Dominant Maternal-Effect Mutations Causing Embryonic Lethality in *Caenorhabditis elegans*

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ABSTRACT

We undertook screens for dominant, temperature-sensitive, maternal-effect embryonic-lethal mutations of *Caenorhabditis elegans* as a way to identify certain classes of genes with early embryonic functions, in particular those that are members of multigene families and those that are required in two copies for normal development. The screens have identified eight mutations, representing six loci. Mutations at three of the loci result in only maternal effects on embryonic viability. Mutations at the remaining three loci cause additional nonmaternal (zygotic) effects, including recessive lethality or sterility and dominant male mating defects. Mutations at five of the loci cause visible pregastrulation defects. Three mutations appear to be allelic with a recessive mutation of *let-354*. Gene dosage experiments indicate that one mutation may be a loss-of-function allele at a haploin sufficient locus. The other mutations appear to result in gain-of-function "poison" gene products. Most of these become less deleterious as the relative dosage of the corresponding wild-type allele is increased; we show that relative self-progeny viabilities for the relevant hermaphrodite genotypes are generally $M/+/+ > M/+ > M/M/+ > M/Df > M/M$, where M represents the dominant mutant allele.

UNDERSTANDING the genetic control of *Caenorhabditis elegans* embryogenesis will require identifying genes involved in patterning of the early embryo. Such genes have so far been sought almost exclusively in screens for recessive mutations causing embryonic lethality (HIRSH and VANDERSLICE, 1976; WOOD *et al.* 1980; SCHIERENBERG, MIWA and VON EHRENSTEIN 1980; MIWA *et al.* 1980; ISNENGHI *et al.* 1983; DENICH *et al.* 1984; KEMPHUES *et al.* 1988). These screens yielded mutations defining about 70 genes, most of which appear to act maternally (for review, see WOOD 1988), and led to an estimate of 200–600 genes that could mutate to alleles that cause embryonic lethality (CASSADA *et al.* 1981). However, based on a survey of probable null alleles, KEMPHUES, KUSCH and WOLF (1988) estimated that there may be as few as a dozen maternally active genes whose products are required only for embryogenesis.

We have undertaken a different kind of screen with different biases, for dominant temperature-sensitive (*ts*) lethal mutations affecting *C. elegans* embryogenesis, based on the following rationale. Dominant gain-of-function mutations, resulting for example in the expression of a normal gene product in the wrong tissue or at the inappropriate time during development, could yield additional information about the function of genes previously identified by recessive mutations (LEWIS *et al.* 1980; AMBROS and HORVITZ 1984; STRUHL 1985). More important, it seems likely that some genes involved in early embryogenesis will be identifiable more easily, or perhaps only, by dominant rather than recessive mutations. There are at least three possible classes of such genes.

First, members of redundant multigene families are likely to be identified only by dominant, gain-of-function "poison" mutations (SUZUKI 1970; WATERSTON, HIRSH and LANE 1984; see PARK and HORVITZ 1986 for review). Null mutations in these genes will have a wild-type phenotype, because other members of the family can supply the function of the defective locus. Gene families such as these may be common in *C. elegans*. PARK and HORVITZ (1986) examined dominant behavioral and morphological mutations at nine loci and found that homozygotes for apparent loss-of function alleles of four of the genes showed no mutant phenotype. They estimated that half the genes in *C. elegans* may have wild-type loss-of-function phenotypes. Similar estimates have been made for other organisms as well (GOEBL and PETES 1986; DOVE 1987). In *C. elegans*, dominant gain-of-function mutations have been found in known or suspected members of several multigene families, including an actin gene, *unc-92* (WATERSTON, HIRSH and LANE 1984), a tRNA gene, *sup-7*, (WATERSTON 1981), four known or probable collagen genes, *sqt-1*, *sqt-2*, *sqt-3*, and *rol-8* (KUSCH and EDGAR 1986; KRAMER *et al.* 1988), and a gene for an unidentified muscle component, *unc-93*

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(GREENWALD and HORVITZ 1980, 1982).

A second class of genes more easily identifiable in screens for dominant mutations are haploinsufficient loci, which are required in two normal doses. Loss-of-function mutations in essential haploinsufficient genes must be isolated as conditional alleles (or in the presence of a duplication). Such genes could encode components of morphogenetic gradients or other positional signalling systems that would likely be sensitive to dosage, such as *bicoid* in Drosophila (DRIEVER and NUSSLEIN-VOLHARD 1988). Several mutations at haploinsufficient loci that result in disruption of embryonic patterns have been found in this organism (DENELL 1978; SIMPSON 1983; GELBART *et al.* 1985; FROHNHOFER and NUSSLEIN-VOLHARD 1986; GERGEN and WIESCHAUS 1986; MOHLER and WIESCHAUS 1986). In *C. elegans*, *unc-108* (PARK and HORVITZ 1986), *tra-2* (SCHEDL and KIMBLE 1988), and *fem-3* (BARTON, SCHEDL and KIMBLE 1987) may be examples of haplo-insufficient loci.

A third class, likely to overlap the first, includes genes whose products form multimers in which one defective subunit could inactivate the complex (KEMPHUES *et al.* 1980; WATERSTON, HIRSH and LANE 1984; KUSCH and EDGAR 1986; HERSKOWITZ 1987; ALBERS and FUCHS 1987; STACEY *et al.* 1988), or for which the stoichiometry between different types of subunits must be strictly regulated (ORKIN and KAZAZIAN 1984; MEEKS-WAGNER and HARTWELL 1986; HOMYK and EMERSON 1988).

We report here on the isolation and initial characterization of eight dominant *ts* maternal-effect embryonic-lethal mutations in *C. elegans*. For each mutation we have determined the genetic map position, the site of production of the corresponding gene product (egg, sperm, and/or embryo) based on genetic tests for parental effects, and its time of action as determined by temperature-shift experiments. We have also determined the resulting embryonic phenotype as observed by differential interference contrast (Nomarski) microscopy, and whether the mutation results in a gain or loss of function based on gene dosage experiments. Although simple genetic complementation tests are not feasible in all cases because of dominance, the map positions and other properties of the eight mutations suggest that they represent six genes. Two of these, *let-354* (HOWELL *et al.* 1987; HOWELL 1989) and *mei-1* (K. KEMPHUES and S. SPRUNGER, personal communication) were previously identified by other mutations; the remaining four have been named *mel-23*, *mel-24*, *mel-25* and *mel-26* (for *m*aternal-*e*ffect-*l*ethality).

MATERIALS AND METHODS

Culture conditions: *C. elegans* (var. Bristol) was maintained according to BRENNER (1974). Unless otherwise stated, all stocks and crosses were maintained at 15°C. The viability of self progeny was determined by scoring complete broods of 5–8 individual hermaphrodites (500–2500 embryos total). Animals were transferred daily to fresh plates preequilibrated to the appropriate temperature, until they stopped laying fertilized embryos. Each plate was scored for unhatched embryos one day after the hermaphrodite was removed. The following day, hatched animals were counted, and the number of arrested larvae (those much smaller than their sibs) was noted. For experiments at 15°, animals were transferred every other day, and unhatched embryos were counted two days after the hermaphrodite was removed.

Genes and alleles: Genetic nomenclature follows that described by HORVITZ *et al.* (1979). LG refers to linkage group. *M* is used to designate the dominant *ts* allele in general genotype descriptions. The following genes and alleles were used (also see Figure 1):

LG I: *bli-4(e937)*, *dpy-5(e61)*, *emb-6(hc65)*, *dpy-14(e188)*, *let-80(s96)*, *let-84(s91)*, *let-354(h79)*, *lin-10(e1438)*, *lin-11(n566)*, *lin-28(n719)*, *mei-1(b284)*, *unc-11(e47)*, *unc-13(e450* and *e1091)*, *unc-15(e73)*, *unc-29(e193* and *e1072)*, *unc-38(x20)*, *unc-73(e936)*, *unc-74(e883)*.

LG II: *zyg-9(b244)*.

LG III: *dpy-17(e164)*, *dpy-18(e364)*, *dpy-28(s939)*, *unc-32(e189)*, *unc-49(e382)*, *unc-69(e587)*, *vab-7(e1562)*.

LG IV: *daf-15(m81)*, *dpy-20(e1282)*, *fem-3(e1996)*, *unc-17(e245)*, *unc-24(e138)*.

LG V: *daf-11(m47)*, *emb-18(g21)*, *him-5(e1490)*, *unc-42(e270)*.

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

Isolation of mutations: Mutagenesis with ethylmethanesulfonate (EMS, Sigma, St. Louis, MO) was carried out according to BRENNER (1974). L4 hermaphrodites were mutagenized with EMS and the F_1 progeny (putative *M*/+) were picked to individual plates at 15°. After embryos were laid at the permissive temperature, worms were shifted to 25°, allowed to self-fertilize for 1d, and then removed. The following day, those pairs of plates that showed hatching at 15°, but not 25°, were selected. Of 2500 F_1 worms screened, five proved to carry dominant *ts* lethal mutations. An alternative screen, where L4 F_1 animals were first cloned at 25° and then shifted to 15°, produced three dominant *ts* mutations among 1500 tested worms. The overall frequency was eight mutations among 8000 mutagenized haploid genomes. Each mutant was derived from a separate mutagenized parent.

All mutations were outcrossed at least ten times to remove extraneous mutations induced by the mutagen and were mapped to intervals of ≤1 cM (Figure 1). For each mutation, an appropriate balancer chromosome, carrying closely linked flanking morphological markers, was chosen.

Genetic mapping: Three-factor map positions were determined by selecting recombinants for the morphological markers from the following heterozygotes. The map positions are shown in Figure 1. The fraction of *ts* recombinants are indicated.

let-354 I: *let-354(ct42)/unc-11 unc-38*: Unc-11 (4/11), Unc-38 (7/11). (These two phenotypes can be distinguished: *unc-11* shows a ratchet-like reverse movement; *unc-38* is sluggish; *unc-11 unc-38* is severely paralyzed).
let-354(ct42)/unc-73 dpy-5: Unc (2/7), Dpy (15/25).
let-354(ct76)/unc-11 unc-38: Unc-11 (1/2), Unc-38 (6/10).
let-354(ct76)/unc-73 dpy-5: Unc (9/16), Dpy (13/17).

let-354(ct77)/unc-11 unc-38: Unc-11 (7/9), Unc-38 (3/8).
let-354(ct77)/unc-73 dpy-5: Unc (4/15), Dpy (9/12).

mel-23 III: *mel-23(ct45)/unc-49 vab-7*: Unc (12/18), Vab (28/33).
mel-23(*ct45*)/*unc-32 dpy-28*: Unc (20/22); because *dpy-28* mutants show maternal-effect lethality, Dpy recombinants were not scored.

mei-1 I: *mei-1(ct46)/lin-10 unc-29*: Lin (1/9), Unc (7/12).
mei-1(ct46)/unc-13 lin-28: Unc (14/17), Lin (2/25).

mel-24 IV: *mel-24(ct59)/unc-24 fem-3*: Unc (18/20); Fem animals are not self fertile, and so were not scored.
mel-24(ct59)/unc-24 daf-15: Unc (13/18); Daf-15 animals do not emerge from dauer, and so could not be scored.

mel-25 V: *mel-25(ct60)/unc-42 daf-11*: Unc (15/22). Because both *ct60* and *daf-11* are *ts*, the cross was carried out as follows. Heterozygous males of the above genotype, grown at 15°, were backcrossed to *unc-42 daf-11*; *lon-2* hermaphrodites at 25°. There is no paternal-effect lethality associated with *ct60* (see RESULTS). Unc non Daf non Lon were tested for maternal-effect lethality. Daf-11 animals emerge from dauer inefficiently, and so these were not scored.

mel-26 I: *mel-26(ct61)/unc-29 lin-11*: Unc (10/24), Lin (14/22).

