Viable Maternal-Effect Mutations That Affect the Development of the Nematode *Caenorhabditis elegans*

Siegfried Hekimi, Paula Boutis and Bernard Lakowski

Department of Biology, McGill University, Montréal, Québec, Canada H3A 1B1

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ABSTRACT

We carried out a genetic screen for viable maternal-effect mutants to identify genes with a critical function relatively early in development. This type of mutation would not have been identified readily in previous screens for viable mutants and therefore could define previously unidentified genes. We screened 30,000 genomes and identified 41 mutations falling into 24 complementation groups. We genetically mapped these 24 loci; only two of them appear to correspond to previously identified genes. We present a partial phenotypic characterization of the mutants and a quantitative analysis of the degree to which they can be maternally or zygotically rescued.

GENETIC screens in the nematode *Caenorhabditis elegans* have identified numerous genes required for normal development. Most of these genes have been identified through screens for viable mutants with abnormal morphology or behavior (*e.g.*, BRENNER 1974; HODGKIN and BRENNER 1977; HODGKIN 1980, 1983; CHALFIE and SULSTON 1981; RIDDLE *et al.* 1981; TRENT *et al.* 1983; FERGUSON and HORVITZ 1985; PARK and HORVITZ 1986; DESAI *et al.* 1988; MANSER and WOOD 1990; AVERY 1993; STARICH *et al.* 1995). However, work in other organisms, in particular, Drosophila, has amply demonstrated that full loss-of-function mutations in genes controlling the earliest aspects of development have such profound consequences on later development that they almost invariably lead to embryonic lethality. To identify such genes in *C. elegans*, genetic screens for maternal-effect and zygotic embryonic lethal mutations have been carried out and a number of genes required for specific aspects of early development have been identified (*e.g.*, ISNENGHI *et al.* 1983; PRIESS *et al.* 1987; KEMPHUES *et al.* 1988a,b; SCHNABEL and SCHNABEL 1990a,b; MAINS *et al.* 1990; BUCHER and GREENWALD 1991; MELLO *et al.* 1992; BOWERMAN *et al.* 1993; WILLIAMS and WATERSTON 1994). However, essential genes can also be identified through the isolation of viable hypomorphic mutations, that is, mutations that do not fully abolish the activity of the gene but produce a visible phenotype (*e.g.*, FERGUSON and HORVITZ 1985).

One class of genes that has not been systematically pursued to date is that for which mutational disruption does not necessarily lead to lethality but for which the requirement is sufficiently early and/or for sufficiently small amounts that there would be a maternal effect. Such genes and mutations would not have been automatically found in previous screens for viable mutants, as in those screens in general only the first generation of homozygotes was examined.

Indeed, relatively few maternal-effect viable mutations have been characterized to date. However, some particularly interesting genes display this type of heritability, among them, genes in the dosage compensation and sex-determination pathways, such as *sdc-1* (VILLENEUVE and MEYER 1987), *tra-3* (HODGKIN and BRENNER 1977), and *dpy-27* (MEYER and CASSON 1986; CHUANG *et al.* 1994). Other such genes include *mes-1*, which is involved in germ line formation (CAPOWSKI *et al.* 1991); *eat-3*, which affects pharyngeal pumping (AVERY 1993); and genes involved in dauer larva formation, such as *daf-1* (SWANSON and RIDDLE 1981), *daf-21*(THOMAS *et al.* 1993) and *daf-23* (GOTTLIEB and RUVKUN 1994).

To identify new genes involved in the development of the worm, we carried out a screen for maternal-effect viable mutants and identified 41 mutations leading to morphological or behavioral defects.

MATERIALS AND METHODS

General methods and strains: *C. elegans* strains were cultured as described by BRENNER (1974). Standard genetic methods for *C. elegans* were used (BRENNER 1974; SULSTON and HODGKIN 1988). Animals were cultured at 20° unless otherwise stated. Wild type was the N2 Bristol strain. Mutations and rearrangements used were as follows:

LGI: *dpy-5(e61), unc-40(e271), bli-4(e937), dpy-14(e188ts), che-3(e1124), unc-29(e1072), daf-16(m26), dpy-24(s71), unc-75(e950), unc-54(e190), nDf24, nDf23, eDf3.*

LGII: *dpy-10(e128), rol-6(e187), unc-4(e120), unc-53(e404), rol-1(e91), eat-2(ad465), unc-52(e669su250ts).*

LGIII: *dpy-1(e1), daf-2(e1370ts), emb-32(g58), emb-2(hc58), emb-1(hc57), unc-79(e1030), emb-7(hc66), dpy-17(e164), gro-1(e2400), mel-31(s2438), mel(jb7), lon-1(e185), sma-4(e729), emb-16(g19), lin-39(n1760), lin-13(n387), ncl-1(e1865), emb-25(g45), mab-5(e1239), unc-36(e251), unc-32(e189), emb-34(g62), emb-24(g40), dpy-18(e364), tDf1.*

Corresponding author: Siegfried Hekimi, 1205 Doctor Penfield Ave., Montréal, Québec, Canada H3A 1B1.
E-mail: hekimi@notung.biol.mcgill.ca

LGIV: *dpy-9(e12), lin-1(e1777), dpy-13(e184sd), bli-6(sc16), eat-12(ad695), unc-24(e138, e1172), unc-43(e408), let-657(s1254), dpy-20(e1282ts), unc-31(e169, e928), unc-26(e205), tra-3(e1767), dpy-4(e1166sd), nDf41, eDf19, eDf18, mDf7, sDf2.*
LGV: *dpy-11(e224), unc-42(e270), rol-4(sc8), lin-25(n545), him-5(e1467), unc-76(e911), dpy-21(e428), unc-51(e369), sDf32, sDf28, ctDf1, arDf1.*
LGX: *unc-7(e5), lin-15(n309), sdc-1(n485), sup-10(n983)*
Balancers: *mnC1 II, nT1 (IV;V)*
Strains used: *CB4622 unc-26(e205) tra-3(e1767) dpy-4(e1166)/ nT1 IV; +/nT1 (V)* (BARNES 1991).

Isolation of mutants: Wild-type animals were mutagenized with ethyl methane sulfonate (EMS) following the standard protocol (SULSTON and HODGKIN 1988). Groups of five mutagenized hermaphrodites (P0) were plated on 9-cm Petri dishes and allowed to self-fertilize. They were removed after having laid ~200 eggs (40 eggs each). The resulting F_1 animals were left on the plate until they had laid a total of 6000 to 10,000 F_2 eggs and were then removed. When most of the resulting F_2 animals had grown to adulthood, 50 adults with no detectable morphological or behavioral mutant phenotype were picked and transferred onto individual small plates. Those animals, which produced an entire brood of morphologically, behaviorally or developmentally (slow growth) mutant worms, were analyzed further. Thirty thousand F_2 animals were screened as described above, and 41 independent mutations were isolated, tested for complementation and mapped.

Complementation tests: Complementation tests between newly isolated mutations were performed when they appeared to produce similar phenotypes. Most commonly, heterozygous males (m_1/+) of one mutation were mated to homozygous hermaphrodites of the other strain (m_2/m_2) and their progeny scored for mutant males. Complementation to alleles of known genes was tested in the same way. This type of complementation test checks for the failure of zygotic rescue of m_2 by m_1. However, in the case of mutations for which zygotic rescue by a wild-type allele either failed entirely or was incomplete, failure of zygotic rescue would not have been a sufficiently discriminating criterion to establish noncomplementation. In these cases, the mutations were first individually mapped and then complementation tests were carried out using strains with linked markers. Three mutations of this type (*qm16, qm31* and *qm35*) were found to map to the same genetic location, and their allelism was then established in the following way: 10 or more hermaphrodite cross-progeny from a cross of m_1/+ males to marked m_2/m_2 hermaphrodites were individually picked and their self-progeny scored. Hermaphrodite cross-progeny producing only mutant self-progeny were found; because maternal rescue by the wild-type allele is complete for all three mutations, we concluded that they were allelic.

Three mutations with a similar Mau phenotype (*qm18, qm19* and *qm45*) are *X*-linked. Their allelism was established by taking advantage of the ability of *tra-2(q276)* to transform *XX* animals into phenotypic males that are able to mate efficiently (L. AVERY, personal communication). *q276/q276; qm45/qm45* males were constructed and used in complementation tests to *qm18* and *qm19*.

