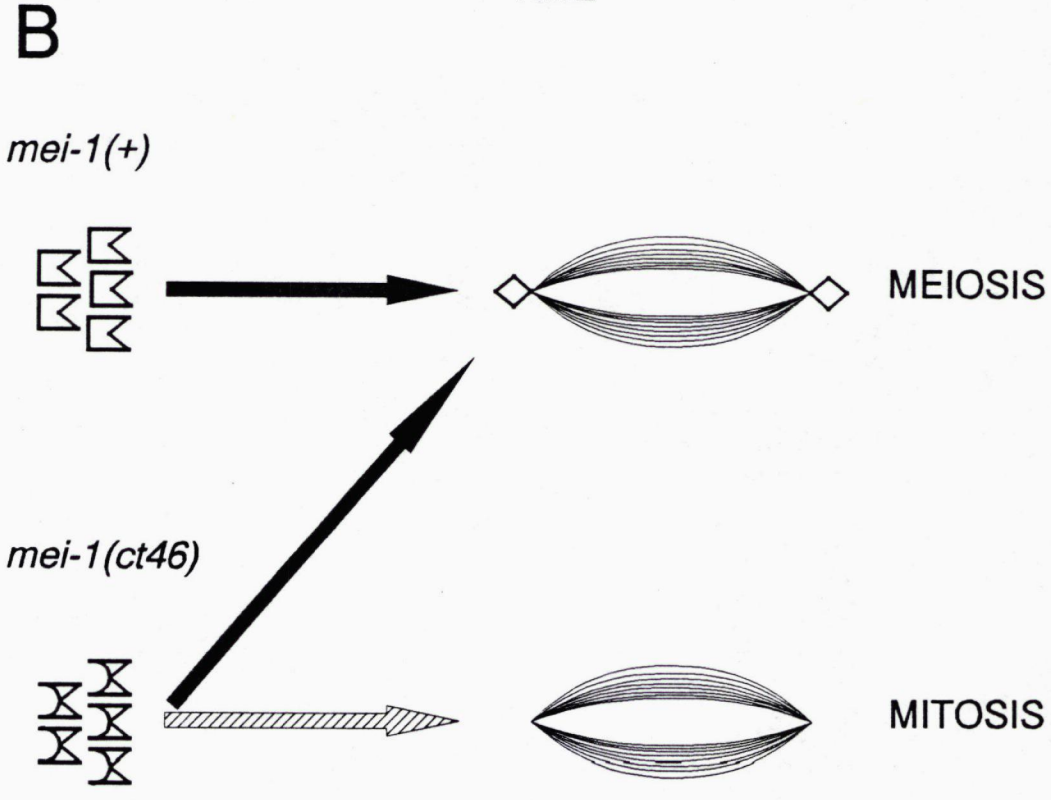
We postulate that the *mei-1* gene product is a meiotic spindle component, or a regulator of such components, with a functional analog in the mitotic spindle. Previous work demonstrated that maternal *mei-1(+)* activity was required for normal embryonic meiosis. However, a dominant allele, *mei-1(ct46)*, caused defects in mitosis rather than in meiosis. Intragenic suppressors of *ct46* were isolated at high frequency and fell into two classes. The first group of these

suppressors displayed characteristics consistent with null alleles, acting in *cis* to convert a *gf* mutation into a *lf* allele. The second group of intragenic suppressors were anomalous; they could dominantly suppress *ct46* both in *cis* and in *trans* and, with one exception, lacked functional *mei-1* activity. All mutations in both classes disrupted meiotic spindle formation when homozygous; mitotic divisions in these embryos were normal. This information and the results described in this work suggest the following model: *mei-1* gene product may be a component of a multimeric structure required for meiosis that must be inactivated prior to mitosis. The dominant allele, then, produces a product which is functional but whose activity is abnormally stable, preventing post-meiotic elimination (Figure 7A). Failure to reduce *mei-1* activity below a threshold level prior to mitosis results in inappropriate positioning of the mitotic spindle and aberrant cell division. *Trans*-suppressors produce a defective product capable of complexing with *ct46* or wild-type protein; such complexes retain some activity but are inactivated prior to mitosis. Class 3 alleles reported in this work represent modifications of *ct46* which have reduced activity (at both meiosis and mitosis) but have retained ectopic mitotic expression of this reduced activity.

Our evidence demonstrates that intragenic *mei-1* alleles capable of *trans*-suppressing *ct46* inhibit *gf mei-1* gene products in a dominant negative manner. Furthermore, these antimorphic alleles similarly affect *mei-1(+)* activity and do so prior to the completion of meiosis; the strongest *trans*-suppressor available, *ct46ct82*, produces defects in meiosis caused by a reduction in *mei-1(+)* activity

Figure 7. Two alternative models of the nature of the *mei-1(ct46)* mutation. (A) 'Hyper-stability'- *mei-1* activity is normally eliminated immediately after meiosis; *mei-1(ct46)* product is refractory to inactivation. (B) 'Specificity'- *mei-1* activity is normally only compatible with the meiotic spindle; *mei-1(ct46)* product can incorporate into both meiotic and mitotic spindles.