Allelism with other loci: Since our mutations are dominant, complementation with previously identified recessive mutations that map to the same region (based on the 1989 *C. elegans* genetic map; M. EDGLEY, personal communication) would be ambiguous. Allelism was often excluded by further mapping.

ct42, *ct76*, *ct77*: The recessive *ts* maternal-effect lethal mutations of *emb-6* map to the same interval as *ct42*, *ct76* and *ct77*. However + +*dpy-5*/+*emb-6*+ recombinants were readily recovered from *M* + *dpy-5*/+ *emb* + hermaphrodites as non-*ts* progeny (5/500 for *ct42*, 4/435 for *ct76*, and 4/280 for *ct77*). A map distance this large (2 cM) excludes allelism and indicates a probable map order of *M emb-6 dpy-5*. This region has been saturated for zygotic-lethal mutations (HOWELL *et al.* 1987; HOWELL 1989), and *ct42*, *ct76*, and *ct77* are likely to be alleles of *let-354* (see RESULTS). Allelism was shown by crossing *M dpy-5/unc-11 bli-4* males to *sDp2(I;f)*; *let-354(h79) dpy-5 unc-13* hermaphrodites at 15°. The number of wild-type outcross progeny was noted, and Dpy larvae were picked to new plates and scored 3 days later for growth. Crosses using *dpy-5*/+ males were used to determine the control frequency of Dpy offspring relative to their wild-type sibs.

ct46: Apparent loss-of-function intragenic revertants complemented *let-80* and *let-84* but did not complement *mei-1(b284)* (P. E. MAINS *et al.* in preparation).

ct60: A recessive *ts* maternal-effect lethal mutation of *emb-18* maps to the same region as *ct60*. The high frequency of recombination between the mutations makes allelism unlikely. A total of five non-*ts* *unc-42* + +/+ *emb-18* + recombinants were found among 1900 non-Unc progeny of *unc-42* + *ct60*/+ *emb-18* + hermaphrodites. This corresponds to a map distance of 0.4 cM.

Male rescue experiments: One L4 hermaphrodite was mated to five L4 and young adult N2 males at 25°. Three to six crosses were done for each mutation. All animals were transferred after one day to fresh plates. The viability of the progeny at 25° was determined as described in *Culture Conditions*. The following day, the hermaphrodites alone were shifted to 15°, and the degree of outcrossing was assessed by scoring the morphological phenotypes of the progeny. These outcross progeny arise from male sperm stored during the mating at 25°.

For male rescue experiments at 15°, two L4 hermaphrodites were mated to five L4 and young adult N2 males. Two to four such crosses were done for each mutation. All animals were transferred every other day, until hermaphrodites ceased producing fertilized eggs. The viability of progeny was scored as described in *Culture Conditions*, and the degree of outcrossing was determined by the morphological phenotype of progeny.

Mating experiments with mutant males: Five young adult males and two L4 hermaphrodites were mated at 25°. Two to three matings each were done for each mutation. All animals were transferred daily until the hermaphrodites stopped laying fertilized eggs. The progeny viability was assessed as described in *Culture Conditions*, except that progeny were allowed to reach adulthood before they were scored. The degree of outcrossing was determined by the morphological phenotype of the progeny (see text and legend to Table 3).

Dosage experiments: The viability of all mutant strains was normalized to that of coisogenic strains lacking the *ts* mutation. Genotypes to be compared were matched for morphological phenotype. Dosage effects were determined by comparing viabilities of the sets of strains listed below under each of the mutant alleles. Only selected strain constructions are described; the others were done in a straightforward manner. The map in Figure 1 shows the extents of the duplications and deficiencies used.

let-354(ct42): *sDp2(I;f)/0*; *ct42 dpy-5 unc-13/dpy-5 unc-15* (*M*/+/+)
ct42 dpy-5 unc-13/unc-15 (*M*/+)
sDp2(I;f)/0; *ct42 dpy-5 unc-13* (*M*/*M*/+)
ct42 dpy-5/ct42 dpy-5 (*M*/*M*)
ct42 dpy-5/hDf6 dpy-5 unc-13 (*M*/*Df*).

Balanced strains containing *sDp2* (one copy per diploid genome; two copies are lethal) are similar to those described by HOWELL *et al.* (1987). *ct42 dpy-5/ct42 dpy-5* were Dpy-5 self progeny of *ct42 dpy-5/unc-73 dpy-14* hermaphrodites. The number of sibs exhibiting the phenotype resulting from homozygosity of the balancer chromosome was used as the control value for the viability of these animals. The deficiency strain was constructed by crossing *ct42 dpy-5/unc-73 dpy-14* males to *hDf6 dpy-5 unc-13/unc-11 bli-4* hermaphrodites. Dpy larvae were picked to new plates and scored for growth three days later. The number of Unc-73 progeny (*unc-73 dpy-14/hDf6 dpy-5 unc-13*) was also noted to control for the viability of the deficiency.

let-354(ct76 and *ct77)*: As described for *ct42*.

mel-23(ct45): *mnDp37[unc-32](III;f)/0*; *ct45 vab-7/dpy-17 unc-32 vab-7* (*M*/+/+)
ct45/dpy-17 unc-32 vab-7 (*M*/+)
mnDp37[unc-32](III;f)/0; unc-32 ct45 (*M*/*M*/+).

M/+/+ animals were constructed by crossing *ct45 vab-7/unc-32 dpy-18* males to *mnDp37[unc-32]/0*; *dpy-17 unc-32 vab-7* hermaphrodites (which are Unc-32 because *mnDp37* carries a mutant *unc-32* allele) and selecting wild-type offspring. The F_2 progeny were checked for the absence of Dpy-18 segregants to exclude the possibility of a crossover

eliminating *ct45* in the male parent. Two copies of *mnDp37* per diploid genome is lethal.

M/M/+ animals were produced by crossing *unc-32 ct45/dpy-17 vab-7* males to *mnDp37[unc-32]/0; dpy-17 vab-7* hermaphrodites. Wild-type progeny were selfed, and Unc-32 segregants were chosen. These should be *mnDp37[unc-32]/0; unc-32 ct45* (*unc-32 ct45* homozygotes without the duplication are inviable).

mei-1(ct46): sDp1(I;f)/0; ct46 unc-29/dpy-5 unc-29 (M/+/+)
dpy-5 ct46 unc-29/+ (M/+)
sDp1(I;f)0; dpy-5 ct46 unc-29 (M/M/+)
ct46/dpy-5 ct46 unc-29 (M/M)

nDp4(I;?)/0; ct46 unc29/+ unc-29; lon-2/+ (M/+/+)
ct46 unc-29/+; lon-2/+ (M/+)
nDp4(I;?)/0; ct46 unc-29; lon-2/+ (M/M/+)
ct46 unc-29/ct46 + (M/M)

ct46 unc-29/unc-13 ct46 unc-29 (M/M)
unc-13 ct46 unc-29/nDf23 (M/Df).

Two copies of either duplication is lethal. *M/+/+* animals were constructed by crossing *ct46 unc-29* males to *sDp1/0; dpy-5 unc-29* hermaphrodites and choosing wild-type progeny that segregated many Unc-29 non-Dpy-5 animals, or by crossing *nDp4/0; ct46 unc-29; lon-2* hermaphrodites by *ct46 unc-29* males and choosing wild-type progeny. *M/Df* animals were generated as the Unc-29 progeny of *ct46/unc-13 ct46 unc-29* males crossed to *nDf23/unc-13 lin-11* hermaphrodites.

mel-24(ct59): ct59 unc-24/mnT12 (IV;X)/mnT12 (IV;X) (M/+/+)
ct59/unc-24 dpy-20 (M/+)
unc-24/mnT12 ct59 (IV;X)/mnT12 ct59 (IV;X) (M/M/+)
ct59/ct59 (M/M)
ct59 unc-24/eDf18 (M/Df)

Strains trisomic for LG IV were constructed by making use of a fusion chromosome of LG IV and LG X, *mnT12 (IV;X)*, as described by SIGURDSON *et al.* (1986). These authors have shown that *mnT12* homozygotes are viable and fertile. Heterozygous hermaphrodites (*IV/mnT12/X*) show high levels of meiotic nondisjunction between *mnT12* and the *X* and so have a Him phenotype (*h*igh *i*ncidence of *m*ales) among self progeny. The *IV/mnT12/0* males are fertile. In addition, SIGURDSON *et al.* (1986) showed that hermaphrodites heterozygous for *mnT12* show high levels of meiotic nondisjunction between *mnT12* and LG IV, often resulting in *IV/mnT12/mnT12*. These animals are characterized as being Him, which is indicative of continued heterozygosity for *mnT12* and a normal LG IV. However, they will not segregate viable progeny showing the phenotypes for recessive markers carried on the normal LG IV, since these animals would lack *mnT12* and consequently be nullo-*X*. The presence of markers on the normal LG IV can be confirmed by test crossing the *IV/mnT12/0* male progeny of *IV/mnT12/mnT12* hermaphrodites.

M/+/+ animals were constructed as follows. *ct59/ct59 unc-24* males were crossed to *mnT12* hermaphrodites. The F_1 *ct59 unc-24/mnT12/X* hermaphrodites segregate both males and Unc-24 F_2 self progeny. Hermaphrodites of the genotype *ct59 unc-24/mnT12/mnT12* were recognized among the F_2 as being Him and *not* segregating Unc-24 animals. The genotype was confirmed by crossing the F_3 *ct59 unc-24/mnT12/0* males to *unc-24 dpy-20; him-5* hermaphrodites. Because the hermaphrodite contained *him-5*, a high level of diplo-*X* gametes (HODGKIN, HORVITZ and BRENNER 1979) would result in *ct59 unc-24/dpy-20 unc-24; X/X* hermaphrodite outcross progeny. These are Unc non Dpy and show *ts* maternal-effect lethality. The appearance of these progeny confirmed the presence of the *ct59 unc-24*-bearing chromosome in the *ct59 unc-24/mnT12/mnT12* animals. Each of the animals used in the experiments reported in Table 5 was tested in this manner.

Coisogenic *unc-24/mnT12/mnT12* control animals were constructed and tested in a similar manner.