Identification of alleles of known genes: Alleles of two known genes were identified in the screen: three alleles of *sdc-1* and two alleles of *tra-3*. The two mutations (*qm44* and *qm52*) with a Tra-3 phenotype were tested for noncomplementation with *tra-3(e1767)* and balanced over *nT1* simultaneously, by crossing *qm44*/+ or *qm52*/+ to the strain CB4622 and recovering the appropriate progeny classes. Alleles of other genes for which maternal-effect viable mutations are known were not isolated, probably because of the experimental conditions of the screen. The screen was carried out at 20° or 15°, and only strains in which at least 90% of the F_3 shared a phenotype were kept. We also did not keep strains that were so sick they would have required special measures or were difficult to propagate. These conditions probably excluded or made unlikely the recovery of alleles of *dpy* genes involved in dosage compensation. Alleles of these genes are mostly near lethal and sometimes cold sensitive and would probably have been discarded for that reason. The *daf-1, daf-21* and *daf-23* maternal-effect mutations are temperature sensitive, as is dauer formation itself (GOLDEN and RIDDLE 1984). A *daf* allele producing substantially <90% dauer larvae at 15° or 20° would not have been kept or possibly not even noticed, as F_3 plate carrying a substantial number of wild-type looking worms would have been immediately discarded. Finally, mutants in *eat-3*, a gene defined by a single mutation, are very sick, and would not have been kept for that reason. Furthermore, *eat-3(ad426)* might itself be a hypomorphic mutation, and possibly, more usual alleles are stronger and thus lethal.

Maternal rescue: To score for maternal rescue, wild-type males were mated to homozygous mutant hermaphrodites and 10 of the resulting wild-type F_1 hermaphrodites were pooled and allowed to lay eggs for a 24-hr period. All resulting F_2 animals were scored. When maternal rescue was incomplete, it was noted which aspect of the phenotype was not rescued or how the animal's phenotype deviated from the wild type. All progeny found on a given plate were scored to identify possible dead eggs or larvae, but no dead animals were ever observed.

Zygotic rescue: Wild-type males were mated to homozygous mutant hermaphrodites marked with a recessive mutation, and the anatomical and behavioral phenotypes of the cross-progeny (m/+) were scored. All mutations that showed incomplete zygotic rescue also displayed some embryonic and larval lethality in the absence of maternal and zygotic rescue. Lethality in these heterozygotes, however, was not scored because it would have been too difficult to distinguish such animals from dying self-progeny. Therefore, only animals that had survived embryogenesis and the earliest stages of larval development were scored.

Paternal rescue: The possibility of a paternal effect was investigated by testing whether sperm carrying a mutant copy of the gene but produced by a heterozygous male carrying a wild-type copy of a gene could contribute enough wild-type gene activity to the developing zygote to result in a wild-type phenotype. Heterozygous males (m/+) were mated to homozygous mutant hermaphrodites (m/m) and cross-progeny males were scored. In the absence of paternal rescue, half of the males should be phenotypically mutant and half should be wild type. When marked strains were used as a source of hermaphrodites, both male and hermaphrodite cross-progeny were scored. In no case did we see paternal rescue leading to a detectable amelioration of the phenotype at the level of the dissection microscope.

Penetrance: For the reference allele of each novel gene, the degree of embryonic lethality and larval lethality was scored, as well as the penetrance of visible phenotypes in surviving adults. To determine lethality, 10–20 animals of a given genotype were pooled and allowed to self-fertilize for a 12-hr period. Approximately 200 of the eggs laid during this time were then transferred to a fresh plate and counted. Twenty-four hours later, the number of unhatched (assumed to be dead) eggs was counted. Several days later, the number of animals that had reached adulthood was scored.

Phenotypic analysis: Three mutant strains suspected by overall phenotype to have muscle defects were stained with fluorescein isothiocyanate-labeled phalloidin to visualize F-actin in muscle cells. 4′,6-Diamidino-2-phenylindole (DAPI) was also used to stain nuclei. Mutant and control N2 worms

were harvested in M9 buffer and pelleted. The animals were frozen for 10 min at −80° and thawed to permeabilize the worms. For fixation, the worms were immersed in acetone for 10 min and pelleted again. The acetone was aspirated, and the animals were air dried. The concentrations used for phalloidin and DAPI were 0.1 and 1 μg/ml, respectively. The animals were stained in the solutions for 3 hr, rinsed three to four times in M9 buffer, and then mounted and examined by fluorescence microscopy. All three strains were also observed by polarized light and differential interference contrast microscopy.

Animals carrying *mau* mutations were also examined with the carbocyanide dye DiI to stain the sensory neurons (HEDGECOCK *et al.* 1985; E.M. HEDGECOCK, personal communication).

RESULTS

A genetic screen for viable maternal-effect mutations: Wild-type (N2) worms were mutagenized with EMS and allowed to self-fertilize for two generations. Animals resulting from the second round of self-fertilization (F_2) are likely to be homozygous carriers of a number of new mutations with or without visible phenotypic effects. Such animals were examined, and those that displayed wild-type overall morphology and behavior were picked onto individual fresh plates and allowed to self-fertilize. Those strains that then produced an entirely mutant brood (F_3) were analyzed further in the following way. After the initial isolation, all mutants were crossed to wild-type (N2) males and three to five phenotypically wild-type hermaphrodites from the resultant progeny (F_1) were picked onto individual plates. The self-progeny (F_2) of these individuals were then examined for the presence of phenotypically wild-type animals. When ~25% of these F_2s showed the mutant phenotype, the strain was discarded because it suggested that the animal picked originally was an escaper, that is, an animal carrying a fully zygotic mutation, which was not strongly expressed. When substantially fewer than 25% of the animals displayed a mutant phenotype, 24 phenotypically wild-type F_2s were picked onto individual plates and their F_3 progeny examined. At this stage, in most cases, some of the F_3 broods were composed almost entirely of animal displaying a mutant phenotype. When none of the F_3 broods displayed a fully mutant phenotype, the procedure was repeated. When the repeated procedure was still unsuccessful at obtaining fully mutant F_3 broods, we considered that the phenotype of the original mutant strain was synthetic in origin, that is, due to more than one independently segregating mutation, and the strain was discarded.

Strains that appeared generally sick without displaying specific movement defects or anomalies of body shape were not kept. We analyzed 30,000 F_2 animals and their progeny in this way and isolated 41 mutations representing 24 complementation groups.

The screening procedure selects for mutations for which the presence of a wild-type copy of the gene in the mother is sufficient to produce a wild-type phenotype in the offspring. However, it neither selects for, nor against, mutations for which the presence of a wild-type copy in the zygote is insufficient for a wild-type phenotype (strict maternal effect).

Summary of the mutant phenotypes: The 24 complementation groups fall into seven phenotypic classes: (1) *mau* (maternal-effect uncoordinated), eight genes; (2) *mum* (maternal-effect uncoordinated and morphologically abnormal), three genes; (3) *mal* (maternal-effect morphologically abnormal), four genes; (4) *mad* (maternal-effect dumpy), three genes; (5) *mud* (maternal-effect uncoordinated and dumpy), one gene; (6) *clk* (abnormal function of biological clocks), three genes; and (7) genes involved in sex determination and/or dosage compensation, two genes (whose phenotypes are not discussed further here).

Table 1 gives short summaries of the phenotype, expressivity and penetrance of the reference mutation for each gene. In the text below, we describe the mutant phenotypes in more detail. The phenotypic descriptions given in Table 1 and in the main text refer to the adult phenotypes, unless explicitly stated. Most of the phenotypic defects of all the mutants can be observed in L1 larvae, indicating that the genes are required during embryogenesis as suggested by the fact that their phenotype can be rescued by a maternal effect. However, at this stage of the characterization of these genes, we have not attempted to determine whether the gene products were also required during postembryonic development.