A
mei-1(+)
mei-1(ct46)
MEI-1
ACTIVITY
MEIOSIS
TIME
MITOSIS


B
mei-1(+)
MEIOSIS
mei-1(ct46)
MITOSIS

as seen in *ct46ct82/ct46ct82/+* animals (Table 2). We propose that the dominant negative effect of *mei-1 trans*-suppressors results from interactions between active and inactive gene products forming an inactive complex. Similar dominant negative interactions have been described in *C. elegans* for certain functionally redundant loci involved in cuticle formation (Kusch and Edgar, 1986; Kramer *et al.*, 1988). Since the *mei-1(null)* phenotype can involve defects in meiosis I, *mei-1* gene product may assemble early in meiosis; *trans*-suppressors may inactivate these complexes as they form.

Since *trans*-suppressors appear to act early in meiosis, why are meiotic defects not observed in embryos produced by *ct46ct82/+* or *ct46ct82/ct46* hermaphrodites? One possibility is that these animals produce just enough *mei-1(+)* activity to support normal meiosis; further reductions cause significant phenotypic effects. An alternative is that *mei-1(+)* activity is normally produced in excess; *ct46ct82* interacts with enough *ct46* product to eliminate the dominant effect but does not significantly reduce the excess present at meiosis. Two observations support the former explanation. First, embryos produced by *ct46ct82/ct46ct82/+* parents display meiotic defects; a relatively small change in gene dosage reduces *mei-1* activity below the critical threshold. Second, an additional *lf* mutation at a second locus in the same genetic pathway, *mei-2*, causes meiotic defects in *ct46ct82 trans*-heterozygotes. This observation also establishes an interaction between the products of the *mei-1* and *mei-2* loci.

Many of our inferences assume that class 3 alleles represent *lf*

modifications of *mei-1(ct46)* which reduce protein activity at both meiosis and mitosis. Given this hypothesis, properties of class 3 alleles should reflect properties of *ct46*. One alternative is that these mutations create new properties; inferences about properties of *ct46* are then difficult. However, since class 3 alleles were identified at relatively high frequency (10 suppressors isolated in a screen of approximated 22,750 *mei-1(ct46)* bearing chromosomes), a frequency similar to previous estimates of *lf* mutation rate at "average" *C. elegans* loci after standard EMS mutagenesis (Brenner, 1974; Anderson and Brenner, 1984; Park and Horvitz, 1986; Rogalski and Riddle, 1988; Johnsen and Baillie, 1991), and since their embryonic phenotype is similar to that produced by *ct46*, we believe this alternative unlikely.

The observation that class 3 alleles are phenotypically enhanced by wild-type activity and are capable of causing wild-type activity to function inappropriately at mitosis is striking. The classical interpretation (Muller, 1932) of this type of phenotypic enhancement is that class 3 alleles represent hypermorphic mutations that increase protein activity. In this interpretation, additional activity enhances phenotypic severity by exaggerating the initial excess. However, gene dosage experiments show that *ct46* does not behave like a hypermorphic mutation (Mains *et al.*, 1990a) and since class 3 alleles arise frequently, we prefer an alternate explanation.

One plausible model is based on the idea that like *ct46*, the product of class 3 alleles is unusually stable or refractory to post-meiotic inactivation. The

phenotypic enhancement of class 3 alleles by wild-type activity could then result from the dominant product artificially stabilising wild-type product in a complex (i.e. conferring its unusual stability in *trans*). One apparent anomaly in this interpretation is why wild-type activity did not enhance the *mei-1(ct46)* phenotype. If *ct46* produces a protein that is unusually stable but retains normal activity, complexes between *ct46* and wild-type product might be unusually stable but would possess the same activity as complexes containing only *ct46* product; as a result, no phenotypic enhancement would result.

While models in which dominant *mei-1* mutations cause *mei-1(+)* product to become unusually stable seem plausible, alternative frameworks are also possible (Figure 7B). For example, perhaps *mei-1* protein activity normally persists after meiosis; mitotic mis-incorporation might be prevented because the mitotic spindle might distinguish between *mei-1* and its mitotic analog; *mei-1(ct46)* might be altered such that this discrimination becomes more difficult. In this model, the postulated mechanism of *trans*-suppression can remain unaffected; incorporation of defective product into a multimeric complex inactivates the assembly.

Dramatic rearrangements of intracellular components are required during cell division: a complex series of nuclear and cytoplasmic migrations take place, intracellular organelles are partitioned and cleavage culminates a temporally precise series of steps (reviewed in Hartwell *et al.*, 1974; McIntosh, 1991). Research in many systems has defined a number of the steps involved and has

provided evidence for the importance of temporal coordination (for reviews Hartwell and Weinert, 1989; Murray, 1991). Three basic mechanisms maintain the temporal order of cell division events. The primary ordering mechanism is the intrinsic time required to complete each step in the pathway. Second, a strict temporal hierarchy in any multi-step process can be maintained when the product of one step is an essential substrate for a subsequent stage. Finally, to compensate for variations in the intracellular environment, somatic cells in a number of organisms have evolved a system of 'checkpoints' which inhibit certain processes until a series of conditions are fulfilled (for examples see Weinert and Hartwell, 1988; Hoyt *et al.*, 1991; Li and Murray, 1991; Schimke *et al.*, 1991).