M/M/+ animals were constructed as follows. *ct59/ct59 unc-24* males were crossed to *mnT12* hermaphrodites, and F_1 animals which segregated males but *not* Unc-24 offspring were selected (*ct59/mnT12/X*). Recombinants between the *ct59* on the normal LG IV and *mnT12* were identified by showing the *ts* maternal-effect lethality characteristic of *ct59/+*, but not being Him (*mnT12* ct59/*mnT12*). These were allowed to self, and animals with few viable embryos, a characteristic of *ct59* homozygotes, were chosen. These *mnT12 ct59/mnT12 ct59* animals were crossed to *unc-24/+* males, and progeny of the genotype *unc-24/mnT12 ct59/X*, which segregated male and Unc-24 offspring, were chosen. The self progeny which were Him, but did *not* segregate Unc-24 (*unc-24/mnT12 ct59/mnT12 ct59*) were the desired *+/M/M* animals. The genotype of each animal reported in Table 5 was confirmed by crossing their male progeny (*unc-24/mnT12 ct59/0*) to *unc-24 dpy-20* hermaphrodites. The *unc-24* chromosome was confirmed by the presence of Unc non-Dpy offspring. Their wild-type hermaphrodite sibs were tested for *ts* maternal-effect lethality to confirm the presence of *ct59*.

ct59 unc-24/eDf18 animals were the wild-type progeny of the cross of *eDf18/unc-24 dpy-20* males to *ct59 unc-24* hermaphrodites. Recombinant wild-type animals which had not received *eDf18* would be *dpy-20/ct59 unc-24* and would be recognized by the segregation of Dpy progeny.

mel-26(ct61): sDp1 (I;f)/0; unc-29 ct61/unc-11 unc-29 lin-11 (M/+/+)
ct61/unc-11 unc-29 lin-11 (M/+)
sDp1 (I;f)/0; unc-29 ct61 lin-11 (M/M/+)
ct61/unc29 ct61 lin-11 (M/M)

nDp4 (I;?)/0; unc-29 ct61/unc-29 ct61; lon-2/+ (M/+/+)
unc-29 ct61/unc-29+; lon-2/+ (M/+)
nDp4(I;?)/0; unc-29 ct61/unc-29 ct61; lon-2/+ (M/M/+)
unc-29 ct61/unc-29 ct61; lon-2/+ (M/M)

unc-29 ct61 (M/M)
unc-29 ct61/nDf23 (M/Df)

M/+/+ animals were constructed by crossing *unc-29 ct61* males to *sDp1/0; unc-11 unc-29 lin-11* and picking wild-type progeny, or by crossing *unc-29* males to *nDp4/0; unc-29 ct61; lon-2* and picking wild-type progeny. *M/Df* animals resulted from crossing *unc-29 ct61* males to *nDf23/unc-13 lin-11* hermaphrodites and choosing Unc-29 offspring.

Microscopic observations: Living embryos were dissected from gravid hermaphrodites in embryo buffer (EDGAR and MCGHEE 1986), mounted on agar pads on a microscope slide and observed at 25° with a Zeiss Universal Microscope equipped with Nomarski optics. At least five embryos were observed for each genotype. A Panasonic AG6050 videorecorder with a DAGE MTI camera was used for time-lapse observation. Embryos were flash-photographed using Kodak Tech Pan 2415 film. In all figures, embryos are oriented anterior-left, dorsal-up.

Temperature-sensitive period (TSP): One- to four-cell embryos were dissected at 15° or 25° from gravid hermaphrodites in embryo buffer. The embryos were mounted

on an agar pad on a microscope slide and the Nomarski images were briefly recorded (≤1 min) to assess the developmental stage. After incubation for a specified period, the slides were shifted to the appropriate temperature where they would equilibrate within 1–2 min, and the embryos were scored the next day for hatching or for development to a well-formed larva within the eggshell. In cases where the temperature shift was performed before dissection, gravid hermaphrodites were transferred to plates or embryo buffer pre-equilibrated at 15° or 25°. Each time point represents 20–60 embryos. All reported times are normalized to 25°.

ct45 and *ct60* were treated differently. Embryos were staged by picking two-cell embryos for *ct45* or one-cell embryos for *ct60* (since the two-cell stage was grossly abnormal for this mutant). The embryos were placed on plates at the appropriate temperature. For *ct60* the number of hatching embryos that failed to grow was noted.

The following developmental times, in min, at 25° were used (KEMPHUES *et al.* 1986; DEPPE *et al.* 1978), and it was assumed that the growth rate at 15° is half that at 25° (BYERLY *et al.* 1976): fertilization, 0; appearance of pronuclei, 20; pseudocleavage, 27; pronuclear meeting, 32; AB-P division (first cleavage), 35; mid-2-cell, 40; $^{1}AB_{2}$ division, 45; mid 4-cell, 52; $^{2}AB_{4}$ division, 55; $^{4}AB_{8}$ division, 68; $^{8}AB_{16}$ division, 95. Embryos dissected prior to pronuclear formation rarely survive due to incomplete shell formation and so were disregarded.

RESULTS

Initial characterization of mutations: A total of eight independent dominant *ts* embryonic-lethal mutations were isolated from among 8000 EMS-mutagenized haploid genomes (see MATERIALS AND METHODS). This frequency is fivefold higher than that found by PARK and HORVITZ (1986) for dominant morphological and behavioral mutations in *C. elegans*, and it is comparable to that found by SUZUKI (1970) for dominant zygotic *ts* lethal mutations in Drosophila. The mutations were given names and assigned to six genes as follows, based on tests to be described below: *mel-23(ct45)*, *mel-24(ct59)*, *mel-25(ct60)*, *mel-26(ct61)*, *mei-1(ct46)* and *let-354(ct42, ct76, ct77)*. Figure 1 shows the genetic map positions of the six loci, derived from three-factor crosses as described in MATERIALS AND METHODS.

Hermaphrodites heterozygous for these mutations (*M*/+) were initially identified as animals producing a much higher percentage of hatching embryos at the permissive temperature of 15° than at the restrictive temperature of 25° (Table 1). In addition, animals homozygous for *mel-23(ct45)* or for any of the *let-354* mutations *ct42*, *ct76* or *ct77* were inviable at all temperatures, while homozygotes for *mel-25(ct60)* showed non-conditional sterility. Homozygous *mei-1(ct46)*, *mel-24(ct59)*, and *mel-26(ct61)* strains were viable and fertile at 15°, although progeny embryos showed decreased hatching relative to those produced by heterozygous hermaphrodites. Therefore, all eight mutations are semidominant, but will be referred to as dominant for convenience.

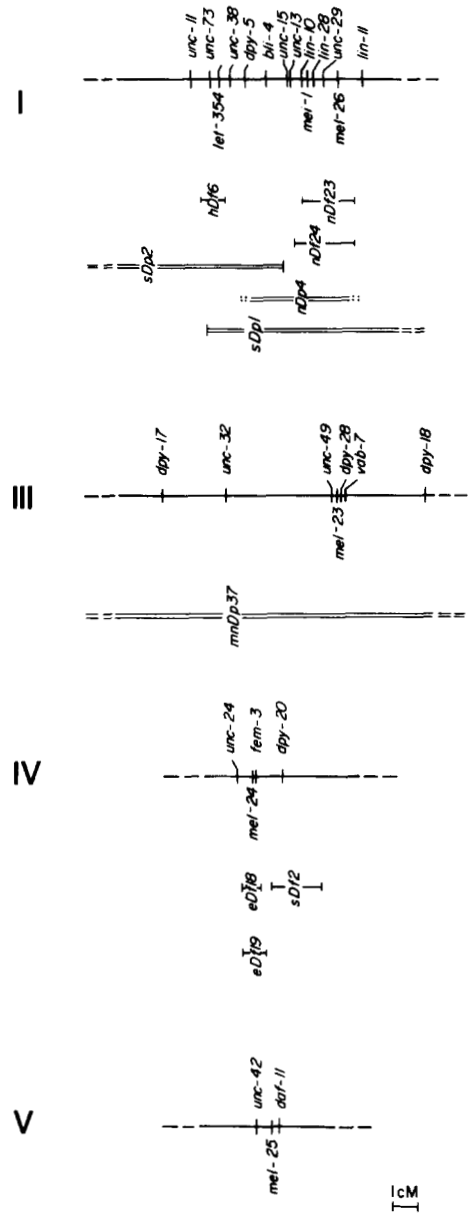

FIGURE 1.—Genetic map of selected regions of the *C. elegans* genome. The positions of the six genes defined by the dominant *ts* mutations (*let-354*, *mei-1*, *mel-23*, *mel-24*, *mel-25*, and *mel-26*), are shown below the lines representing linkage groups. Extents of deficiencies are indicated by single lines and of duplications by double lines. Dashes indicate that these rearrangements extend beyond the regions of the map presented; dots indicate that their boundaries have not been precisely determined.

The screens employed could potentially yield both parental-effect (maternal or paternal) and nonparental-effect (zygotic) dominant *ts* lethal mutations. For the latter, 25% of the self-progeny embryos produced by heterozygous hermaphrodites should survive at 25°; for the former, none should survive (assuming complete expressivity). As shown in Table 1, all mutations except *ct59* resulted in much less than 25% survival of embryos from *M*/+ hermaphrodites at 25°, indicating parental-effect dominance with high expressivity. As will be shown below, *ct59* is also a

TABLE 1

Temperature-sensitive viability of self-progeny embryos from mutant hermaphrodites

Mutant	15°	20°	25°
let-354:			
ct42/unc-11 dpy-5	72/84[a] (0.32)[b]	11/12[a] (0.36)[b]	2/2[a]
ct76/unc-11 dpy-5	18/25 (0.36)	0.9/1.0	0/0
ct77/unc-11 dyp-5	56/71 (0.33)	3.3/4.2 (0.36)	0.9/1.1
+ */unc-11 dpy-5*	99/99 (0.25)	ND[c]	98/98 (0.26)[b]
mel-23:			
ct45/unc-32 vab-7	24/36 (0.33)	0.1/0.2	0/0.1
+ */unc-32 vab-7*	96/98 (0.24)	ND	98/99 (0.26)
mei-1:			
ct46	16/19	2.6/2.8	0/0
ct46/dpy-5 unc-29	88/90 (0.24)	61/63 (0.25)	0.3/0.4
+ */dpy-5 unc-29*[d]	99/99 (0.25)	99/99 (0.26)	99/100 (0.28)
mel-24:			
ct59	1.0/1.2	0/0	ND
ct59/unc-24 dpy-20	96/97 (0.26)	60/61 (0.28)	22/27 (0.23)
+ */unc-24 dpy-20*	99/99 (0.24)	ND	99/99 (0.26)
mel-25:			
ct60/unc-42	17/26 (0.33)	3.0/38	1.0/13
+ */unc-42*	100/100 (0.24)	100/100 (0.26)	98/98 (0.24)
mel-26:			
ct61	12/12	1.2/1.2	0/0
ct61/dpy-5 unc-29	99/99 (0.24)	90/91 (0.27)	4.0/4.2
+ */dpy-5 unc-29*[d]	99/99 (0.25)	99/99 (0.26)	99/100 (0.28)

[a] Percent of total embryos hatching and surviving to at least late larval stages/percent of total embryos hatching (including those that arrested as larvae). The remaining embryos failed to hatch. Based on 500–2500 total embryos for each strain.

[b] Fraction of animals that grew to at least late larval stages showing the phenotype of the morphological markers carried by the balancer chromosome. These animals are homozygous for the balancer chromosome and therefore are +/+ at the locus defined by the *ts* mutation. Only those cases in which >50 animals were scored are presented.

[c] Not determined.

[d] Same control used for both *ct46* and *ct61*.

parental-effect mutation, but with incomplete expressivity. For several of the mutations, *M*/+ hermaphrodites at 25° gave an appreciable number of embryos that hatched, but produced larvae that failed to survive (Table 1).

Self progeny of *M*/+ hermaphrodites were scored for dominant or recessive zygotic phenotypes, in addition to parental effects. As described above, five of the mutations resulted in a recessive zygotic lethal or sterile phenotype at 15°. If there were an additional dominant zygotic effect on the viability of *M*/+ embryos, then the fraction of +/+ animals among the progeny of *M*/+ hermaphrodites should be >0.25 for those mutations that are homozygous viable, or >0.33 for those known to be homozygous inviable. (The +/+ progeny could be identified by morphological markers on the balancer chromosome.) This test was most easily performed at 15° or 20° where a significant number of progeny hatched. Only *ct60* showed an additional dominant zygotic effect on viability (Table 1). For *ct59*/+ hermaphrodites, the fraction of +/+ animals among surviving self progeny was about 0.25 at all temperatures, indicating that this mutation, like the others, causes a dominant parental-effect lethality with incomplete penetrance rather than simply a dominant zygotic lethality.