Description of mau genes: mau genes can be subdivided in three groups: (1) *mau-1, mau-2* and *mau-4,* mutations in which result in neuroanatomical defects; (2) *mau-3, mau-5* and *mau-6,* mutations in which result in defective muscle function; and (3) *mau-7* and *mau-8,* mutations that result in generalized locomotory defects that we ascribe to defects in synaptogenesis. Among the *mau* mutants, only *mau-7* and *mau-8* show significant embryonic lethality. Dying embryos, however, appear fully differentiated and not deformed.

mau-1 (two alleles): For both *mau-1* alleles, the mutant phenotype is extremely variable. The most affected animals are almost totally paralyzed at hatching, grow very slowly and many die before reaching adulthood. The most mild phenotype is shown by a small proportion of animals that grow at a normal rate but kink severely when attempting backward movement. Between these extremes, individual *mau-1* mutants display qualitatively distinct phenotypes. For example, although most of the animals appear to lay their eggs normally, a proportion (1/5) of the mutants are strongly egg-laying defective (Egl). Similarly, a subset of the mutants resembles *unc-6* and *unc-5* mutants in their locomotory pattern, suggesting abnormal function of their dorsal cord (HEDGECOCK *et al.* 1985). A different subset of the animals tends to lie with a stiff ventral bend but with mobile

TABLE 1

Phenotypic characterization of 22 genes

Gene	Alleles	Phenotypes	Lethality and penetrance	Zygotic and maternal rescue
mau-1	*e2507* *qm6*	Variabily uncoordinated, including kinking, coiling and paralysis. Egg-laying defective. Neuroanatomical defects.	4% of embryos die (8/206)[a]. 25% of larvae die (49/198) 100% of adult survivors show a mutant phenotype (150/150).	Full zygotic rescue (0/257). Full maternal rescue (0/288).
mau-2	*qm4* *qm5* *qm40*	Kinky uncoordination. Egg-laying defective. Neuroanatomical defects. Short excretory canals.	5% of embryos die (5/106). 71% of larvae die (72/101). 100% of adult survivors show a mutant phenotype (169/169).	Full zygotic rescue (0/135). Sporadic failure of maternal rescue (3/363). No maternal rescue of the egg-laying defect (70/363).
mau-3	*qm10*	Uncoordinated: progressive paralysis. Abnormal muscle anatomy.	8% of embryos die (20/251). 4% of larvae die (10/231). 100% of adult survivors show a mutant phenotype (221/221).	Full zygotic rescue (0/279). Full maternal rescue (0/183).
mau-4	*qm45* *qm18ts* *qm19ts*	Uncoordinated: stiff posterior body, somtimes lethargic. Withred tail, *qm18* and *qm19* mutants are wild type at 15°, mutant at 22° and dead at 25°.	2% of embryos die (7/285). 4% of larvae die (11/278). 100% of adult survivors show a mutant phenotype (267/267).	Full zygotic rescue (0/104). Sporadic failure of maternal rescue (1/314).
mau-5	*qm17*	Uncoordinated: progressively worsening sluggishness and saccadic forward movement. Egg-laying defective. Abnormal muscle anatomy.	3% of embryos die (4/159). 9% of larvae die (14/155). 100% of adult survivors show a mutant phenotype (141/141).	Full zygotic rescue (0/247). Full maternal rescue (0/324).
mau-6	*qm50*	Uncoordinated: progressive paralysis leading to full paralysis and rapid death after the 4th larval moult.	3% of embryos die (6/211). 10% of larvae die (20/205). 100% of adult survivors show a mutant phenotype (185/185).	Full zygotic rescue (0/144). Sporadic failure of maternal rescue (1/294).
mau-7	*qm56*	Uncoordinated: lethargic, kinky jerky, ratchet-like movements in reverse. Constipated due to frequent absence of the expulsion contraction.	20% of embryos die (66/328). 16% of larvae die (43/262). 100% of adult survivors show a mutant phenotype (219/219).	Full zygotic rescue (0/221). Full maternal rescue (0/199)
mau-8	*qm57*	Similar to *qm56*.	15% of embryos die (29/193). 16% of larvae die (26/164). 100% of adult survivors show a mutant phenotype (110/110).	Full zygotic rescue (0/251). Full maternal rescue (0/293).
mum-1	*qm32*	Variably deformed but with no prominent single feature. Deformed pharynx. Very severely uncoordinated: from strongly kinky to complete paralysis. Neuroanatomical defects. abnormal excretory system. Abnormal gonads.	32% of embryos die (71/223). 80% of larvae die (116/144). 100% of adult survivors show a mutant phenotype (123/123). *qm32/mDf7* animals all die as embryos unless maternally rescued. When maternally rescued such animals can produce a few *qm32/qm32* progeny.	Sporadic failure of zygotic rescue: (5/262) of F1s show a mutant phenotype. Sporadic failure of maternal rescue: (4/413) of F2 animals are transiently uncoordinated during L3/L4 stages.
mum-2	*qm33*	Similar to *mum-1 (qm32)*	5% of embryos die (10/203). 54% of larvae die (91/170). 82% of adult survivors show a mutant phenotype (65/79). *qm33/sDf9* are not Unc nor morphologically abnormal when maternally rescued but are sterile.	Sporadic failure of zygotic rescue (21/429) of F1s show a mutant phenotype. Sporadic failure of maternal rescue: (6/348) of F2 animals are transiently uncoordinated during L3/L4 stages.
mum-3	*qm46*	Similar to *mum-1 (qm32)*	15% of embryos die (38/252). 26% of larvae die (56/214). 100% of adult survivors show a mutant phenotype (267/267).	Full zygotic rescue (0/210). Full maternal rescue (0/371).
mal-1	*qm27*	Dorsal protrusions on head and tail. Head frequently twisted.	30% of embryos die (64/213). 86% of larvae die (102/118). 44% of adult survivors show a mutant phenotype (28/63).	Full zygotic rescue (0/326). Full maternal rescue (0/747).

TABLE 1

Continued

Gene	Alleles	Phenotypes	Lethality and penetrance	Zygotic and maternal rescue
mal-2	*qm31* *qm16* *qm35*	Variably deformed; frequently hypertrophic ventral side of the head.	41% of embryos die (91/220). 37% of larvae die (28/75). 43% of adult survivors show a mutant phenotype (78/180).	No zygotic rescue: (78/183) 43% of surviving adult F1s show the mutant phenotype which is identical to the penetrance of the phenotype without zygotic rescue. Full maternal rescue (0/137).
mal-3	*qm34*	Head frequently deformed because the buccal cavity does not open at the tip of the head but ventrally, dorsally or laterally.	18% of embryos die (60/330). 94% of larvae die (102/109) 100% of larvae show a phenotype (270/270). Very few animals survive to adulthood and only few of those show the mutant phenotype (2/7).	Full zygotic rescue (0/275). Full maternal rescue (0/118).
mal-4	*qm36*	Hypertrophic left side of the head, generally anterior of the terminal bulb.	4% of embryos die (12/290). 46% of larvae die (69/150) 53% of adult survivors show a mutant phenotype (43/81).	Full zygotic rescue (0/156). Full maternal rescue (0/463).
mad-1	*qm39*	Very poor embryonic elongation. Hatchlings are short and deformed, similar to *dpy-17* mutants. In later stages the anterior half of the body is short but well developed while the posterior half is thin.	4% of embryos die (10/225). 46% of larvae die (69/150). 100% of larvae show a mutant phenotype (234/234). 74% of adult survivors show a mutant phenotype (142/192).	Full zygotic rescue (0/236). Partial maternal rescue (5/285).
mad-2	*qm62* *qm58*	Adults are short but early larvae are not. Animals are lethargic but display hyperactive foraging movement and very rapid pumping when stimulated by light touch. Hermaphrodite and male tails are abnormal. Very slow development.	53% of embryos die (117/219). 40% of larvae die (41/102). 100% of adult survivors show a mutant phenotype (201/201).	Full zygotic rescue (0/244). Full maternal rescue (0/269).
mad-3	*qm64*	Hatchlings are of variable length and the shortest invariably die. Adults are short and appear unhealthy.	39% of embryos die (88/245). 37% of larvae die (39/105). 100% of adult survivors show a mutant phenotype (224/224).	Full zygotic rescue (0/257). Full maternal rescue (0/288).
mud-1	*qm21* *qm22*	Hatchlings are of variable length, with very poor movement. Adults are short and show kinky uncoordination.	70% of embryos die (159/227). 97% of larvae die (66/68). 100% of adutl survivors show a mutant phenotype (110/110).	Full zygotic rescue (0/274). Full maternal rescue of Unc phenotype (0/153). Partial maternal rescue of Dpy phenotype (9/153).
clk-1[b]	*e2519* *qm11* *qm30* *qm47* *qm51*	Slow and variable embryonic and post-embryonic development; slow cell cycle; long life span; slow and irregular behavioral rhythms including swimming, pumping and defecation.	No lethality; fully penetrant.	Profound zygotic and maternal rescue.
clk-2	*qm37*	Similar to *clk-1*; embryonic lethal at 25°.	Some larval and embryonic lethality at all temperatures; fully penetrant for timing phenotypes.	Profound zygotic and maternal rescue.
clk-3	*qm38* *qm53*	Similar to *clk-1*.	No lethality; fully penetrant for timing phenotypes.	Profound zygotic and maternal rescue.