Unlike 'adult' somatic cell division, embryonic cell cycles appear to have few checkpoint controls (reviewed in Murray and Kirshner, 1989). Our observations are consistent with this idea; the switch from meiosis to mitosis during *C. elegans* embryogenesis may not be checkpoint dependent. Embryos lacking maternal *mei-1* activity fail to assemble a meiotic spindle and segregate maternal chromosomes aberrantly; however, polar body formation, albeit aberrant, is often attempted and subsequent mitotic divisions in these embryos are normal and occur at appropriate times.

In conclusion, *mei-1* activity appears to display a precise temporal threshold; a certain level of activity is required to support meiosis but ectopic activity can cause mitotic defects. Dominant *mei-1* alleles may stabilise a multimeric complex of *mei-1* product, causing mitotic cleavage defects. Alleles

of *mei-1* that produce defective products can incorporate into these multimers, inactivate the complex and block the dominant effects. If *mei-1* dominant mutations do indeed cause *mei-1* protein to become unusually stable, an additional hypothesis can be made. If specific mechanisms are necessary to eliminate post-meiotic 'debris', these systems must rapidly inactivate meiosis-specific components and replace them with mitotic counterparts. In this manner, meiosis may constrain the embryonic cell cycle: mitosis can not occur properly until meiotic products have been eliminated. Molecular characterisation of the *mei-1* gene product should clarify the underlying mechanisms behind these genetical observations.

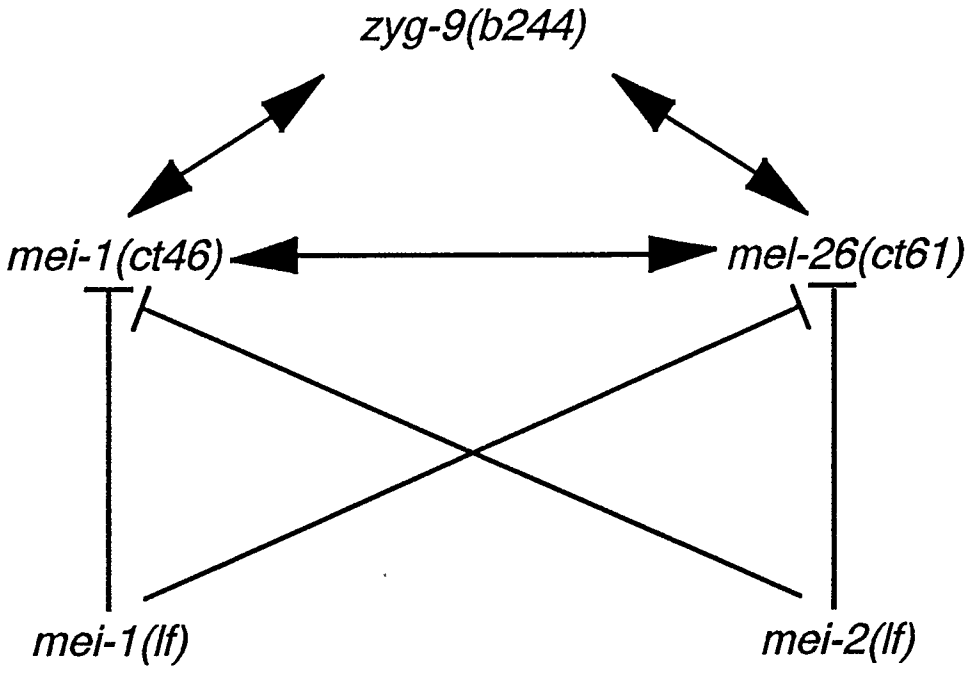
CHAPTER III

Genetic Identification and Analysis of the *sml-1* Locus

INTRODUCTION

Previous work demonstrated that *mei-1* alleles display complex genetic interactions with mutations at three other loci: *mei-2, mel-26* and *zyg-9* (Figure 8). One of these genes was identified in a screen for dominant suppressors of the dominant mutation *mei-1(ct46)* (Mains *et al.*, 1990b) but saturating for extragenic dominant suppressors using this procedure was impractical; the high frequency of intragenic pseudo-revertants was inconvenient. Using the alternative screening procedure described in Chapter II, we hoped to isolate more extragenic suppressors which might identify new genes involved in the *mei-1* pathway and establish additional genetic interactions between the four known loci. As outlined in Chapter II, this alternate screen identified ten intragenic pseudo-revertants and three putative extragenic suppressors; properties of the latter are described below. These latter mutations define the *sml-1* (suppressor of mitotic lethality) locus, act as dose dependent suppressors of *mei-1(ct46)* and reduce the activity of the *mei-1* genetic pathway.

Figure 8. Schematic illustration of the genetic interactions between alleles of *mei-1*, *mei-2*, *mel-26* and *zyg-9* (Mains *et al.*, 1990b).