Male mating experiments: Because *C. elegans* is a self-fertilizing hermaphrodite, the observed parental effects could be mediated by the sperm rather than the oocyte, and thus would represent paternal effects rather than maternal effects. This possibility can be tested by providing non-mutant sperm from wild-type males crossed to mutant hermaphrodites at 25°. The outcross progeny of a paternal-effect mutant should show full viability in such a test. As shown in Table 2, this was not the case for any of the mutations; outcross progeny of *M*/+ hermaphrodites at 25° failed to survive, showing that the effects are maternal. Likewise, mating did not increase the frequency of surviving embryos produced at 15° by *M/M* hermaphrodites homozygous for *mei-1*(*ct46*), *mel-24*(*ct59*), or *mel-26*(*ct61*). However, for hermaphrodites heterozygous for *let-354* mutations, *mel-23*(*ct45*), or *mel-25*(*ct60*), mating at 15° did increase the percentage of hatching embryos that gave viable larvae, suggesting that the inviable larvae produced by these animals from self-fertilization (Tables 1 and 2) represented predominantly homozygous mutant progeny.

Because the mutations are dominant, paternal effects could also be detected by introducing the mutation into the zygote through sperm, in crosses at 25°

TABLE 2

Effects of mating to wild-type males on viability of progeny embryos from mutant hermaphrodites

Mutant	15°		25°	
	Mated	Self	Mated	Self
let-354:				
ct42 dpy-5/unc-11 dpy-5	86/86[a] (99)[b]	65/70	4.5/6.6[a] (98)[b]	4.4/7.2[a]
ct76 dpy-5/unc-11 dpy-5	21/22 (99)	23/27	0/0 (100)	0/0
ct77 dpy-5/unc-11 dpy-5	59/61 (100)	43/50	3.1/3.3 (99)	1.7/1.7
mel-23:				
ct45 unc-69/unc-69 vab-7	ND[c]	ND	0/0 (100)	0/0
ct45/unc-32 vab-7; lon-2	26/31 (99)	25/36	ND	ND
mei-1:				
ct46 unc-13	21/21 (99)	26/29	0/0 (100)	0/0
ct46 unc-13/dpy-5 unc-13	ND	ND	2.4/2.4 (98)	4.7/4.7
mel-24:				
ct59 unc-24	6.5/6.7 (99)	3.5/3.9	ND	ND
ct59 unc-24/dpy-20 unc-24	ND	ND	13/17 (89)	13/16
mel-25:				
ct60/unc-42; lon-2	25/36 (97)	11/27	1.0/8.9 (98)	2.3/9.6
mel-26:				
ct61 unc-29	6.3/6.4 (70)	15/15	ND	ND
ct61 unc-29/unc-13 unc-29	ND	ND	0.1/0.1 (100)	0.4/0.4

[a] Percent of total embryos hatching and surviving to at least late larval stages/percent of total embryos hatching (including those that arrested as larvae). The remaining embryos failed to hatch. Based on 500–2500 total embryos for each strain.

[b] Percent outcross progeny among surviving progeny, determined by the morphological phenotype (see text). At 15°, progeny were scored directly on the mating plate. At 25°, after 2 days of mating, males were removed, and the mated hermaphrodites were shifted to 15° and allowed to produce progeny, which were scored for morphological phenotype.

[c] Not determined.

of *M*/+ males (raised at 15°) to wild-type hermaphrodites. A dominant paternal-effect mutation should cause inviability of outcross progeny. However, the data presented in Table 3 show that few unhatched embryos were seen in such crosses.

Introducing these mutations to the zygote through male sperm by mating at 25° bypasses the maternal-effect lethality, allowing easy detection of any additional dominant *ts* zygotic effects without requiring temperature-shifts of embryos to avoid maternal effects. Using appropriately marked chromosomes, the progeny that inherited the mutant allele can be distinguished morphologically. In all cases shown in Table 3, hermaphrodites conceived and reared at 25° in this manner were fully viable and grew to adulthood. They showed no morphological defects, and were self-fertile when shifted to 15° (data not shown). However, males heterozygous for any one of three *let-354* mutations, *mel-23*(*ct45*), or *mel-25*(*ct60*) and reared at 25° were unable to mate efficiently at 25°. These are the same five mutations that resulted in recessive zygotic inviability or hermaphrodite infertility. In some cases, mating efficiency improved when males were shifted as adults to 15° and tested at that temperature. The tails of *M*/+ males of the five strains that showed poor mating when reared at 25° often exhibited defects visible in the dissecting microscope, yielding a Mab (*m*ale *ab*normal) phenotype.

Two of the mutations caused additional maternal effects at 15°. The progeny of *mei-1*(*ct46*), *mei-1*(*ct46*)/+, and *mel-26*(*ct61*) hermaphrodites showed, as adults, a Vab (*v*ariable *ab*normal) morphological phenotype with low penetrance. The defects seen included an absent or abnormal tail whip, a truncated tail, thickened body (usually posteriorly), and Dpy or Roller phenotypes. The penetrance of these defects among outcross progeny was the same when the above hermaphrodites were mated to mutant or wild-type males (Table 4a), demonstrating that this was a maternal-effect phenotype. Male and hermaphrodite progeny were affected equally (data not shown). Some of the Vab hermaphrodite offspring from the cross of *ct46*/+ hermaphrodites by +/+ males were tested for maternal-effect temperature-sensitivity, and 6/12 were found to be +/+, confirming the maternal-effect nature of the Vab phenotype. The effect for *ct46* was semidominant and slight *ts* (Table 4b). The effects are not due to a second linked mutation, based on the following arguments. *ct46* and *ct61* had been outcrossed 12 times following mutagenesis, so the presence of an unlinked *vab* mutation is highly unlikely. The *mei-1*(*ct46*) chromosome present in the hermaphrodites listed in Table 4 was derived from crossover events within 1 cM on both sides of *ct46*, and the *mel-26*(*ct61*) chromosome was derived from a crossover event within 1 cM to the left of *ct61*. The possibility of linked *vab* mutations is therefore remote. In addition, suppressors of the *mei-1*(*ct46*) maternal-

TABLE 3

Mating experiments with males carrying dominant *ts* mutations

Male genotype at *ts* locus	Reared 16°, mated 25°[a]			Reared 25°, mated 25°[b]		Reared 25°, mated 16°[c]	
	Surviving progeny[d] (%)	Proportion outcross progeny[e] (%)	Progeny carrying *ts*[f] (%)	Surriving progeny (%)	Proportion outcross progeny[e] (%)	Surviving progeny (%)	Proportion outcross progeny[e] (%)
let-354(ct42)/+	96/96	76	55	100	0	95	35
let-354(ct76)/+	95/96	62	50	100	0	101	6
let-394(ct77)/+	98/98	60	57	100	9	100	63
mel-23(ct45)/+	90/97	103	50	101	0	100	0
mei-1(ct46)	105/106	101	—[g]	100	130	ND[h]	ND
mel-24(ct59)	102/102	89	—[g]	99	70	ND	ND
mel-25(ct60)/+	89/90	98	50	103	0	100	6
mel-26(ct61)	98/98	62	—[g]	100	90	101	108

[a] Values are normalized to those obtained in crosses using N2 males, in which percent survival ranged from 94/94 to 99/100, with the exception of the *ct60* control which was 90/90. Percent outcross in the control crosses was 94 to 99. The following crosses were performed (male parent listed first): *ct42*, *ct76*, or *ct77/unc-11 dpy-5* × *unc-13 dpy-5*, *ct45/unc-69 vab-7* × *dpy-18 vab-7*, *ct46* × *unc-13 dpy-5*, *ct59* × *unc-24 dpy-20*, *ct60/unc-42 sma-1* × *unc-42 dpy-11*, *ct61* × *unc-13 dpy-5*.

[b] The morphologically wild-type F_1 males from the crosses whose results are shown in the first three columns were mated to *unc-17* hermaphrodites. Note that the males carrying *ct46*, *ct59*, and *ct61* are no longer homozygous for the *ts* mutation. Values are normalized as above to controls, in which percent survival varied from 95 to 100% and outcross from 68 to 83%.

[c] Crosses were carried out as in footnote *b*. Values are normalized to controls in which percent survival varied from 99 to 100% and outcross from 70 to 84%.

[d] Percent of total embryos hatching and surviving to at least late larval stages/percent of total embryos hatching (including those that arrested as larvae). The remaining embryos failed to hatch. Based on 500–2500 total embryos for each strain.

[e] Some mutant-bearing males showed greater mating efficiency than the N2 controls; hence some values are >100%.

[f] Based on morphological phenotype of progeny, see footnote *a*.

[g] All outcross progeny carry the *ts* mutation.

[h] Not determined.

effect lethality have been isolated, and these also suppress the Vab phenotype (P. E. MAINS *et al.*, in preparation).

ct60 also shows a dominant Him phenotype resulting in 4% male self progeny at 15° (data not shown). The normal frequency of male self progeny is <0.2% (HODGKIN, HORVITZ and BRENNER 1979).

In summary, the mutations *mei-1(ct46)*, *mel-24(ct59)*, and *mel-26(ct61)* behaved as strict dominant *ts* maternal-effect lethal mutations. The mutations *let-354(ct42, ct76,* and *ct77)*, *mel-23(ct45)*, and *mel-25(ct60)* also resulted in strict *ts* dominant maternal-effect lethality. These latter five mutations were also associated with nonconditional recessive zygotic lethality (or sterility in the case of *ct60*) and caused specific dominant *ts* zygotic male mating defects.

The allelism of the *let-354* mutations *ct42*, *ct76*, and *ct77* was established as follows. All mapped to the same small region on LG I (MATERIALS AND METHODS), and their phenotypic effects were very similar. They failed to complement one another for the recessive zygotic lethality and also failed to complement the previously identified recessive lethal *let-354(h79)* (data not shown) as described more fully in a subsequent section. Also as described below, their responses in gene dosage experiments, their TSPs, and their embryonic phenotypes were similar. Allelism for the remaining mutations was ruled out by genetic mapping or complementation tests using intragenic revertants of the dominant mutation (see Figure 1 and MATERIALS AND METHODS).

Nature of the gene defects: The nature of dominant mutations may be inferred from gene dosage experiments (MULLER 1932). In interpreting such experiments, it is assumed that the concentration of a mutant or wild-type gene product is roughly proportional to the number of copies of the respective alleles that are present.

A dominant loss-of-function mutation (a haploinsufficient locus) should show complete suppression of the mutant phenotype upon addition of a duplication of the wild-type locus to give the genotype *M/+/+*. A deficiency uncovering an essential haploinsufficient locus (*Df/+*) should be impossible to recover in a diploid.

A dominant mutation that results in over-expression or hyperactivity of the normal wild-type gene product should show suppression of the mutant phenotype when a wild-type allele is replaced with a deficiency for the region (*M/Df*), while addition of a duplication (*M/+/+*) should enhance the mutant phenotype.