[a] For the entire table, the numerator in the fraction given in brackets corresponds to the number of mutant animals observed and the denominator to the total number of animals scored.

[b] The phenotype of *clk-1* mutants has been described in detail in a previous publication (WONG *et al.* 1995); the two alleles not previously described (*qm47* and *qm51*) have phenotypes similar to the previously described alleles. No quantitation of the phenotypes and heritability of the *clk* mutations is given here as an adequate description would be beyond the scope of this article, given the quantitative nature of the phenotypes.

heads and tails. This phenotype resembles that of *unc-4* (WHITE *et al.* 1992), suggesting abnormal function of ventral cord motor neurons.

mau-1 mutants were examined by staining with the carbocyanide dye DiI, which stains sensory neurons in the head and in the tail. In most animals, the projections of amphid (those in the head) and phasmid (those in the tail) neurons appeared normal. In a few cases, however, the processes that the phasmids send into the ventral cord appeared twice as long as in the wild type.

mau-2 (three alleles): All three alleles of this gene lead to a similar phenotype. When attempting backward movement, these animals kink severely. In addition, *mau-2* mutants are strongly Egl. The egg-laying defect is not maternally rescued: a homozygous *mau-2* mutant originating from a mother carrying a wild-type allele of the gene displays fully wild-type locomotion but is severely Egl (see Table 1). Approximately 15% of *mau-2* mutants die during larval development. Examination of these worms by DIC (differential interference contrast) microscopy showed that the excretory canal was invariably very short and strongly convoluted in places (Figure 1B).

Examination of *mau-2* mutants with DiI did not reveal any defects in the amphid neurons, but dye filling of the phasmid neurons failed entirely in >90% of the animals. In most of the remaining cases, they stained on only one side and only very rarely on both sides.

mau-4 (three alleles): All mutant animals are severely Unc when attempting backward movement. These mutants have a partially wasted posterior body similar to the phenotype of some mutants with abnormal migrations of the canal associated neuron (MANSER and WOOD, 1990). Wasting is generally confined to the most posterior one-fourth of the body. This wasted part of the posterior body can appear stiff or flaccid, but it is always unable to propagate normal waves of muscle contraction. Two observations suggest that the normal substrates necessary for proper cell and axonal migrations are altered in the wasted part of the body. First, the posterior gonad arm generally reflexes back prematurely toward the anterior of the animal instead of entering the wasted region. Second, all amphid and phasmid neurons could be normally stained with DiI and the amphid projections always appeared wild type. However, the anteriorly directed processes of the phasmids into the ventral nerve cord were always very short in these mutants, whereas the posterior projection into the tail spike appeared normal.

Two alleles of the gene (*qm18ts* and *qm19ts*) are temperature sensitive with a steep temperature dependence: most animals are almost fully wild type when raised at 20°, when raised at 22° are fully mutant, and die as larvae at 25°. One allele (*qm45*) is Unc at all temperatures, although it is also subviable at 25°.

The phenotypes seen in *mau-1, mau-2* and *mau-4* strongly suggest that these genes are required for normal neuroanatomy and that their movement defects (in particular for *mau-1* and *mau-2*) probably arise from abnormal or missing neuronal connections.

mau-3, mau-5, mau-6 (one allele each): Mutants in each of these genes appear to have defective muscles. The phenotype of all three mutants worsens throughout development. *mau-5* mutants are Egl and only become fully paralyzed after filling up with eggs. The progression of the paralysis of *mau-3* mutants is very gradual during postembryonic life, becoming fully paralyzed only during adult life. By contrast, the phenotype of *mau-6* mutants worsens to full paralysis very rapidly after reaching adulthood, leading rapidly to death.

When worms are examined under polarized light, muscles can be visualized by their birefringent properties. The muscle bands in all three mutants appear very narrow and ragged compared with wild-type worms, and some muscle cells appear to be degenerating. In some *mau-6* mutants, muscle cells are found in isolation and not aligned into a band nor attached to each other. All other aspects of anatomy appear normal at the level of DIC microscopy. To date, it is unclear which aspect of muscle development or function is affected in these mutants; however, the maternal effect makes it unlikely that a structural molecule directly involved in muscle contraction is affected.

mau-7, mau-8 (one allele each): Mutants in these two genes display the same pattern of phenotypes. They are lethargic and, when undisturbed, tend to lie on the plate with a fully extended body posture. However, they are also jerky; even when undisturbed, they display frequent very rapid forward or backward movements over very short distances that are not followed by a more continuous movement. In addition, they display a number of distinct movement defects. For example, when moving forward, the head and the anterior half of the body propagate only very shallow waves. At the extreme of the phenotypic continuum, the anterior one-third of the body is fully stiff and pushed forward by the rest of the body in a manner that is very reminiscent of the locomotory defects of levamisole-resistant uncoordinated mutants like *unc-29* (LEWIS *et al.* 1980). Furthermore, when induced to move backward, these mutants frequently kink but also display a discontinuous ratchet-like movement reminiscent of mutants of *unc-11*, a gene shown to be required for normal cholinergic neurotransmission (CULOTTI *et al.* 1981; HOSONO and KAMIYA 1991; NGUYEN *et al.* 1995). Finally, all animals are strongly constipated and show defects in the defecation cycle, including very long periods and the frequent absence of expulsion contractions, a behavior known to require the normal function of GABAergic neurotransmission (MCINTIRE *et al.* 1993a,b). These observations suggest that some general aspect of synaptic function is affected. As these mutants display a maternal effect,