Suppression

Enhancement

MATERIALS AND METHODS

Culture Conditions: General procedures for animal husbandry have been described in Chapter II; similar methods were used in these experiments.

Genes and Alleles: The nomenclature of Horvitz *et al.*, (1979) is followed. The following genes and alleles were used (Figure 9).

LG I: *dpy-5(e61), lin-11(n566), mel-26(ct61ts), mei-1(ct46ts, ct46ct99), unc-13(e1091), unc-29(e1072).*

LG II: *rol-6(e187), unc-4(e120), zyg-9(b244ts).*

LG III:*daf-2(e1370ts), dpy-17(e164), lon-1(e185), unc-32(e189), unc-45(e286ts), unc-79(e1068), unc-93(e1500), vab-6(e697).*

LG IV: *dpy-20(e1282ts), unc-24(e138).*

LG V: *dpy-11(e224), sma-1(e30).*

LG X: *lon-2(e678).*

Isolation and Characterisation of Suppressors of *mei-1(ct46):* The methods and rationale for the suppressor screen employed in this work have been described in detail in Chapter II. Three independent lines bearing suppressors not linked to marker mutations on LG I were identified in a screen of approximately 45,500 F_1 animals. All of these mutations were outcrossed at least

five times, displayed no apparent phenotype in the absence of *ct46*, and were maintained as homozygous *mei-1(+)* stocks at 15°.

Genetic Mapping: Three-factor map positions were determined by selecting recombinants for the morphological markers from the following heterozygotes. Each recombinant strain was then tested for the presence of the suppressor by backcrossing to *mei-1(ct46)* homozygotes and assaying *ct46/+; sup?/+* outcross progeny for the ability to produce viable embryos at the non-permissive temperature (25°). The fraction of recombinant lines bearing the suppressor are indicated. No linkage was detected to LG II, IV, V or X (data not shown).

sml-1 (III): *sml-1(sb26)/lon-1 unc-32*: Lon (0/8).

sml-1(sb26)/vab-6 daf-2: Vab (5/5), Daf (0/1).

sml-1(sb26)/dpy-17 unc-79: Dpy (1/1).

sml-1(sb26)/dpy-17 unc-93: Dpy (13/17),
Unc (3/10).

sml-1(sb27)/dpy-17 unc-93: Dpy (4/13),
Unc (3/15).

RESULTS

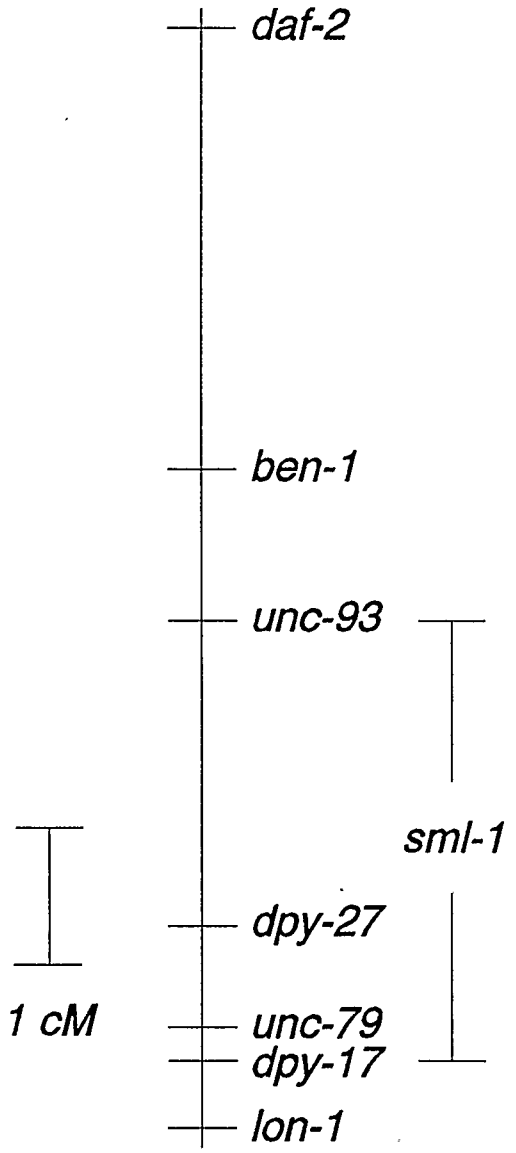
Rare extragenic suppressors of *ct46* define a new locus: As described in Chapter II, three suppressors of *ct46* were identified which were unlinked to

markers on LG I. Three-factor mapping experiments place two of the extragenic suppressors within a small (approximately 3cM) region of LG III (Figure 9). Given the relatively small genetic size of this region and the few known genes within it, we hypothesise that these two suppressors are allelic, defining a new locus, *sml-1*. The third suppressing mutation has not yet been mapped. Note that complementation testing would likely be uninformative; *sup/sup trans*-heterozygotes would probably display no phenotype.