A dominant "gain-of-function" mutation resulting in a gene product that competes with the wild-type product (e.g. a "poison") should show at least partial suppression of the resulting phenotype by addition of an extra dose of the wild-type allele (*M/+/+*). A gain-of-function mutation that shows no competition with the normal gene product (*e.g.*, resulting in inappro-

TABLE 4

***mei-1(ct46)* and *mel-26(ct61)* cause dominant maternal-effect Vab phenotypes**

Hermaphrodite	Male	Percent Vab among surviving progeny	Temperature
a) Maternal effects on outcross progeny[a]			
mei-1:			
unc-13 ct46 unc-29	*ct46*	10.0	15°
unc-13 ct46 unc-29	N2	11.2	15°
unc-13 unc-29	*ct46*	0	15°
unc-13 ct46 unc-29 / + + *unc-29*	*ct46*	3.8	15°
unc-13 ct46 unc-29 / + + *unc-29*	N2	3.7	15°
mel-26:			
unc-29 ct61	*ct61*	22.2	15°
unc-29 ct61 unc-29	N2	16.3	15°
+ *unc-29 ct61* / *unc-13 unc-29* +	*ct61*	0.8	15°
+ *unc-29 ct61* / *unc-13 unc-29* +	N2	0.9	15°
b) Maternal effects on self progeny showing slight temperature sensitivity[b]			
mei-1:			
unc-13 ct46 unc-29 / + + +		2.5	15°
unc-13 ct46 unc-29 / + + +		5.5	20°

[a] The indicated hermaphrodites were crossed to the indicated males at 15°; numbers indicate percent Vab animals among outcross progeny only. Totals of 100–1000 progeny were scored.

[b] The indicated hermaphrodites were allowed to produce self progeny at the indicated temperature. Totals of 500–1000 progeny were scored.

priate expression of the wild-type product in time or location) should not be affected by the dosage of the wild-type locus. Dosage tests were carried out on alleles at five of the six loci as follows.

let-354(ct42, ct76, ct77): For each of these three mutations, as shown in Table 5, heterozygous hermaphrodites carrying an extra dose of the wild-type locus as a duplication (*M*/+/+) produced 5–10-fold more embryos that hatched at 25° than did heterozygotes lacking the duplication (*M*/+). Although the values for the strong allele, *ct76*, were low at 25°, a clear increase was seen at 15°. For all three mutations, *M*/*M*/+ hermaphrodites were much less fertile than the corresponding *M*/+ hermaphrodites, showing very low if any progeny viability even at 15°, and indicating a strong dosage dependence of the mutant product in the presence of one dose of the wild-type product.

At 15° all three mutations caused recessive zygotic lethality as noted earlier: *M*/*M* embryos often survived to mid-larval stages, and very rarely to the L4 stage. However, *M*/*Df* embryos arrested at a much later point in larval development than the *M*/*M* progeny of *M*/+ hermaphrodites. Table 5 shows that most such *ct42*/*Df*, *ct76*/*Df*, and *ct77*/*Df* animals reached the L4/adult stage, at both 15° and 25°. However, these animals never produced embryos (except for a few that all proved to be recombinants lacking the *ts* mutation). These data showed that *ct42*, *ct76*, and *ct77* displayed a non-*ts* zygotic dose dependency in the absence of wild-type gene product and that wild-type product was required for fertility in the presence of these mutations. Therefore, the dominant *let-354* mutations seem to result in gain-of-function poisons that can compete with the wild-type gene product and that result in dosage-dependent phenotypes in the presence or absence of wild-type gene product.

The recessive mutation *let-354(h79)*, which results in zygotic early larval arrest (HOWELL *et al.* 1987; HOWELL 1989), behaves similarly, but not identically to a deficiency of the region. When *ct42*, *ct76* or *ct77*/+ males were crossed to hermaphrodites carrying *let-354(h79)* (see MATERIALS AND METHODS), the resulting *M*/*let-354* animals showed larval arrest, but unlike *M*/*Df* animals produced in similar crosses, fewer than 5% grew to the L4 stage (data not shown). Therefore, it is likely that *ct42*, *ct76*, and *ct77* are alleles of *let-354*, but *h79* is apparently not a true null.

mel-23(ct45): The dominant maternal-effect lethality resulting from this mutation was dramatically suppressed by the presence of an extra wild-type allele in the form of a free duplication. *ct45*/+ hermaphrodites produced only one hatched larva (which arrested) out of 4455 embryos at 25° (Table 5). In contrast, *ct45*/+/+ hermaphrodites produced 86% hatching progeny embryos at 25°. However, there was also an additive effect of an additional dose of *ct45* relative to the heterozygote. While *ct45*/+ hermaphrodites produced 36% hatching embryos at 15° (Table 1), 27/27 *ct45*/*ct45*/+ animals produced either no hatched embryos or no embryos at all. This was also true of 29/29 animals reared at 14° (data not shown).

Therefore, the *ct45* gene product could be a gain-of-function poison that is very efficiently competed by the wild-type product. Alternatively, *mel-23* could be a haploinsufficient locus, as suggested by the nearly complete suppression by a single extra copy of the wild-type gene. However, it would then be puzzling that the self progeny embryos from *ct45*/*ct45*/+ hermaphrodites exhibit much reduced hatching relative to those of *ct45*/+, since the opposite would be expected (see DISCUSSION).

mei-1(ct46): As shown in Table 5, presence of a maternal duplication (*M*/+/+) resulted in a four-to ten-fold increase in progeny viability at 25° relative to the heterozygote (*M*/+), depending on the duplication used. In addition, *M*/*M*/+ hermaphrodites constructed with either duplication produced a lower

TABLE 5

Allele dosage effects on viability of self-progeny embryos from hermaphrodites carrying dominant *ts* maternal-effect mutations[a]

Mutation	Temperature	(+/+/+)[b]	M/+/+	M/+	M/M/+	M/M	M/Df	(+/Df)[b]
let-354(ct42)	15°	(92)	89	86	0	2.2[c]	130[c]	(68)
	25°	(96)	14	3.0	—[d]	—[d]	81[c]	(76)
let-354(ct76)	15°	—[e]	62	37	2.6	4.2[c]	84[c]	—[e]
	25°	—[e]	3	0.4	—[d]	—[d]	90[c]	—[e]
let-354(ct77)	15°	—[e]	88	79	0.4	3.7[c]	84[c]	—[e]
	25°	—[e]	12	1.2	—[d]	—[d]	54[c]	—[e]
mel-23(ct45)	25°	(93)	86	0.02	0[f]	—[g]	—[h]	—[h]
mei-1(ct46)	15°	(89) *sDp1*	—[d]	83	12[i]	25		(74)
	20°	(92) *sDp1*	63	50	1.3	6.4/2.7[j]	13[k]	(73)
	25°	(73) *sDp1*	26	1.5	0	0		(74)
mei-1(ct46)	15°	(93) *nDp4*	—[d]	91	17[i]	34		
	20°	(94) *nDp4*	68	54	0.6	7.7		
	25°	(88) *nDp4*	14	3.8	0	0		
mel-24(ct59)	15°	(91)	99	96	14	4.1	51[l]	(71)
	25°	(66)	38	24	—[d]	—[d]	—[d]	(71)
mel-26(ct61)	15°	—[m] *sDp1*	—[d]	94	—[d]	13	12[n]	(74)
	25°	—[m] *sDp1*	46	1.3	—[d]	—[d]	—[d]	(74)
mel-26(ct61)	15°	—[m] *nDp4*	—[d]	98	27	14		
	20°	—[m] *nDp4*	99	63	2.3	0.7		
	25°	—[m] *nDp4*	56	0.7	0	0		

[a] Headings indicate maternal genotypes. All values are percent of self-progeny that hatched (except for those indicated by footnote *c*), normalized to the results obtained with isogenic strains lacking the *ts* mutation (footnote *b*). See MATERIALS AND METHODS for complete genotypes of the strains compared and their construction.

[b] The values for the isogenic +/+/+ strains are shown in parentheses, but note that the values for M/+/+ and M/M/+ are already normalized to these. The values for the +/*Df* isogenic strains are similarly shown and normalized.

[c] Percent of the expected animals of the given genotype that grew to L4 or adulthood. All were sterile except for recombinants that lost the mutation, which are not included.

[d] Not determined.

[e] Same as for *let-354(ct42)*.

[f] See text.

[g] Nonconditional recessive lethal.

[h] No deficiency is available for this region (see text).

[i] The large free duplications used (*sDp1* and *nDp4*) cover most of LG I and by themselves showed some inviability (although the survival values shown are corrected for these effects). The brood sizes of +/+/+ hermaphrodites for both duplications was reduced by about ⅔ compared to +/+ at 25° (data not shown).

[j] The value of 6.4%, for progeny of *ct46/dpy-5 ct46 unc-29* hermaphrodites, is for comparison to morphologically wild-type animals in the three columns to the left; the value of 2.7%, for progeny of *ct46 unc-29/unc-13 ct46 unc-29* hermaphrodites, is for comparison to morphologically Unc-29 animals in the last column (*unc-29/nDf23*). Both values are shown so that animals of the same morphological phenotype can be compared. See MATERIALS AND METHODS.

[k] Because there are no flanking markers to the left of *ct46* uncovered by the deficiency (*nDf23*) used in the experiment shown in Table 5, it is possible that *ct46* also is not uncovered. However, a second larger, overlapping deficiency, *nDf24*, which does uncover a flanking marker on the left, yielded the same results (data not shown). The increase in progeny viability compared to the homozygote was seen in six separate experiments using both deficiencies and at various temperatures. Chromosomes were used that had undergone crossovers within 1 cM on either side of *ct46*. This would make it unlikely that these results were due to a second deleterious recessive mutation closely linked to *ct46* that was not uncovered by the deficiency and therefore was heterozygous in *ct46/Df* but homozygous in *ct46/ct46* animals.

[l] It is possible that the deficiency used (*eDf18*) did not uncover *ct59*. There is no flanking marker to the left of *ct59* uncovered by this deficiency. The same is true for a second deficiency (*eDf19*) that gave similar results (data not shown). The result may then be the nonspecific decrease in the viability of embryos from *ct59/+* hermaphrodites (97% at 15°, Table 1) beyond the 25% zygotic lethality expect from the deficiency. (Note that the data presented is corrected for this decrease.) However, *sDf2 IV*, which does not cover *ct59*, showed no decrease in viability relative to *ct59/+* beyond the expected 25% when heterozygous with *ct59* (data not shown). Chromosomes were used that had undergone crossovers within 1 cM on either side of *ct59*. This would make it unlikely that these results were due to a second deleterious recessive mutation closely linked to *ct59* that was not uncovered by the deficiency and therefore was heterozygous in *ct59/Df* but homozygous in *ct59/ct59* animals.

[m] Same as for *mei-1(ct46)*.

[n] It is possible that the deficiency used (*nDf23*) did not uncover *ct61*, since it uncovered no flanking markers to the right of *ct61*. Therefore, this result could represent a nonspecific decrease in the viability of progeny from *ct61/+* hermaphrodites (99% at 15°, Table 1) beyond the 25% zygotic lethality expected from the deficiency. This is unlikely, since a second deficiency, *nDf24*, which removes a region overlapping *nDf23*, behaved similarly to *nDf23* (data not shown). In addition, *ct61/+ I; eDf18/+ IV* showed no nonspecific decrease (data not shown).

percentage of hatching progeny than the heterozygote, indicating a dosage effect of *ct46* in the presence of a wild-type allele.

At 15° and 20°, *M/M/+* hermaphrodites produced a lower percentage of hatching embryos than did homozygous (*M/M*) hermaphrodites, suggesting that the mutant gene product may function better alone than in the presence of wild-type product. However, it is also possible that this result could be caused by non-specific effects of the large duplications used (see footnote *i*, Table 5 and DISCUSSION). Finally, *M/Df* hermaphrodites produced a higher percentage of viable progeny than *M/M* hermaphrodites (Table 5), indicating a dosage effect of the mutation in the absence of the wild-type allele (see footnote *k*, Table 5).