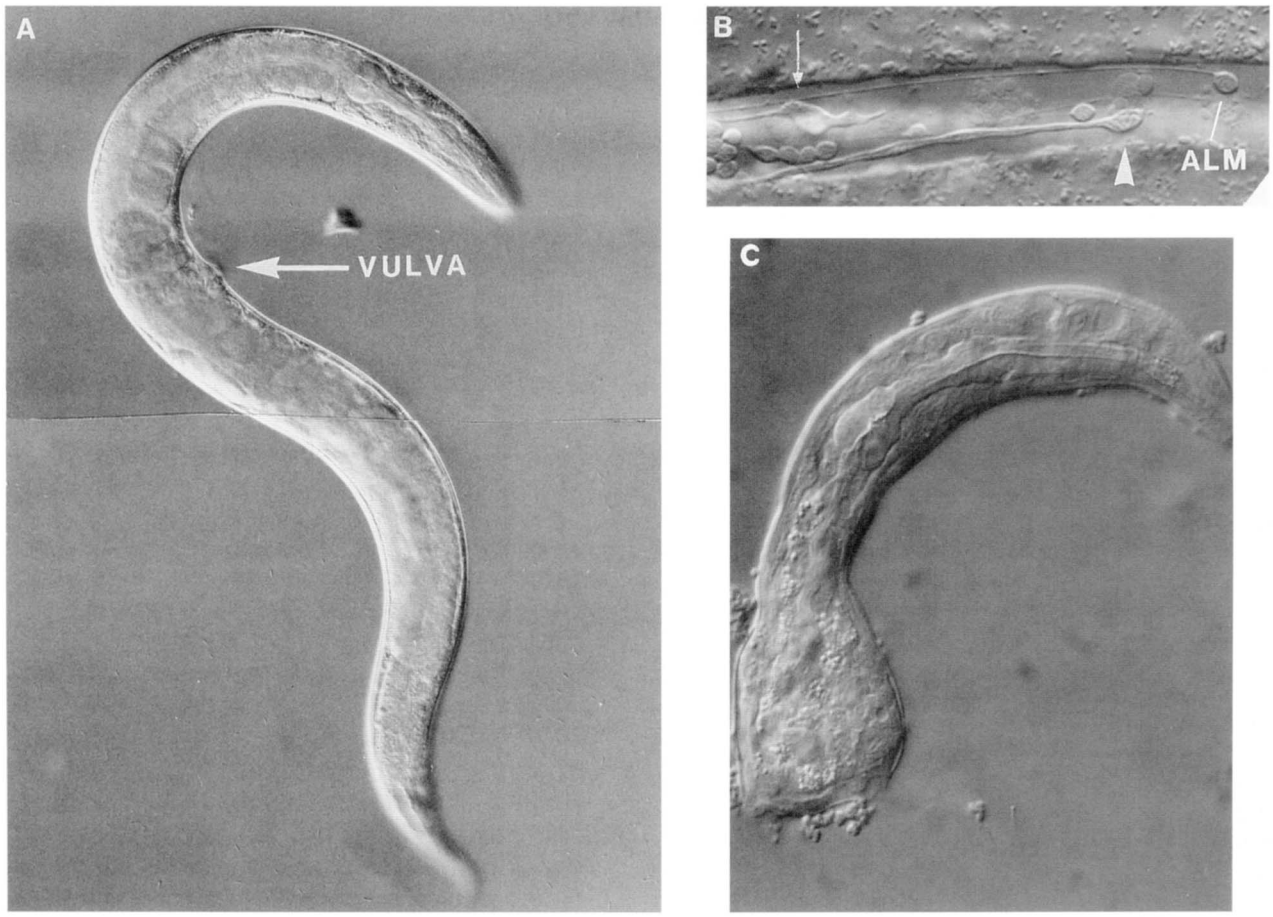

FIGURE 1.—Morphological phenotypes of uncoordinated mutants. *mum-1* and *mau-2* mutants. (A) A *mum-1* mutant with an anteriorly mispositioned vulva. (B) Part of a *mau-2* mutant at the L3 stage (between the posterior of the pharynx and the ALM (anterior lateral microtuble) neuron cell body; anterior to the left). On the left, several nuclei of head neurons can be seen (round structures), as well as a cell with a complex shape (indicated by an arrow) that is the amphid sheath cell. In this animal, the excretory canal stops prematurely in a club-shaped structure indicated by a large arrowhead. In the wild type, the excretory canal extends normally to the anus of the animal, which would be well to the right of this picture. The cell lying on the canal, just left of the point of termination, is probably the BDU neuron. (C) A *mum-1* mutant displaying a normally developed head and an undifferentiated posterior body.

it suggests that their altered synaptic function could result from a defect in synaptogenesis.

Descriptions of mum genes: Each of the three *mum* genes is defined by a single mutation, which results in similar phenotypes, and they are discussed as a single class. Mutants are variably uncoordinated, ranging from classic kinking to total uncoordination. The most severely affected animals are unable to propagate contraction waves along their body; instead different regions of the body appear to contract independently. All three *mum* mutations result in a high level of lethality (see Table 1). In all three mutants, severe defects in embryogenesis can be observed. In almost all cases, the dying embryos are severely deformed, but with no simple or obvious pattern to the abnormalities. However, in some of the dying embryos, the head developed normally, whereas the rest of the body remains a relatively undifferentiated ball of cells (Figure 1C). This phenotype resembles that of known mutants [(*e.g., nob-1*) (K. L. VAN AUKEN, L. G. EDGAR and W. B. WOOD, personal communication)].

Animals that survive to adulthood display a number of distinct phenotypes. A variably deformed head is frequently observed, ranging from a relatively rounded rather than a pointed head, to hypertrophic dorsal and ventral bulges. The pharynx is often highly abnormal, ranging from abnormal relative sizes of its parts to apparent total absence of parts (*e.g.,* no anterior bulb or no isthmus). Deformations of other parts of the body were also observed, albeit more rarely.

The most reproducible and interpretable abnormalities we observed in these mutants are those affecting the reproductive and excretory systems. In the wild type, the posterior gonad arm is on the left of the gut and the anterior arm is on the right. In all three mutants, one gonad arm is frequently on the wrong side of the gut, resulting in animals that have both gonad arms on the same side of the gut. More rarely, the gonad arms are fully inverted relative to wild type (Figure 2). These defects are highly penetrant; in *mum-3,* for example, 80% of worms that reach adulthood have their posterior gonad on the right rather than on the

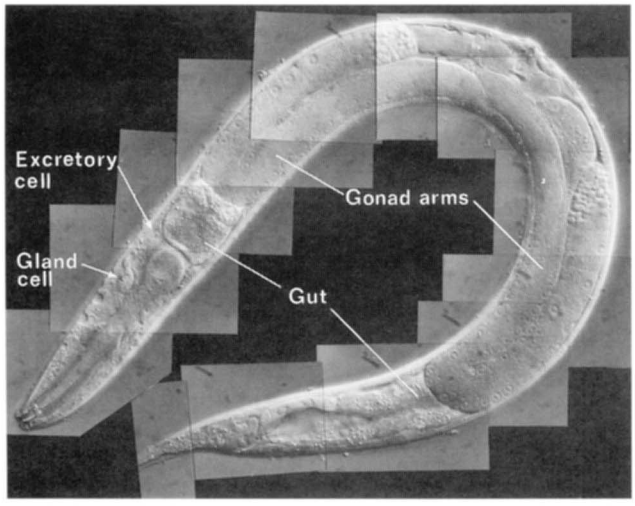

FIGURE 2.—A *mum-2* mutant showing two of the phenotypes frequently found in *mum* mutants: the posterior gonad is positioned on the right of the gut, resulting in an animal with both gonad arms on the same side, and the right excretory gland cell is also positioned anteriorly and the excretory cell is positioned too posteriorly. Although the mispositioning of these two cell is independent, in this particular animal, these two cells have almost exactly inverted their respective positions.

left. Abnormally positioned and shaped gonad primordia can be observed in L1 and later larval stages. Moreover, we frequently observe an abnormal placement of the vulva (either posterior or anterior of its normal position) (Figure 1A). Together, these observations suggest that in these mutants, the cells forming the gonad primordium migrate abnormally.

We also observed highly stereotypical anomalies in the positions of the cells of the excretory system, in particular, the excretory cell and the excretory glands (Figure 2). In the wild type, the excretory cell is on the left, ventral and anterior to the terminal bulb of the pharynx, whereas the excretory glands, a bilaterally symmetric pair of cells, are located ventrally and posterior to the terminal bulb and send thick processes anteriorly where they meet and fuse. In the mutants, individual excretory glands are frequently located more anteriorly, midway between the pharyngeal bulbs, and send a process posteriorly to meet the process of the contralateral gland cell. In all three mutants, the right gland cell is affected more frequently than the left. For example, in *mum-1,* 60% of the right and 15% of the left gland cells are positioned anteriorly. In addition, the excretory cell is also frequently positioned too posteriorly and/or on the right side (Figure 2). For example, in *mum-3,* 35% of the excretory cells is in the abnormal posterior position and 69% is on the right.

The canals produced by the excretory cells are almost invariably abnormal in these animals. We observe very frequently one or both posterior canals in the ventral cord or both posterior canals on the same side of the animal. In rare cases, we observed more than two posterior canals, suggesting that the normally anteriorly directed canals are directed posteriorly in these animals.

Taken together, our observations suggest that in these mutants some fundamental aspect of cell movement is impaired, resulting in misplacement of cells throughout development, including those cells that do not undergo long distance migrations.

Descriptions of mal genes: mal-1 (one allele): This mutation produces extensive embryonic and larval lethality. About half of the surviving mutants show one or more of a few stereotypical defects. These include a dorsal anteriorly directed protrusion on the head and a posteriorly directed dorsal protrusion over the anus (Figure 3). The same defects, but much more severe, can frequently be observed in dying early-stage mutant larvae and embryos. The defects in these mutants do not resemble those of any previously known mutant.

mal-2 (three alleles): The three alleles of this gene are very similar and produce most frequently a severe ventral hypertrophy located under the pharyngeal isthmus and posterior bulb (Figure 4A). Other less-circumscribed defects are also observed, presenting a general and not obviously stereotyped deformation of the body. These defects often lead to early lethality and can also be observed in dying embryos. The defects seen in *mal-2* mutants are reminiscent of those observed in *vab-3* mutants (LEWIS and HODGKIN 1977).

mal-3 (one allele): The main defect in this mutant appears to be that most of the time the buccal cavity does not open at the tip of the head. Instead, it appears to open at random positions more posteriorly, in the head, including on the dorsal, ventral or lateral sides (Figure 4C). Embryonic and larval lethality is high in this mutant because many dying embryos and larvae appear to have the pharynx and the buccal cavity detached from the hypodermis. The few animals that survive to adulthood generally show only a relatively mild phenotype (Table 1).

mal-4 (one allele): In this mutant, there is a conspicuous deformation of the head resulting from a severe hypertrophy of the left side of the head only. The hypertrophy is variable in size and anteroposterior extent but mostly is located in the anterior part of the head (Figure 4B). The lineages that form analogous bilaterally symmetrical pairs of hypodermal cells in the head are not homologous by lineage (SULSTON *et al.* 1983). Our view, therefore, is that the defect in *mal-4* mutants is in the specification of only the lineages on the left side.