The frequency with which these alleles arise implies that they do not represent simple *lf* mutations. Approximately 45,500 copies of the *sml-1* locus were screened for suppressors; the total of two (possibly three) mutations identified represents a frequency no greater than 1/15,000. Since estimates of the loss-of-function mutation rate at the average *C. elegans* locus after standard EMS mutagenesis range from 1/2000 to 1/6000 (Brenner, 1974; Anderson and Brenner, 1984; Park and Horvitz, 1986; Rogalski and Riddle, 1988; Johnsen and Baillie, 1991), suppressing *sml-1* alleles are clearly rare.

All three extragenic mutations suppress both *mei-1(ct46)* and *mei-26(ct61)*: Both intragenic *mei-1 trans*-suppressors and the known *mei-2* alleles can suppress both *mei-1(ct46)* and a different dominant mutation at a second locus, *mei-26(ct61)* (discussed in Mains *et al.*, 1990b and Chapter II). As these mutations were selected only for their suppression of *ct46*, suppression of *ct61* was unanticipated. We therefore tested these three new extragenic suppressors

Figure 9. Genetic map of the left region of LG III. The region to which *sml-1* has been mapped is indicated above.


daf-2
ben-1
unc-93
sml-1
dpy-27
1 cM
unc-79
dpy-17
lon-1

for interactions with both *ct46* and *ct61* (Table 4 and data not shown). As shown in Table 4, *sml-1(sb26)* can suppress both mutations in a dosage dependent manner; we hypothesise that *sb25* and *sml-1(sb27)* will behave similarly. Therefore, these mutations do not represent allele specific suppressors of *ct46*; rather they appear to affect a property more general to the *mei-1 - mel-26* pathway.

***sml-1(sb26)* is enhanced by *mei-1(ct46ct82)*:** A mutation which reduces the overall activity of a genetic circuit might interact with a number of mutations in the common pathway. Certain *mei-2* mutations appear to fall into this category (Mains *et al.*, 1990b) and, as discussed in Chapter II, display synergistic enhancement in *trans*-heterozygous combinations with strong *mei-1 trans*-suppressors. When heterozygous to wild type, *mei-1 trans*-suppressors produce barely enough *mei-1* activity to support normal meiosis; additional heterozygosity for a *mei-2(lf)* mutation reduces this activity below a critical threshold causing meiotic defects. We therefore tested whether *sml-1(sb26)* also reduced the activity of the *mei-1* pathway by examining *trans*-heterozygous combinations between this mutation and the strong *mei-1 trans*-suppressor, *mei-1(ct46ct82)* (Table 5). As noted, *sml-1(sb26)* is synergistically enhanced by *mei-1(ct46ct82)*; embryonic viability is decreased and the frequency of spontaneous males is elevated. As discussed in Chapter II, this observation implies that *sml-1(sb26)* functions in meiosis and reduces the overall activity of the *mei-1* genetic pathway.

Table 4. *sml-1(sb26)* dose dependent suppression of *mei-1(ct46)* and *mel-26(ct61)*

Mutation[a]	+/+[h]	*sb26*/+[f]	*sb26/sb26*[g]
1. *ct46*/+[b]	0.5	24	69
2. *ct46/ct46*[c]	0.2	4	nd[i]
3. *ct61*/+[d]	3	36	66
4. *ct61/ct61*[e]	0	5	nd[i]

[a]Headings indicate maternal genotypes. All values are percent of self-progeny that were viable at 25°. Between 100 and 900 embryos of each genotype were scored.

[b]These hermaphrodites were morphologically wild type and coisogenic for the recessive marker *unc-29*.

[c]These hermaphrodites were morphologically Unc-29.

[d]These hermaphrodites were morphologically wild type and coisogenic for the recessive marker *unc-13*.

[e]These hermaphrodites were morphologically Unc-13.

[f]These hermaphrodites were heterozygous for the recessive marker *dpy-17*.

[g]These hermaphrodites were morphologically Dpy-17.

[h]There results were obtained previously (Mains *et al.*, 1990b).

[i]not determined

Table 5. Synergistic interactions between *mei-1* and *sml-1*

Maternal Genotype[a]	Viable Progeny (percent)	Male (percent)
1. *ct46ct82/+; sb26/+*	71	4.0
2. *ct46ct82/+; sb26/sb26*	9.4	20
3. *ct46ct82/+*	96	0.24

[a]All hermaphrodites were coisogenic for the recessive marker *unc-29*, those in lines 1 and 3 were morphologically wild type, those in 2 were morphologically Dpy-17 (which marked the *sb26* chromosome). Hermaphrodites in line 1 were also heterozygous for the recessive marker *dpy-17*, those in 3 were heterozygous for the recessive markers *unc-13* and *lin-11*. Between 300 and 1200 embryos of each genotype were scored.

DISCUSSION

Extragenic suppressors have been used in a variety of systems to identify loci that can interact with a known mutation (for examples in *C. elegans* see Greenwald and Horvitz, 1982; Park and Horvitz, 1986). In many cases, loci identified as genetic suppressors have subsequently been shown to encode proteins with plausible functional interactions with the product of the original gene (in *C. elegans*, for example, see Horvitz and Sternberg, 1991). Ideally one would

hope to identify a 'chain' of revertants in which each mutation is related by a suppressing interaction to a different mutation in the group (discussed in Jarvik and Botstein, 1975; applied in Moir *et al.*, 1982). In this manner, a series of genetically interacting loci involved in a common pathway might be identified. Implicit, however, is the ability to select for 'suppressors of suppressors'; that is, suppressors which correct a mutational defect which itself was selected for suppressing properties. Unfortunately, most genetic suppressors lack selectable phenotypes on their own (Jarvik and Botstein, 1975); suppressor isolation is then typically restricted to a single 'cycle' of screening.