Elsewhere (P. E. MAINS *et al.* in preparation) we will show that *ct46* interacts with mutations at two other loci on LGI, one of which is *mel-26(ct61)*. The duplications used above included both these loci while the deficiencies removed the *mel-26* locus. This could complicate the interpretation of the gene dosage experiments and could be responsible for the unexpectedly decreased viability in *ct46/ct46/+*. A similar situation has been described in Drosophila (KENNISON and RUSSELL 1987). Nevertheless, the *ct46* mutation is likely to result in a gain-of-function poison that can compete with wild-type gene product, and that shows dosage effects on phenotype either in the presence or absence of wild-type gene product. We will present evidence elsewhere (P. E. MAINS *et al.*, in preparation) that the *ct46* mutation is an allele of the *mei-1* gene.

mel-24(ct59): Heterozygous *ct59* hermaphrodites carrying an extra dose of the wild-type gene (*M/+/+*) produced only about 1.5 times more viable embryos than heterozygous *M/+* hermaphrodites at 25° (Table 5) indicating little competition between the mutant and wild-type products. It should be noted that the *ct59/+/+* animals carried an entire extra copy of LG IV (see DISCUSSION).

The dosage of the mutant *ct59* gene product showed effects in both the presence and absence of wild-type gene product. At 15°, *M/M/+* hermaphrodites produced only 14% viable embryos, compared to 96% from *M/+* hermaphrodites. Progeny of *M/Df* hermaphrodites at this temperature showed a ten-fold higher percentage of viability than those of *M/M* hermaphrodites (see footnote *l*, Table 5).

Therefore, *ct59* appears to result in a gain-of-function poison product, which is poorly competed by extra wild-type product and unusually sensitive to dosage in the absence of wild-type product.

mel-25(ct60): Dosage experiments have not yet been carried out on this locus, because of the unavailability of both duplications for this region of LGV and deficiencies that unequivocally uncover the locus.

mel-26(ct61): Viability of self-progeny embryos was 35–80-fold higher for *M/+/+* than for *M/+* hermaphrodites at 25° depending upon the duplication used. Likewise, the viability of embryos from *M/M/+* was intermediate between that of embryos from *M/+* and *M/M* hermaphrodites (Table 5). In contrast to results with the other dominant *ts* mutations, *M/Df* hermaphrodites showed no higher progeny viability than *M/M* hermaphrodites at 15° (see footnote *n*, Table 5).

Therefore, this mutation appears to result in a gain-of-function poison with which the wild-type product competes effectively, but which does not show a dosage effect in the absence of wild-type product. As noted above, *ct61* interacts with mutations in other genes on LGI including *mei-1(ct46)*, and this could complicate the interpretation of the gene dosage experiments.

Embryonic phenotypes: Living embryos produced by hermaphrodites carrying each mutation were examined using Nomarski optics at 25°. For embryos that did not become too disorganized, lineages were observed from shortly after fertilization through the beginning of gastrulation, that is, up to 28 cells at 100 minutes postfertilization (DEPPE *et al.* 1978; SULSTON *et al.* 1983). The position and timing of the first divisions in the MS, E, C, D, and P lineages were noted. After the second round of cell divisions of the AB lineage, only the timing of the AB divisions was followed.

Embryos were also observed briefly at approximately 5, 8, 11, and 24 hr postfertilization to determine the terminal phenotype. The degree of morphogenesis and the expression of differentiation markers for the muscles (twitching) and intestine (gut birefringence) was noted at 24 h postfertilization (LAUFER, BAZZICALUPO and WOOD 1980). Temperature-shift experiments (WOOD *et al.* 1980) were used to define the TSP for each mutation. All reported times are normalized to the 25° rate of development (see MATERIALS AND METHODS).

Wild-type development: For comparison, normal embryogenesis is illustrated in Figure 2. Shortly after fertilization, the maternal and paternal pronuclei become visible at the anterior and posterior of the embryo, respectively, and begin to move toward each other. After a brief pseudocleavage (Figure 2A), the pronuclei come together in the posterior region (Figure 2B). They subsequently fuse, and the embryo immediately begins first cleavage, with the spindle oriented along the anterior-posterior axis (Figure 2C). The division is unequal resulting in the larger anterior AB cell and the posterior P_1 cell (Figure 2D). The second cleavage round begins with the symmetric division of AB, quickly followed by the asymmetric division of P_1 (Figure 2E). The anterior and dorsal

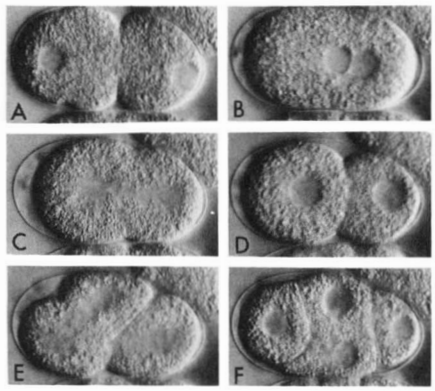

FIGURE 2.—Nomarski photomicrographs of self-progeny embryos from wild-type hermaphrodites. In these and subsequent figures, embryos are oriented so that anterior is leftward and dorsal is upward. A) Pseudocleavage stage, showing maternal (anterior) and paternal (posterior) pronuclei. B) Pronuclear meeting. C) First cleavage. D) Two-cell embryo, showing that anterior AB cell is larger than the posterior P_1 cell. E) Second cleavage showing AB entering mitosis slightly earlier than P_1. F) Four-cell embryo. The AB daughters are the anterior and dorsal cells; the P_1 daughters are the ventral EMS and the posterior P_2 cell.

cells in the four-cell embryo are AB descendants, while the ventral EMS and posterior P_2 cells are the daughters of P_1 (Figure 2F). Gastrulation takes place between 100 and 300 min; most cell proliferation ceases shortly thereafter, and morphogenesis begins (for further details see SULSTON *et al.* 1983; WOOD 1988).

let-354(*ct42*, *ct76*, *ct77*): Self-progeny embryos from *M*/+ hermaphrodites showed similar defects in both mitosis and cytokinesis during the early cleavages (data not shown). Nuclear envelope breakdown was usually on schedule, but incomplete nuclear division and cytokinesis often occurred. Spindles were poorly developed and multiple nuclei within individual cells were often observed. Cleavage furrows were weak and often disappeared. This was especially apparent for *ct77* where embryos still appeared to have only two cells even after the second round of division. The TSP for all three mutations extended from the one-cell to the four-cell stage.

mel-23(*ct45*): In self-progeny embryos from *M*/+ hermaphrodites at 25°, the first observable defect was at the three-cell stage. In wild-type two-cell embryos, the AB cell begins to divide before the P_1 cell, but division of P_1 begins before AB division is completed (Figure 3A). In embryos from mutant hermaphrodites, the P_1 division did not begin until after completion of the AB division (Figure 3B). In the four-cell embryo, the EMS cell normally remains on the ventral midline, and the two AB cells are positioned on the dorsal and anterior sides of the embryo (Figure 3C). In embryos from mutant hermaphrodites, the EMS cell was more anterior than normal (Figure 3D). These embryos appeared to rotate about the longitudinal axis at the four-cell stage, so that the EMS cell took up a ventral-right (4/5 embryos) or a ventral-left

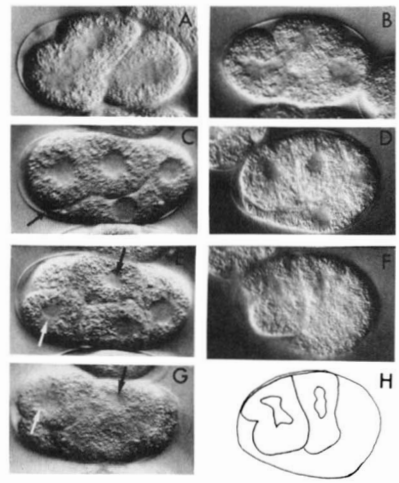

FIGURE 3.—Nomarski photomicrographs of self-progeny embryos from wild-type and *mel-23*(*ct45*)/+ hermaphrodites at 25°. A) The AB cell (anterior) in a wild-type embryo is completing its division as the P_1 cell (posterior) is entering mitosis. B) The P_1 cell in an embryo from a mutant hermaphrodite has yet to enter mitosis when the AB division is nearly completed. C) Late four-cell wild-type embryo. D) Late four-cell embryo from a mutant hermaphrodite. Note that the EMS cell (ventral) is more anterior (arrow) than in (C). E), G) Upper and lower focal planes, respectively, of a wild-type embryo after completion of the second-round AB divisions, which occur left-right in the embryo (perpendicular to the plane of the page). The two anterior cells (white arrows) in both panels are sisters, as are the two dorsal cells (black arrows). F) The second round of AB divisions in an embryo from a mutant hermaphrodite is occurring along the dorsal-ventral axis (rather than the normal left-right division). H) Tracing of (F) showing outlines of the AB cells and their cleavage spindles, which are seen in an oblique cross-section. In normal embryos these spindles would usually be at right angles to the focal plane, yielding a very small cross-sectional area.

(1/5 embryos) position, and the AB cells rotated accordingly. The next AB cleavage round occurred at the normal time, but with a dorsal-ventral rather than the normal left-right orientation (Figure 3E–H). The EMS division was about 50% behind schedule, and subsequent division rounds of all cells were delayed by a factor of about two. Embryos arrested with about 28 cells, near the onset of gastrulation. No twitching or gut birefringence was subsequently observed.

The TSP for embryonic lethality of progeny from *M*/+ hermaphrodites began approximately 2 hrs prior to fertilization and ended about 6 hrs later, at the end of gastrulation (Figure 4). Surprisingly, however, if oocytes developing at 25° were downshifted prior to the beginning of the TSP, up to 95% of the embryos hatched. This result contrasted sharply with the hatching percentage of 36% for embryos that developed entirely at 15° (Table 1) or for embryos upshifted after the end of the TSP (Figure 4). Therefore, being at the restrictive temperature for a period prior to the TSP was beneficial to these embryos. Approximately 25% of the hatching animals arrested as larvae; these were presumably the *ct45*/*ct45* segregants. The animals that grew to adulthood displayed the usual *ct45*/+ *ts* phenotype.

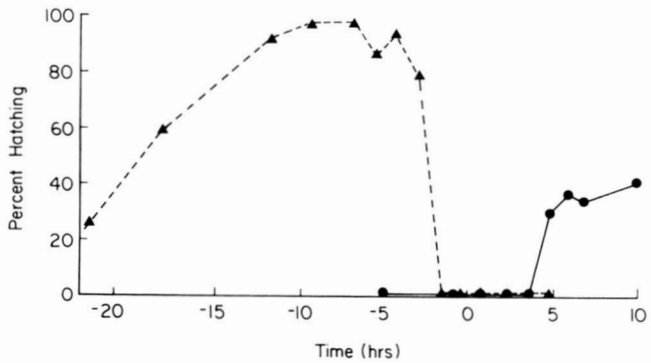

FIGURE 4.—TSP for the viability of self-progeny embryos from *mel-23(ct45)/+* hermaphrodites. Shifts from 15° to 25° or vice versa were performed at the times indicated (normalized to 25° time), as described in MATERIALS AND METHODS. Fertilization is at time 0. Results of downshift experiments are shown by triangles connected with dashed lines and upshift experiments by circles connected with solid lines.