Descriptions of mad and mud genes: The phenotype of *mad* and *mud* mutants have not been extensively studied to date, and the description we can give does not go beyond the descriptive summary given in Table 1. In all cases, males and hermaphrodites are affected, suggesting that these genes are not involved in dosage compensation in any simple way. *mad-2, mad-3* and *mud-1* mutants display significant embryonic lethality. Dying

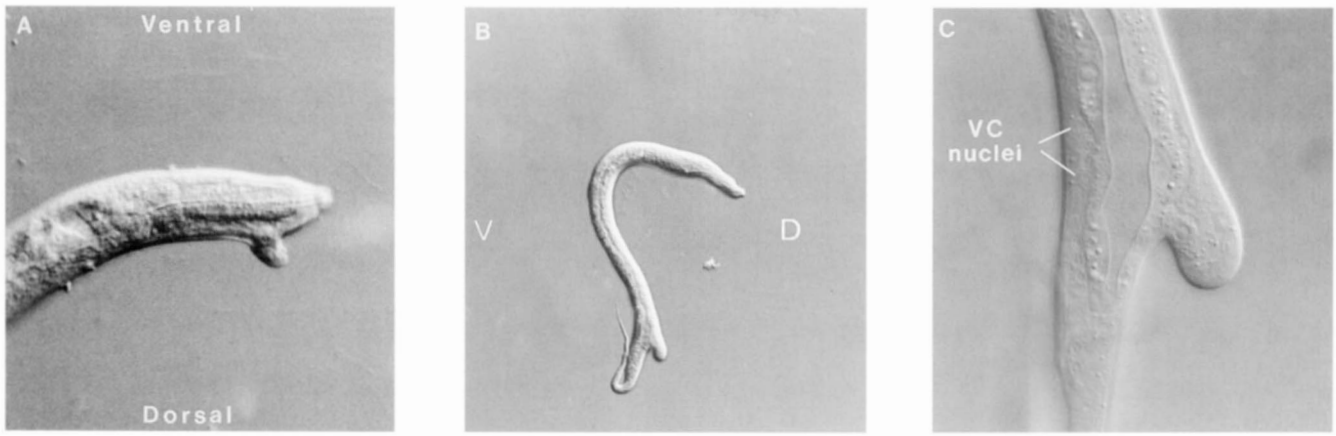

FIGURE 3.—*mal-1* mutants. (A) Head of an animal showing a typical dorsal protrusion. (B) Another animal showing a typical dorsal protrusion in the posterior part of the body. (C) An enlarged view of the protrusion of the animal shown in B, showing the lumen of the gut and the location of ventral nerve cord nuclei. The animals shown are L4 larvae

embryos are frequently poorly elongated but appear fully differentiated and are not highly deformed.

Descriptions of clk genes: clk-1 (five alleles): Three of the alleles of this gene have already been described in detail in a previous paper (WONG *et al.* 1995). In brief, *clk-1* mutations alter the timing of several developmental and behavioral events, including the embryonic cell cycle, the total duration of embryogenesis, the duration of postembryonic development, life span, as well as the length of the defecation, pumping and swimming periods. The two alleles not previously described (*qm47* and *qm51*) display the same pattern of phenotypes as the three alleles previously described (A. WONG and S. HEIKIMI, unpublished data).

The phenotypes of *clk-2* and *clk-3* mutants are mostly similar to those of *clk-1* and are described in detail in a future publication (B. LAKOWSI and S. HEIKIMI, in preparation). *clk-2* is somewhat different from *clk-1* and *clk-3* in that it is defined by a single allele that is temperature sensitive; embryos derived from oocytes produced at 25° fail to hatch and a number of mutant embryos and larvae arrest when transferred to 25°. Some larvae, however, succeed in resuming development when transferred back to 20 or 15°.

Maternal and zygotic rescue: We analyzed the penetrance, expressivity and heritability of the phenotypic effects of the reference allele of each of the novel genes identified in this screen except for the *clk* genes (Table 1). *clk* genes show quantitative alterations of essentially normal development and behavior; a statistically valid quantification of parental effects would be labor intensive (WONG *et al.* 1995) and is beyond the scope of the present work.

Given the nature of our screen, it is not surprising that all mutants display a profound maternal rescue. However, some mutants show partial failure of maternal rescue for all or part of their phenotype. For most mutants in which some failure of maternal rescue has been observed, failure is apparent only in a small percentage of the animals. In Table 1, the fraction given in brackets besides the statement on maternal rescue corresponds to the fraction of the self-progeny from a self-fertilizing heterozygous parent that displayed a mutant phenotype. Complete failure of maternal rescue would therefore correspond to 25% of mutants. The genes for which the reference allele shows partial failure of maternal rescue are the following: *mau-2* (19%), *mud-1* (6%), *mum-2* (2%), *mad-1* (2%), *mum-1* (1%), *mau-4* (0.3%), and *mau-6* (0.3%). In the case of *mau-2*, it is only the Egl phenotype that is mostly not rescued; the Unc phenotype is almost always fully rescued (only 1% of the animals are Unc in the F_2 progeny). Similarly, in the case of *mud-1*, it is only the Dpy phenotype that shows failure of rescue; the Unc phenotype is always rescued. In the cases of *mum-1* and *mum-2*, the meaning of the failure is unclear, as the abnormal phenotype observed in F_2 animals was confined to a transient uncoordination during the L3 and L4 stages.

In our analysis of zygotic rescue (the Table 1), the fraction given in brackets besides the statement on zygotic rescue corresponds to the fraction of mutant cross-progeny observed from a cross of wild-type males into mutant hermaphrodites marked with a fully zygotic recessive mutation. The fraction therefore gives directly the proportion of animals for which the zygotic contribution is not sufficient to give a wild-type phenotype. We observed some failure of zygotic rescue in only three genes: *mum-1*, *mum-2* and *mal-2*. In the case of *mum-1* and *mum-2*, the zygotic genome is sometimes sufficient and sometimes not; this suggests that the genes are required around the time at which the zygotic gene is first being expressed. In the case of *mal-2*, there appears to be no zygotic rescue for the morphological phenotype we observe in survivors (namely, head deformation). For this function, therefore, the gene appears to be required very early, before it is efficiently expressed by the zygotic genome. However, as m^-z^+ *mal-2* worms appear much more healthy than m^-z^- worms, it does