Unfortunately, the suppressors identified in this work lack visible phenotypes. However, analysis of a series of *trans*-heterozygotes bearing these suppressors in combination with other mutations in the same genetic pathway suggests that all *trans*-suppressors of *mei-1(ct46)* (both intragenic and extragenic) may share certain properties.

Like intragenic, *trans*-suppressing *mei-1* mutations and certain *mei-2(lf)* alleles, *sml-1* suppressors are capable of suppressing both *mei-1(ct46)* and *mel-26(ct61)*; clearly none of these mutations represent allele specific suppressors. Formally, therefore, these mutations must affect some property of the genetic pathway common to both dominant mutations. Consistent with this hypothesis, both *mei-1 trans*-suppressors and *mei-2(lf)* alleles appear to reduce the activity of the *mei-1* pathway as a whole. To test the hypothesis that *sml-1(sb26)* might function analogously, we examined *sml-1(sb26)/+;*

mei-1(trans-suppressor)/+ animals for synergistic phenotypic enhancement. As demonstrated in Chapter II, mei-1 (ct46ct82)/+ animals produce just enough *mei-1* activity to support normal meiosis; further loss of activity creates meiotic defects. Since *sb26*/+; *ct46ct82*/+ animals display reduced embryonic viability and increased levels of meiotic non-disjunction (as assayed by the frequency of spontaneous males), it appears that *sb26* reduces *mei-1* pathway activity. Note, however, this reduction in *mei-1* activity may not cause the suppressing effect; *sml-1(sb26)* could suppress the dominant defect through an independent mechanism.

Since *sml-1(sb26)* appears to reduce the activity of the *mei-1* pathway, one possibility is that *sb26* represents a *lf* mutation analogous to suppressing alleles of *mei-2*. However, while a deficiency covering *sml-1* has not been tested for the ability to *trans*-suppress, *sml-1* suppressing alleles are rare and may represent more unusual mutational events. One possibility is that *sml-1(sb26)* represents a *gf* mutation affecting a product which normally inhibits *mei-1* activity; the suppressing product is a more effective inhibitor. In the context of the models discussed in Chapter II, one such product might be a component of a system required to inactivate *mei-1* product after meiosis.

CHAPTER IV

Selection for Mutations Disrupting Embryogenesis

INTRODUCTION

Two loci involved in the genetic pathway discussed in this work were initially identified in a general screen for dominant, temperature-sensitive, maternal-effect lethal mutations (Mains *et al.*, 1990a). This screen was, by design, highly labour intensive; practical considerations prevented large-scale mutagenesis and a number of loci with potential dominant, *ts*, maternal-effect lethal phenotypes remain to be identified. We have, therefore, designed an alternate procedure which should allow positive selection for this type of mutation; if successful, this method should facilitate exhaustive screens.

MATERIALS AND METHODS

Culture Conditions: General procedures used in animal husbandry have been described in Chapter II; similar methods were employed in these experiments.

Genes and Alleles: The nomenclature of Horvitz *et al.* (1979) is followed. The following genes and alleles were used.