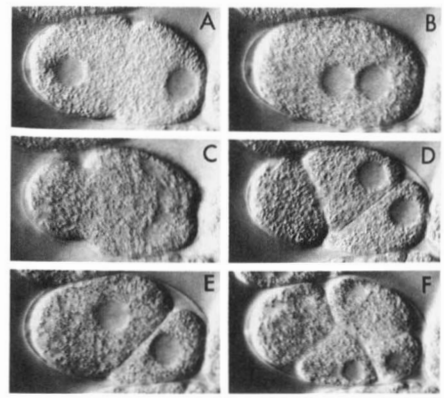

FIGURE 5.—Nomarski photomicrographs of self-progeny embryos from homozygous *mei-1(ct46)* hermaphrodites at 25°. A) Maternal (anterior) and paternal (posterior) pronuclei at pseudocleavage stage. B) Meeting of pronuclei. C) First-cleavage spindle oriented abnormally along the dorsal-ventral axis. D) First cleavage with anterior cytoplast. The boundary between the posterior cells has rotated slightly from its initial longitudinal orientation. E) Anterior cytoplast has fused with the dorsal cell. F) Four-cell embryo.

mei-1(ct46) and mel-26(ct61): Self-progeny embryos from these mutants showed similar phenotypes. For *ct46*, both *M/M* (Figure 5) and *M/+* hermaphrodites produced embryos in which the maternal and paternal pronuclei appeared to migrate normally, but the first cleavage occurred longitudinally rather than transversely (Figure 5A–C). An anterior cytoplast formed, but later re-fused with one of the other cells (Figure 5D–E). The longitudinal cell boundary then rotated to a transverse plane, with an abnormally large AB cell (compare to Figure 2D). Multiple nuclei were sometimes present. Cell boundaries were generally not well defined, and cytoplasmic exchange occurred. The AB and P_1 cells divided simultaneously (rather than AB first, as normally) but a somewhat normal-appearing four-cell embryo often resulted (Figure 5F). No morphogenesis occurred, but variable degrees of twitching and gut birefringence were seen in different embryos.

Embryos from homozygous *ct61* hermaphrodites at 25° were similar to those of *ct46* mutants (compare Figures 5 and 6). The phenotype was often more extreme, in that weak cleavage furrows often disappeared and the nuclei coalesced. An embryo with no distinct cell boundaries but with many nuclei often resulted (Figure 6D).

The phenotype of embryos from *ct61/+* hermaphrodites at 25° was less severe. The first cleavage often appeared normal, except that the mitotic spindles were short and poorly formed. Cleavage furrows were often indistinct and disappeared (data not shown). The embryos arrested prior to morphogenesis and showed gut birefringence and twitching.

The phenotypes resulting from both the *ct46* and *ct61* mutations closely resemble that caused by recessive maternal-effect lethal mutations in the unlinked gene *zyg-9 II*, except that the latter embryos show the additional defect that pronuclear migration does not occur. The TSP resulting from *zyg-9 ts* mutations such as *b244* occurs during first cleavage (KEMPHUES *et al.* 1986). We carefully compared the TSPs of *ct46/+*, *ct61/+*, and homozygous *b244* animals (MATERIALS AND METHODS). As shown in Figure 7, the TSP for all three strains began abruptly during the first division, that of *zyg-9(b244)* homozygotes being delayed slightly. The ending of each TSP was gradual, especially for *zyg-9(b244)*. For all three strains the first survivors were seen in shifts near the end of the first division, and the TSP ended by the onset of gastrulation.

mel-24(ct59): The self progeny from *M/M* hermaphrodites displayed no obvious lineage abnormalities prior to the onset of gastrulation. Terminal embryonic phenotypes varied depending upon the genotype of the hermaphrodite parent. *M/M* animals produced self-progeny embryos that arrested with no morphogenesis, but showed twitching and strong gut birefringence (22/22 embryos). *M/+* hermaphrodites showed incomplete penetrance for maternal-effect lethality (Table 1); 14/31 embryos developed to late morphogenesis or hatched. The remaining embryos arrested while undergoing an abnormal early morphogenesis and showed strong gut birefringence and twitching. One quarter of the 31 embryos should have been homozygous for *ct59*, and yet none arrested as early as those from homozygous hermaphrodites. Therefore, the different arrest points seen for embryos from homozygous and heterozygous hermaphrodites result from maternal, rather than zygotic effects. The TSP for embryos from *ct59/+* hermaphrodites occurred between about 60 to 90 min postfertilization.

mel-25(ct60): Embryos from *M/+* hermaphrodites

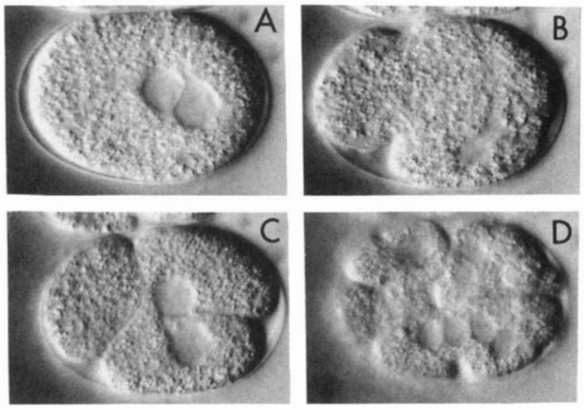

FIGURE 6.—Nomarski photomicrographs of self-progeny embryos from homozygous *mel-26(ct61)* hermaphrodites. A) Pronuclear meeting. B) First-cleavage spindle oriented abnormally along the dorsal-ventral axis. C) Longitudinal first cleavage with anterior cytoplast. D) Later stage with indistinct cell boundaries and multiple nuclei.

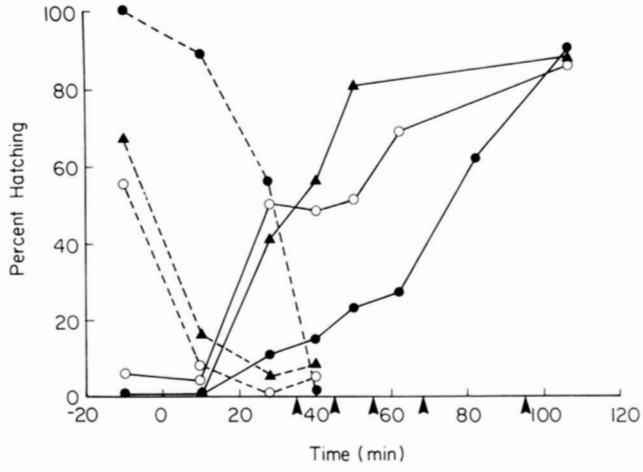

FIGURE 7.—TSP for the viability of self-progeny embryos from *mei-1(ct46)/+*, *mel-26(ct61)/+* and homozygous *zyg-9(b244)* hermaphrodites. Shifts from 15° to 25° and vice versa were performed at the times indicated (normalized to 25° time) as described in MATERIALS AND METHODS. Fertilization is at time 0. Arrows indicate times of synchronous division onset for cells in the AB lineage. The total number of cells in the embryo after each of these division rounds is 2, 3, 6, 12, and 24, respectively. Results of downshift experiments are connected by dashed lines and upshift experiments by solid lines. Triangles, *mei-1(ct46)/+*; open circles, *mel-26(ct61)/+*; solid circles, *zyg-9(b244)*.

(not shown) exhibited defects in both nuclear division and cytokinesis. The first cleavage spindle was usually small and indistinct and cytokinesis often failed. In these cases, the two nuclei coalesced, and the embryo divided simultaneously into four cells. Some embryos never underwent cytokinesis, and resulted in single cells with many nuclei. Embryos arrested at early morphogenesis and showed twitching and gut birefringence.

The TSP for embryonic arrest of these embryos began at about the time of fertilization and ended by the beginning of gastrulation. However, the larvae hatching from embryos shifted up during gastrulation failed to grow. The TSP for larval arrest gradually ended by the beginning of morphogenesis (data not shown).

DISCUSSION

Phenotypic properties of mutants: The properties of the eight *ts* mutations we have analyzed are summarized in Table 6. We have referred to them as dominant for convenience; however, all cause more severe defects when homozygous and are, therefore, semidominant. All show dominant *ts* embryonic lethality. This lethality shows a strict maternal effect, and no paternal or zygotic effects: at the restrictive temperature, +/+ self-progeny embryos from heterozygous hermaphrodites are inviable, as are all embryos from such hermaphrodites when fertilized by wild-type sperm, while embryos from mated wild-type hermaphrodites fertilized by sperm bearing the mutant allele develop normally.

Five of the mutations also show recessive zygotic effects. *let-354(ct42, ct76,* and *ct77)* and *mel-23(ct45)* result in non-conditional recessive lethality, while *mel-25(ct60)* causes gonadogenesis defects resulting in sterility. These recessive effects could result from either the loss of the wild-type function of an essential gene, or from the additive effect of two doses of a poison gene product. These same five mutations also caused dominant nonmaternal-effect *ts* defects in male mating, probably due to apparent abnormalities in the male tail. Therefore, the corresponding gene products could be active in processes occurring during embryogenesis as well as male tail morphogenesis (and perhaps other processes, in which minor defects are not so readily observable).

The remaining three of the eight mutations, *mei-1(ct46)*, *mel-24(ct59)*, and *mel-26(ct61)* result only in strict maternal-effect, *ts* phenotypes. However, the *mei-1(ct46)* and *mel-26(ct61)* mutations also result in a low-penetrance, semidominant, maternal-effect Vab phenotype at permissive temperatures. This may indicate that the corresponding gene products are used twice during embryogenesis, or that slight perturbations in the early embryo, which are themselves not lethal, can lead to other later defects.

The dominant phenotypes resulting from *mei-1(ct46)* and *mel-26(ct61)* are reminiscent of those caused by recessive maternal-effect mutations in the unlinked *zyg-9* gene (WOOD *et al.* 1980; ALBERTSON 1984; KEMPHUES *et al.* 1986). The possible significance of this observation will be discussed in detail elsewhere (MAINS *et al.*, in preparation).

The TSPs of *mei-1(ct46)*, *mel-26(ct61)*, and *zyg-9(b244)* were similar, beginning abruptly just after fertilization. The endings of the TSPs were gradual, but the first survivors were seen among embryos shifted up at the end of the first cleavage. The TSP

TABLE 6

Summary properties of dominant *ts* maternal-effect mutations

	Dominant maternal effects at 25°[a]	Dominant zygotic effects at 25°	Recessive zygotic effects at 15°	Suppression by extra copies of + allele	Dosage dependence: With + allele present	Dosage dependence: Without + allele present	Inferred nature of mutation[b]
let-354(ct42) *let-354(ct76)* *let-354(ct77)*	Lethality	Mab phenotype[c]	Lethality	+	+	+	gf poison
mel-23(ct45)	Lethality	Mab phenotype	Lethality	+++	+	ND[d]	lf haploinsufficient or gf poison
mei-1(ct46)	Lethality Vab phenotype[a]	−	−	+	+	+	gf poison
mel-24(ct59)	Lethality	−	−	±	+	++	gf poison
mel-25(ct60)	Lethality Him[a]	Mab phenotype	Sterility	ND	ND	ND	?
mel-26(ct61)	Lethality Vab phenotype[a]	−	−	+	+	−	gf poison

[a] The maternal-effect Vab phenotypes were seen at 15° and 20° in surviving progeny of *mei-1(ct46)* heterozygotes and homozygotes and of *mel-26(ct61)* homozygotes. The Him phenotype was seen at 15° in progeny of *mel-25(ct60)* homozygotes.
[b] Abbreviations: gf, gain-of-function; lf, loss-of-function.
[c] Male tail abnormalities, mating defects.
[d] Not determined.

for *b244* might be slightly later than for *ct46* and *ct61*. It appears then that the products of these genes are essential during the first cleavage (or for *ct46* and *ct61*, presence of the poison gene products during this time disrupts embryogenesis), but there is also a less stringent requirement for these gene products later in development since the TSP does not end until the onset of gastrulation. KEMPHUES *et al.* (1986) reported that the *zyg-9* TSP was precisely confined to a short period during the middle of the first cleavage, shortly before pronucleus formation, while we find that the ending is gradual. However, KEMPHUES *et al.* (1986) observed embryos *in utero* (allowing a very precise staging of the embryos) and scored only the orientation of the first cleavage. We instead dissected embryos from hermaphrodites and scored hatching. Therefore, a normal first cleavage as observed by KEMPHUES *et al.* (1986) is necessary but not sufficient for subsequent viability. Consistent with this conclusion is the proposal by KEMPHUES *et al.* (1986) that the normal *zyg-9* product is required to produce the very large spindle of the first cleavage. Subsequent cleavages have smaller spindles, but still may have a partial requirement for the wild-type *zyg-9* product.