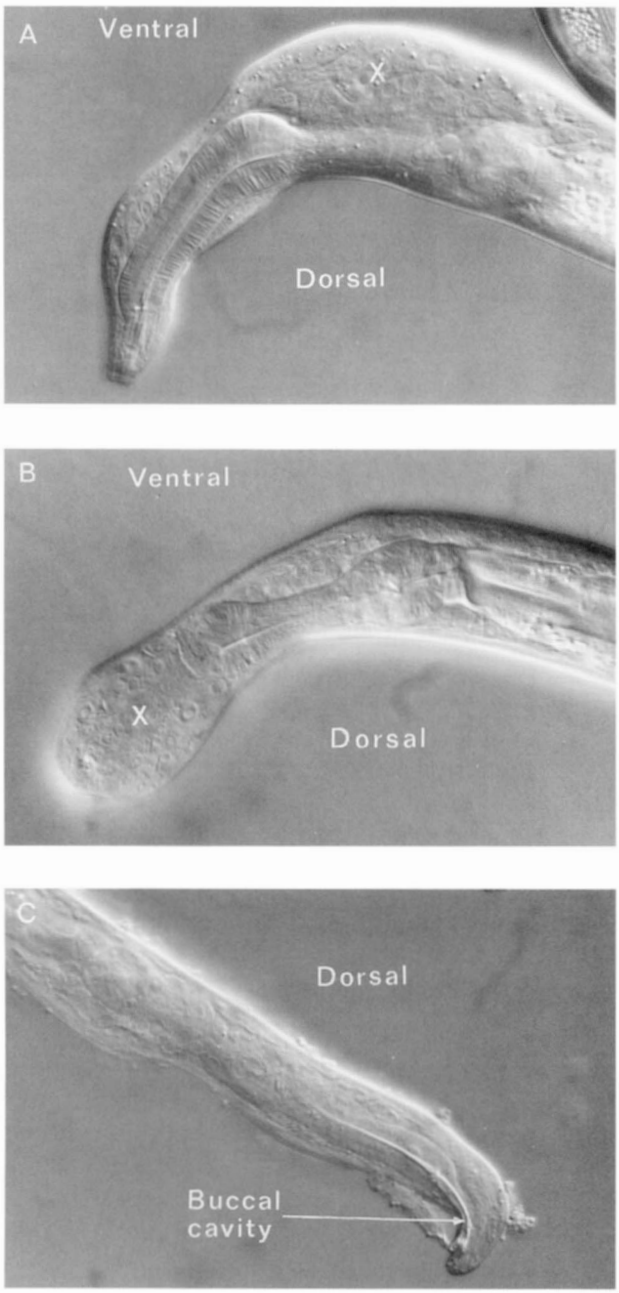

FIGURE 4.—*mal-2*, *mal-3* and *mal-4* mutants. (A) A *mal-2* animal showing a typical ventral hypertrophy (marked by an X). In this animal the pharynx is displaced dorsally by the ventral tissue. (B) A *mal-4* mutant showing typical hypertrophy (marked by an X) on the left side of the head. This animal lies on its left side, and the plane of focus of the micrograph is in the middle of the animal. The pharynx can therefore be seen up to the anterior bulb but then is raised above the plane of focus by the hypertrophy. (C) A *mal-3* animal in which the buccal cavity opens sidewise and ventrally. The animals shown are L4 larvae.

suggest that the requirement for the gene in later development can be partially met by the zygotic genome.

Genetic mapping: We genetically mapped all of the genes; their map position is shown in Figure 5, and the actual corresponding mapping data are given in Table 2.

DISCUSSION

We identified a number of mutations whose phenotypes and mode of transmission suggest that they are involved in the development of the worm. Very few viable maternal-effect mutations had been identified before our screen (see Introduction), probably because only a screen specifically designed to find them would allow their systematic identification.

It is possible that some of the mutations we isolated are not null mutations and that null mutations in these genes would lead to lethality or abolish maternal rescue. However, whatever the effects of null mutations, the phenotypes we identified point to at least some of the processes for which these genes are required. Indeed, the identification and study of hypomorphic mutations to study the processes they affect has been a very successful approach in *C. elegans* (*e.g.*, HILL and STERNBERG 1993). As several of the phenotypic gene classes we identified in this screen show novel phenotypes, we expect they will be useful in the study of the genetic basis of development.

Is there a class of genes defined only by maternal-effect viable mutations? None of our genes map to the vicinity of other viable genes with a similar phenotype. It is likely therefore that most of them have not previously been defined by only strictly zygotic viable mutations. In particular, the *unc* gene class is believed to be close to saturation, reinforcing this argument for the *mau* and *mum* genes (which exhibit uncoordination). Furthermore, for several of our genes, we recovered mutations at the same rate as for *sdc-1* (three alleles) and *tra-3* (two alleles). As all known alleles of these two genes show a maternal effect and are not lethal (albeit *tra-3* mutants are sterile), they were probably recovered near the normal loss-of-function frequency. This suggests that for all genes for which we have obtained at least two alleles (maximum is five for *clk-1*), we might have obtained these alleles at the normal loss-of-function frequency. This would imply that at least a proportion of our genes can be defined only by viable maternal-effect mutations.

However, the possibility remains that many of our mutations correspond to rare hypomorphic alleles of genes whose total loss of function would lead to lethality. Two observations suggest that this could be the case. First, the frequency at which we recovered mutations in our genes is ~10-fold lower than that reported for other screens, which may suggest that only rare events can produce the type of mutations we were isolating (alternative explanations for this observation are discussed below). Second, many of our mutants, in particular those in genes defined by single alleles, show a high incidence of lethality (see Table 1), suggesting that stronger alleles could be fully lethal. Furthermore, the phenotype of both *mum-1* and *-2* over a deficiency is fully lethal, suggesting that null alleles of these two

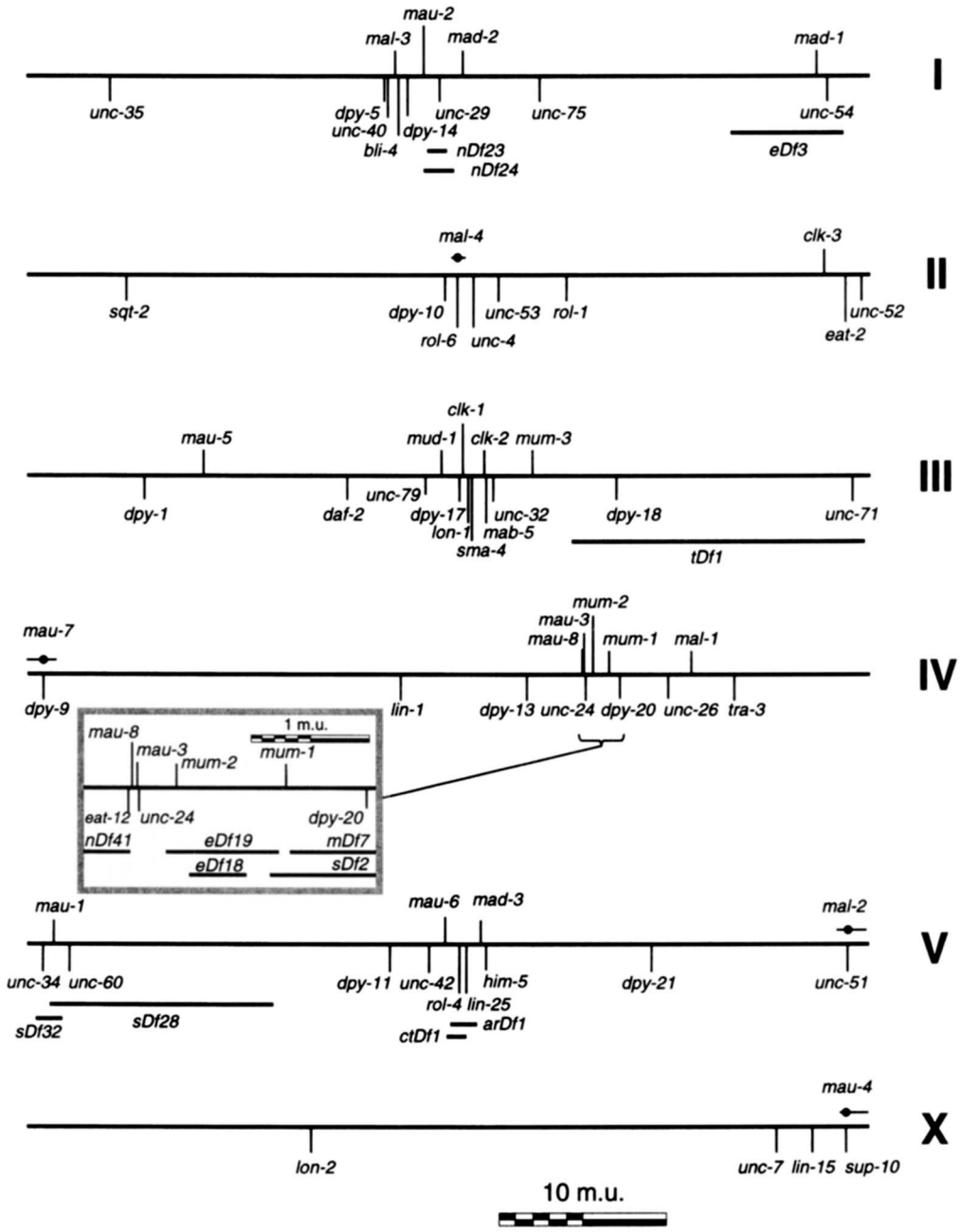

FIGURE 5.—Genetic map positions of the new genes described in this study. This diagram is a summary; for the ultimate mapping data consult Table 2. The full data are available from the Caenorhabditis Genetics Center. Each horizontal line represents an entire linkage group (scale bar is given). Genes defined in this study are drawn above the line, positioned between the reference markers against which they were mapped (drawn below the line). Deficiencies are shown only where complementation data formally narrows the interval for a gene. The inset for linkage group IV shows details not present on the full-scale map. Where a gene was not separated from a marker, its position is marked by a dot above that marker with a bar representing its likely position.

genes would be lethal as well. This impression should, however, be tempered by the observation that we have obtained *mau-1, mau-2* and *mal-2* alleles at relatively high frequency (two to three alleles), and all alleles of these genes also produce very high lethality. Furthermore, the phenotypes of *mau-1, mau-2,* and *mad-1* over deficiencies are not worse than that of mutant homozygotes.