LG I: *daf-8 (e1393ts), dpy-5(e61)* and *lin-11(n566).*

LG IV: *egl-23(n601).*

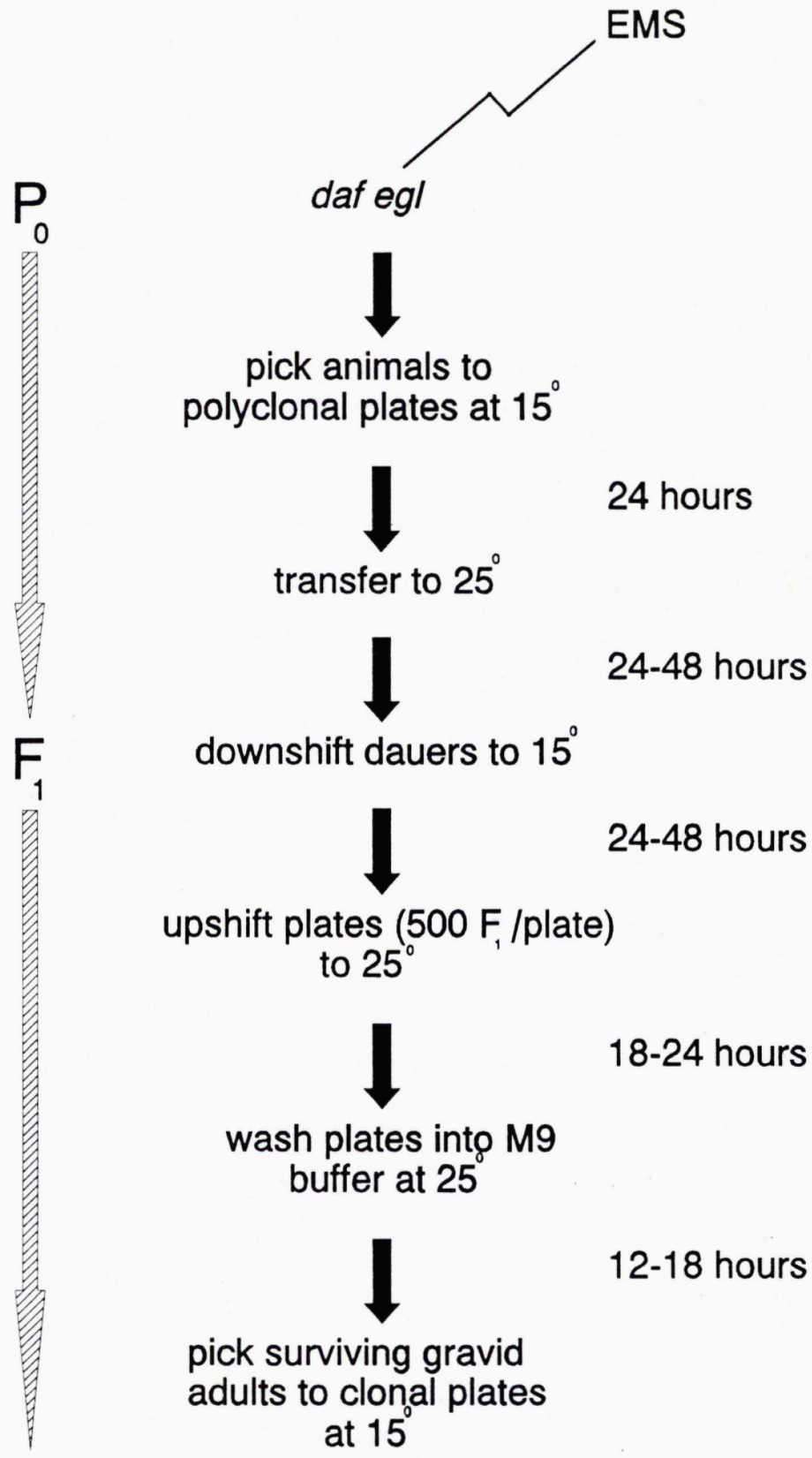
LG X: *lon-2(e678).*

Selection Procedure: A general outline of the genetic screen employed in this study is outlined in Figure 10; a detailed rationale is offered below. Briefly, egg-laying defective (Egl) P_0 animals were mutagenised with EMS under standard conditions (Brenner, 1974) and a synchronous population of F_1 progeny were obtained. These animals were then pooled, transferred to 25° (the non-permissive temperature), and allowed to become gravid; each pool was then transferred to M9 buffer (Wood, 1988) (still at 25°) and incubated until the majority of F_1 animals lysed. Intact F_1 animals were then cloned to 15° and F_2 animals were tested for the presence of dominant, *ts*, maternal-effect lethal mutations.

RESULTS

Screening Rationale: A previous screen for dominant, *ts*, maternal-effect lethal mutations (Mains *et al.*, 1990a) was laborious; F_1 progeny of mutagenised hermaphrodites were cloned to plates at 25°, allowed to lay eggs and then transferred to 15°. Putative mutants were selected by comparing embryonic viability at the two temperatures. As F_1 animals had to be examined individually, the total number of animals that could be screened using this procedure was somewhat limited.

Figure 10. Schematic illustration of the positive selection scheme used to isolate new dominant, *ts*, maternal-effect lethal mutations.


EMS
daf egl
P0
pick animals to polyclonal plates at 15°
24 hours
transfer to 25°
24-48 hours
downshift dauers to 15°
F1
24-48 hours
upshift plates (500 F1 /plate) to 25°
18-24 hours
wash plates into M9 buffer at 25°
12-18 hours
pick surviving gravid adults to clonal plates at 15°

To facilitate saturation for dominant, *ts*, maternal-effect lethals, a method allowing F_1 animals to be screened in large pools was required. The procedureoutlined here relies on five observations. First, by employing strains unable to lay eggs, the viability of a given clutch of embryos can be correlated with their parent, even within a large population of adult animals. Second, embryos which hatch inside an animal produce larvae that rapidly destroy their parent. Third, upon removal of food, adult animals immediately cease egg production; embryos produced prior to this time, however, develop normally and can kill their parent. Fourth, upon return of food, starved animals usually re-initiate egg production. Finally, this screen depends on the ease with which large, synchronous populations of worms can be obtained.

Applying this information to our screening procedure, we anticipate that most F_1 animals would produce viable embryos during the initial incubation at 25^0; hatching of these embryos during the incubation in buffer would destroy the F_1 animal. Rare F_1 animals bearing dominant, *ts*, maternal-effect lethal mutations, however, would produce inviable embryos during the initial period at 25^0 and would, therefore, survive incubation in buffer. Transferring these animals to plates at 15^0 (the permissive temperature) should recover viable F_2 animals bearing the putative dominant mutation.