Nature of dominant mutations: The mode of action of dominant mutations can be inferred from gene dosage experiments (see RESULTS and MULLER 1932). The *let-354* mutations *ct42*, *ct76*, and *ct77*, as well as *mei-1(ct46)* and *mel-26(ct61)* appear to result in gain-of-function poisons that compete with the corresponding wild-type products in a dose-dependent manner. With a few exceptions, the viabilities of self-progeny embryos from mutant hermaphrodites decrease with increasing dosage of the mutant allele relative to the wild-type allele, according to the series $M/+/+ > M/+ > M/M/+ > M/Df > M/M$. The relative severities resulting from the latter two genotypes, showing dose-dependency of *M* in the absence of the + allele, may indicate that the poison also affects the products of other genes in a dose-dependent manner. The exceptions to these results included *ct61/Df*, which showed the same progeny viability as *ct61/ct61*, and *ct46/ct46/+*, which exhibited lower progeny viability than *ct46/ct46* (see RESULTS and footnote *i*, Table 5). In all cases where a deficiency was available, results obtained with the *+/Df* genotype showed that one dose of wild-type product was sufficient for full viability; that is, these loci are not haploinsufficient.

The above observations would suggest that these mutations result in antimorphic gene products as defined by MULLER (1932). However, by this definition the phenotypes resulting from such mutations should resemble those caused by loss-of-function alleles at the same locus, because the antimorphic products inhibit wild-type function. Instead, the phenotype resulting from recessive (presumed loss-of-function) alleles of *let-354* (zygotic larval lethality; HOWELL 1989) differs from that caused by the apparent dominant alleles *ct42*, *ct76* and *ct77* (maternal-effect embryonic lethality). Moreover, we will show elsewhere that loss-of-function alleles of *mei-1* result in embryonic defects that differ markedly from *ct46* (P. E. MAINS *et al.*, in

preparation). Therefore, MULLER's antimorphic definition may be too specific or simplistic for these mutations.

In contrast to the mutations discussed above, the *mel-24(ct59)* mutation showed little effect of dosage relative to the wild-type allele, arguing against gene-product interaction. This mutation may be neomorphic by MULLER's (1932) definition, resulting in a gene product that has a novel function (or site or time of expression) and does not compete with the wild-type product. The results with *ct59* could be complicated because the *ct59/+/+* animals used were trisomic for the entire LG IV. Animals trisomic for wild-type LG IV are morphologically normal (SIGURDSON *et al.* 1986), but one third of their self-progeny embryos failed to hatch at 25° in our control experiments, presumably due to aneuploidy in the zygotes. The viability of embryos from *ct59/+/+* animals was normalized to this value, but the large correction makes somewhat questionable the significance of the slight viability increase over embryos from *ct59/+* hermaphrodites.

The *mel-23(ct45)* mutation may represent loss-of-function at a haploinsufficient locus. The viability of self-progeny embryos increased almost 4000-fold when the relative dose of the wild-type allele was increased from 1/2 (*M/+*) to 2/3 (*M/+/+*) by addition of the free duplication *mnDp37*; 86% of the embryos from *ct45/+/+* hermaphrodites hatched. This result represents nearly complete rescue, because about 10% of the embryos from these hermaphrodites should be homozygous for *ct45* and lacking the free duplication (40% of the progeny of *mnDp37*-bearing hermaphrodites did not inherit the duplication; data not shown). Because these *ct45/ct45* embryos are inviable, the maximum expected survival of *ct45/+/+* progeny embryos should be about 90%, even if the maternal-effect lethality were completely suppressed. It seems surprising that such complete rescue would result from simple competition between the wild-type and mutant products. As predicted for a maternal-effect lethal haploinsufficiency, no deficiency has been isolated for the region containing *ct45*. Moreover, there is indirect evidence for a haploinsufficient region on the right half of LG III. JOHNSEN and BAILLIE (1988) reported that fewer γ-ray-induced lethal mutations (many of which should be deficiencies) were recovered in this region relative to EMS-induced lethal mutations (presumably mostly point mutations) compared to other regions of the genome. J. PLENEFISCH (personal communication) was unable to isolate a deficiency of the *dpy-28* locus, which maps 0.4 cM from *ct45*.

However, arguing against the haploinsufficiency hypothesis for *mel-23* is the lower progeny viability of *ct45/ct45/+* hermaphrodites compared to *ct45/+* hermaphrodites at 15°. If the *ct45* product showed only partial activity at permissive temperature (consistent with the 36% viability of embryos from *ct45/+* hermaphrodites) then an extra dose of the *ct45* allele should increase progeny viability. Possible reasons for the observed opposite effect could be associated with the single wild-type allele being present on the free duplication in *ct45/ct45/+* animals: the gene might function less efficiently there due to position effects (*e.g.*, it is now closer to the chromosome end), or the tissue where the gene is expressed might often be mosaic due to duplication loss. Alternatively, the inviability could result from the zygotic effects of *ct45* noted earlier (recessive lethality and dominant male mating defects), which could be independent of a maternal haploinsufficiency for the gene.

There are reported cases of autosomal dosage compensation in both maize (BIRCHLER 1981) and Drosophila (DEVLIN, HOLM and GRIGLIATTI 1988). If the *mel-23* locus were dosage compensated, the expression of each allele would be decreased by ⅓ in *ct45/ct45/+* relative to the diploid. The benefit of the additional *ct45* allele (which is hypomorphic at 15°) could be outweighed by the ⅓ decrease in activity of the wild-type allele in *ct45/ct45/+*, resulting in the lack of viable progeny. (The possibility of autosomal dosage compensation should not change the interpretations discussed for the other mutations. The relative amounts of the gene products, rather than absolute amounts, are more important when mutant and wild-type products compete.)

Therefore *mel-23(ct45)* could be a loss-of-function mutation at a haploinsufficient locus based on the observed results. Alternatively, *ct45* could result in a gain-of-function poison whose effects are extremely sensitive to an increase of the relative proportion of wild-type product. To distinguish these alternatives, we will try to isolate a deficiency for the region in the presence of a duplication so that progeny viability can be tested for *+/Df* hermaphrodites. If there proves to be a maternal haploinsufficiency in the region, the deficiency could be maintained as *+/+/Df*.

The TSP of *mel-23(ct45)* revealed an additional unusual property of this mutation. The viability of embryos from *M/+* hermaphrodites reared early at restrictive temperature but downshifted prior to the beginning of the TSP was dramatically improved relative to embryos from *M/+* hermaphrodites reared entirely at the permissive temperature (95% vs. 36%). This could indicate that the *ct45* product is cold-sensitive for a period prior to its time of heat sensitivity (*e.g.*, cold sensitive for assembly, heat sensitive for function). Alternatively, the mutant product could interact with other products that are induced at 25° which enable it to function more efficiently when later downshifted (*e.g.*, heat shock proteins).

The mutations *ct42*, *ct76* and *ct77* are apparently alleles at the *let-354* locus. Their properties, although quantitatively different, are qualitatively similar. They result in similar embryonic phenotypes, have similar TSPs, and respond similarly in gene dosage experiments. They all map to the same 1-cM interval and fail to complement for recessive zygotic lethality. They all show the same behavior when combined with a deficiency of the region or with a recessive point mutation of *let-354*.

It might seem unusual to have isolated three gain-of-function alleles at the same locus among only 4000 F_1 progeny of mutagenized animals. The frequency of loss-of-function alleles in an "averaged" *C. elegans* gene after standard EMS mutagenesis is estimated to be 1/2000 gametes (BRENNER 1974; GREENWALD and HORVITZ 1980; ANDERSON and BRENNER 1984; PARK and HORVITZ 1986), and dominant gain-of-function mutations are generally expected to be rare. However, dominant mutations resulting in gain-of-function poisons can be more frequent among gene products that form large supramolecular structures (KUSCH and EDGAR 1986). Dominant alleles of *unc-54* (the major myosin heavy-chain gene) occur at the same frequency as recessive mutations, probably because of the ability of a defective subunit of myosin to disrupt muscle structure (BEJSOVEC and ANDERSON 1988; MACLEOD *et al.* 1977; WATERSTON, HIRSH and LANE 1984). Dominant alleles also occur frequently in other *C. elegans* structural proteins, including paramyosin (*unc-15*, WATERSTON, FISHPOOL and BRENNER 1977), actin (*unc-92*, WATERSON, HIRSH and LANE 1984), and known or probable members of the collagen gene family (*sqt-1*, *sqt-2*, sqt-3, and *rol-8*, KUSCH and EDGAR 1986; KRAMER *et al.* 1988). A dominant nonsense allele has been described in the flight muscle actin gene of Drosophila (KARLIK, COUTU and FYRBERG 1984), and deletion mutations in keratin genes can also be dominant (ALBERS and FUCHS 1987). The *let-354* gene product may likewise function in some large supramolecular structure.

Interestingly, *let-354* also mutates to recessive alleles at an unusually high frequency (HOWELL 1989). However the recessive allele *let-354(h79)* did not behave identically to a deficiency of the region when heterozygous combinations with any one of the three dominant mutations were examined for recessive zygotic lethality. *M/let-354(h79)* and *M/M* animals arrested as early larvae while *M/Df* animals arrested much later. This implies that *h79* is not a true null mutation, and in view of our evidence that wild-type activity decreases the effects of the dominant mutations, *h79* might even be a weak gain-of-function allele. While *h79* did not exhibit any *ts* dominant maternal-effect lethality, one of seven other tested alleles, *let-354(h482)*, did so (HOWELL 1989; A. M. HOWELL, personal communication). These observations, further substantiating the high frequency of gain-of-function alleles at this locus, support the likelihood that the *let-354* gene product functions in a large supramolecular array.

In summary, we have isolated eight dominant *ts* maternal-effect lethal mutations, defining six loci. Mutations at three of the loci show only strict maternal effects; the others cause associated zygotic phenotypes. Mutations at four of the loci appear to result in gain-of-function poisons, while one may be a loss-of-function mutation in a haploinsufficient locus. Two of the loci appear to encode gene products that interact with each other and with products of the *zyg-9* locus during early embryonic cleavage, as will be further described elsewhere. Thus screening for dominant maternal-effect mutations has proven to be an effective means of identifying loci that may encode components of supramolecular structures in the early embryo. Further analysis of these genes and the corresponding mutant phenotypes should be helpful in understanding the morphogenetic events of the first cleavages.

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