Mutation frequency: We isolated alleles of only two previously known genes with viable alleles: *sdc-1* and *tra-3.* For both of these genes, all previously known alleles exhibit maternal rescue (HODGKIN 1985; VILLENEUVE and MEYER 1987, 1990). The frequency at which we recovered mutations in these genes therefore provides a baseline to evaluate the significance of the frequencies we observe with other genes. From 30,000 mutagenized genomes screened, we obtained three *sdc-1* alleles and two *tra-3* alleles. This frequency appears to hold for all

TABLE 2

Summary of genetic mapping for 22 genes

Gene	Genetic mapping data[a]
mau-1 V	Inside *sDf28, sDf32*
mau-2 I	[*dpy-14 unc-29/mau-2*] *dpy-14* (22/36) *mau-2* (14/36) *unc-29* inside *nDf24;* outside *nDf23* complements *che-3*
mau-3 IV	[*bli-6 eat-12 unc-24/mau-3*] *bli-6* (16/31) *eat-12* (13/31) *mau-3* (2/31) *unc-24* outside *nDf41*
mau-4 X	[*lin-15 sup-10/mau-4*] *lin-15* (5/5) *mau-4* (0/5) *sup-10*
mau-5 III	[*dpy-1 daf-2 unc-32/mau-5*] *dpy-1* (3/10) *mau-5* (7/10) *daf-2*
mau-6 V	1) [*unc-42 dpy-21/mau-6*] *unc-42* (3/9) *mau-6* (6/9) *dpy-21* 2) [*rol-4 lin-25 him-5 unc-76/mau-6*] *rol-4* (0/7) *mau-6* (1/7) *lin-25* (2/7) *him-5* (4/7) *unc-76* outside *ctDf1, arDf1*
mau-7 IV	[*dpy-9 lin-1/mau-7*] *dpy-9* (0/11) *mau-7* (11/11) *lin-1*
mau-8 IV	[*dpy-13 unc-24/mau-8*] *dpy-13* (30/31) *mau-8* (1/31) *unc-24* outside *nDf41*
mum-1 IV	1) [*dpy-13 unc-31/mum-1*] *dpy-13* (36/40) *mum-1* (4/40) *unc-31* 2) [*dpy-20 unc-31/mum-1*] *dpy-20* (0/11) *mum-1* (11/11) *unc-31* inside *mDf7;* outside *eDf19, sDf2* complements *let-657, unc-43*
mum-2 IV	[*unc-24 dpy-20/mum-2*] *unc-24* (4/13) *mum-2* (9/13) *dpy-20* inside *eDf19;* outside *eDf18*
mum-3 III	[*unc-32 dpy-18/mum-3*] *unc-32* (8/24) *mum-3* (16/24) *dpy-18* outside *tDf1*
mal-1 IV	[*unc-26 tra-3 dpy-4/mal-1*] *unc-26* (6/17) *mal-1* (10/17) *tra-3* (1/17) *dpy-4*
mal-2 V	[*dpy-11 mal-2/unc-51*] *dpy-11* (35/35) *unc-51* (0/35) *mal-2* [*dpy-11 qm31/++*][b] 58/100 *dpy-11 mal-2* :24 cM
mal-3 I	[*unc-40 bli-4/mal-3*] *unc-40* (5/6) *mal-3* (1/6) *bli-4*
mal-4 II	1) [*dpy-10 unc-53/mal-4*] *dpy-10* (6/16) *mal-4* (10/16) *unc-53* 2) [*rol-6 unc-4/mal-4*] *rol-6* (0/23) *mal-4* (23/23) *unc-4*
mad-1 I	[*dpy-24 unc-54/mad-1*] *dpy-24* (52/53) *mad-1* (1/53) *unc-54* inside *eDf3*
mad-2 I	[*dpy-5 daf-16 unc-75/mad-2*] *dpy-5* (7/12) *mad-2* (5/12) *unc-75* [*unc-29 mad-2/++*][b] 111/118 *unc-29 mad-2* :3.0 cM
mad-3 V	[*rol-4 lin-25 him-5 unc-76/mad-3*] *rol-4* (0/8) *lin-25* (3/8) *mad-3* (1/8) *him-5* (4/8) *unc-76* outside *arDf1*
mud-1 III	[*unc-79 lon-1/mud-1*] *unc-79* (4/10) *mud-1* (6/10) *lon-1*
clk-1 III	[*dpy-17 clk-1 lon-1/gro-1*] *dpy-17* (7/18) *gro-1* (0/18) *clk-1* (11/18) *lon-1* complements *mel-31, mel* (*jb7*)
clk-2 III	[*sma-4 mab-5 unc-36/clk-2*] *sma-4* (35/52) *clk-2* (3/52) *mab-5* (14/52) *unc-36* complements *emb-1, emb-2, emb-7, emb-16, emb-24, emb-25, emb-32, emb-34, ncl-1, lin-13, lin-39*
clk-3 II	[*rol-1 clk-3 unc-52/eat-2*] *clk-3* (3/4) *eat-2* (1/4) *unc-52* [*clk-3 unc-52/++*][b] 430/439 *clk-3 unc-52* : 1.0 cM

[a] Only the data critically determining the position of the gene on the genetic map, as shown in Figure 5, is given; the results of other mapping experiments can be obtained from the CGC (Caenorhabditis Genetic Center) gopher. The genotypes given in square brackets are the relevant genotype of the animals whose descendants were scored to obtain multifactor or two factor data.

[b] Two factor data; the numerator of the fraction represents the number of self-progeny from animals of the given genotype which were homozygous for the doubly mutant chromosome. Only animals homozygous for the marker mutation were scored (the denominator).

the genes in our screen, as only *clk-1* has yielded more alleles with five mutations isolated (Table 1). The standard EMS mutagenesis protocol (SULSTON and HODGKIN 1988) is known to have an approximate 5×10^{-4} probability of inactivating an average gene (BRENNER 1974; GREENWALD and HORVITZ 1980). The average frequency of isolation in our screen is ~10-fold lower (6×10^{-5}). We believe it is unlikely that this low mutation frequency is due to maternal-effect genes being on average smaller targets for mutagenesis or that our mutagenesis conditions, although standard, might have been subtly different and produced a lower level of mutagenesis. Instead, we suggest two alternative explanations. First, the criteria of our screening method might be responsible. We chose F_2 animals that looked as fully wild type as possible to avoid picking animals carrying phenotypically weak but nonetheless fully zygotic mutations. When visible recessive mutants are selected directly from a plate of mutagenized F_2 animals, such discrimination is not necessary. We might thus have been selecting indirectly for genomes that, for some reason, were not strongly mutagenized. The viable ma-

ternal-effect loci would then also be hit more rarely in such genomes. Second, our screen allowed us to recover only relatively severe mutations because of the type of heritability of the genes in which we were interested. For most of our genes, a maternal or zygotic contribution from a wild-type allele is sufficient for a fully wild-type phenotype (Table 1). It is possible, therefore, that when such a gene is only partially inactivated by mutation, the double (maternal and zygotic) contribution is sufficient to produce a wild-type phenotype.

We thank LEON AVERY, who suggested to S.H. that he should stop claiming *mau* genes exist and just carry out the screen instead. We thank JULIA PAK, SHIN TAKAGI, DENISE AU-LEUNG and CLAIRE BÉNARD, for some mapping data, as well as TOM BARNES for advice on mapping and for Figure 5. We thank TOM BARNES and JONATHAN EWBANK for critically reading the manuscript. This work was supported principally by the Canadian National Science and Engineering Research Council (NSERC Operating grant 0121351), the Medical Research Council of Canada (MRC Operating grant MT 12085) and a J. W. McConnell Foundation fellowship to B.L. The earliest phase of this work was supported by the Medical Research Council's Laboratory of Molecular Biology in Cambridge and the Swiss National Science Fund. Some nematode strains used in this work were provided by the Caenorhabditis Genetic Center, which is funded by the National Institutes of Health (NIH) National Center for Research Resources (NCRR). Some other strains used in this work were provided by the *C. elegans* Genetic Toolkit Project, funded by the NIH NCRR. We also thank DON MOERMAN, VICTOR AMBROS, ANDREW CHISOLM and LEON AVERY for strains.

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