Current Status: This screening procedure has been applied twice; neither trial has been completed but the current results are outlined below.

In the first attempt, animals of the genotype *dpy-5 lin-11; lon-2* were mutagenised with EMS and approximately 1800 L4 F_1 animals were screened. All intact animals after the buffer incubation step were cloned to plates at 15°; the 91 clones obtained were divided into three distinct classes (described below).

class 1. (54 animals) failed to produce viable offspring at 15°.

class 2. (30 animals) produced 3-5 viable F_2 offspring.

class 3. (5 animals) produced 20-30 viable F_2 offspring.

The animals in class 3 were subsequently shown to be normal; these probably represent animals which had been too young to produce embryos during the initial 25° incubation and had therefore survived the buffer incubation. All class 2 lines were lost prior to characterisation.

Three problems emerged from this initial work. First, the presence of escaping animals (class 3) suggested that selecting all intact animals after the buffer incubation was not sufficiently stringent; in the second screen, only surviving F_1 animals which contained inviable embryos were cloned. Second, the genotype of the parent strain was unsuitable; *lin-11(n566)* completely blocks egg laying and gravid *lin-11* animals are often damaged by unhatched eggs. In the second screen an alternate strain bearing the mutation *egl-23(n601)* was used; this strain releases embryos when the gonad becomes extremely full. Finally, the first screen required manual selection of a synchronous population of F_1 animals; to avoid this labour intensive step a strain bearing the mutation *daf-8(e1393ts)* was employed. *daf-8* animals arrest as dauer larvae when grown at 25°; large

populations can be prepared at 25^0 and, by downshifting the cultures to 15^0, released from their arrest point simultaneously.

In this second screen, approximately 30,000 F_1 animals were tested and a total of 35 independent lines were isolated. Analysis of these clones is underway.

DISCUSSION

The screening procedure outlined in this work is conceptually similar to a previous screen employed to identify non-conditional, recessive, maternal-effect lethal mutations causing embryonic lethality (for one application, see Kemphues *et al.*, 1988a). In this screen, progeny of individual F_1 Egl animals were examined. Clones which had acquired a recessive maternal-effect lethal mutation would segregate F_2 animals incapable of producing viable embryos; such animals produce 'bags of eggs', a phenotype that is readily scored. Putative mutations were then recovered by selecting siblings from such clones. In the selection scheme outlined in this work, instead of recovering the mutation of interest from sibs of the 'bag of eggs', the mutation is recovered from the bagged worm itself. This modification greatly reduces the number of animals that must be handled individually, facilitating large screens.

CHAPTER V

Future Directions

The work described herein suggests a number of potential experimental approaches. For the sake of brevity the following discussion will focus on genetic methods; molecular approaches are both powerful and straightforward.

The observation that heterozygous animals bearing strong *trans*-suppressors produce barely enough *mei-1* activity to support normal meiosis suggests two approaches to identifying additional genes in the *mei-1* pathway. First, genetic enhancers of *ct46ct82*/+ animals might identify mutations which further reduce the activity of the *mei-1* pathway. However, this approach is somewhat laborious; individual F_1 animals must be examined for phenotypic enhancement. Alternatively, existing collections of recessive mutations that cause increased levels of meiotic non-disjunction (high incidence of males, Him mutants) might include mutations that interact with *mei-1(ct46ct82)*; increased meiotic non-disjunction in *ct46ct82*/+; *him*/+ animals would suggest synergistic enhancement. However, such simple *lf* mutations might not reduce pathway activity sufficiently to create a visible genetic enhancement; the only known *lf* mutation *mei-2(ct102)* known to interact with *mei-1(ct46ct82)* may actually be antimorphic (Mains, personal communication).

Suppressor screens employed to date have clearly failed to saturate for extragenic suppressors of the dominant mutation *mei-1(ct46)*. Two loci in the

mei-1 pathway, *mei-2* and *sml-1*, have been identified through reversion analysis; an alternative screening procedure which does not detect intragenic suppressors may identify additional loci. Furthermore, since all known suppressors of *mei-1(ct46)* also suppress *mel-26(ct61)*, an alternative screen would simply revert the dominant defect caused by *ct61*. Even if such a screen failed to identify interacting loci, intragenic pseudo-revertants of *ct61* would probably clarify the role of the *mel-26* gene product in the *mei-1* pathway.

If successful, the positive selection scheme for the isolation of dominant, *ts*, maternal-effect lethal mutations offers a great deal of potential. While a number of such mutations might lack easily scorable terminal phenotypes, examination of early stage embryos for the presence of defects like those observed with *mei-1(ct46)* should allow rapid classification of the mutant collection. Reversion studies of such a collection would not only identify hierarchies of genetically interacting genes but would also clarify the relative roles of genetically redundant versus essential genes during early embryogenesis (in a manner similar to work on dominant mutations affecting morphology and behaviour, Park and Horvitz, 1986) Finally, minor modifications of this screening procedure should facilitate analogous screens for both recessive maternal-effect lethal and recessive zygotic lethal mutations.

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