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Genetics

A selection for myosin heavy chain mutants in the nematode *Caenorhabditis elegans*

(*unc-54*/deletion mutations/1,2,7,8-diepoxyoctane/free duplications)

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ABSTRACT **The *unc-54* gene of *Caenorhabditis elegans* encodes an abundant myosin heavy chain protein expressed in body-wall muscle cells. We have designed genetic techniques that select directly for *unc-54* mutants. This selection is based upon properties of the *unc-54* dominant allele *e1152*. Mutations that eliminate dominance of *e1152* are null alleles of *unc-54*. Deletions have been identified by their genetic properties. We have defined mutationally a number of essential genes near *unc-54*, and we have described the genetic fine structure of this region of linkage group I. As much as 27% of the *unc-54* mutations induced by the bifunctional alkylating agent 1,2,7,8-diepoxyoctane are multisite deletions. Extrachromosomal free duplications that include *unc-54* are also described.**

The synthesis and assembly of muscle in the small soil nematode *Caenorhabditis elegans* is especially suited to both genetic and biochemical analysis. Muscle proteins constitute a reasonable fraction of the animals' mass, and many mutants defective for motility (1) are biochemically and/or ultrastructurally abnormal in muscle cells (for review see ref. 2). *C. elegans* contains several electrophoretically distinguishable forms of myosin heavy chain (3, 4). The most abundant of these isozymes is encoded by the *unc-54* gene. This protein, termed the "B" form of myosin heavy chain, is synthesized in body-wall but not pharyngeal muscle cells. *unc-54* mutants are paralyzed, and the 95 body-wall muscle cells of the adult lack most (but not all) of their thick myofilaments. Body-wall cells express another heavy chain isozyme, type "A," in addition to *unc-54*. Myosin heavy chain A is the product of as yet unidentified gene(s).

Because body-wall muscle is an abundant tissue, detailed analysis is possible for the *unc-54* protein (4, 5), messenger RNA (6), and gene (6, 7). Indeed, much of our understanding of the *unc-54* gene and its myosin heavy chain product derives from the judicious use of *unc-54* mutations as source material for these analyses. Our ability to dissect the *unc-54* gene may be limited only by the supply of interesting mutations available. As part of a combined genetic and biochemical approach to the *unc-54* gene, we have devised a selective technique that allows the isolation of large numbers of independent *unc-54* mutants (up to 50 per day). This selection is based on the genetic principles elaborated by Muller (8). Recessive mutations ("amorphs" as described by Muller) revert the mutant phenotypes caused by "antimorphic" dominant mutations. We have applied this approach to the *unc-54* gene of *C. elegans*. Mutations that delete *unc-54* plus nearby essential genes have been identified by their genetic properties. These deletions allow us to describe the genetic fine structure of the region surrounding *unc-54*.

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MATERIALS AND METHODS

General Procedures. The conditions for growth, maintenance, and ethyl methanesulfonate (EMS) mutagenesis of *C. elegans* have been described (1). All strains were cultured at 20°C. For mutagenesis with 1,2,7,8-diepoxyoctane (DEO; purchased from Aldrich), nematodes were suspended for 3 hr in M9 buffer (1) containing 2 mM DEO. DEO must be kept anhydrous and solutions made freshly. The uniform genetic nomenclature for *C. elegans* (9) has been used throughout.

Construction of the Balancing Chromosome Let(*r202*). The chromosome Let(*r202*) contains an x-ray-induced recessive-lethal mutation (*r202*) designed to balance the *unc-54* region of linkage group I. The strain with this chromosome was isolated as follows: The triple heterozygote *dpy-5(e61) unc-13(e51) +/+ + unc-54(e1152)* was constructed. This strain is paralyzed due to the dominance of *unc-54(e1152)*. Wild-type recombinants [*+ +/dpy-5(e61) unc-13(e51)*] are easily scored; they compose 15% (74/478) of this strain's progeny. Young adult hermaphrodites of genotype *dpy-5(e61) unc-13(e51) +/+ + unc-54(e1152)* were x-irradiated with 7000 rads (dose rate = 530 rads/min; 1 rad = 0.01 gray). After treatment, 2397 F_1 animals having the characteristic motility phenotype of *e1152/+* heterozygotes were picked individually and transferred to Petri dishes. Fertile strains that did not produce wild-type offspring were retained. Strain CB2792 is one such isolate. It proved to be of genotype *unc-54(e1152) +/+* Let(*r202*). The balancer chromosome Let(*r202*) is lethal when homozygous. Surprisingly, this chromosome does not contain *dpy-5(e61)* or *unc-13(e51)*. Let(*r202*), therefore, arose in a recombinant chromosome. Although the chromosome containing Let(*r202*) was constructed in a manner designed to suppress crossing-over on the right arm of linkage group I, genetic tests indicate that Let(*r202*) does not act as a cross-over suppressor in the *unc-59–unc-54* interval (data not shown). The balancing properties of Let(*r202*) are restricted to the *unc-54* region. Let(*r202*) has not been extensively characterized. Its ability to balance the *unc-54* region may be due to the close proximity of *r202* to *unc-54* (see below).

Defining Essential Genes Near *unc-54*. *e1092* is a recessive allele of *unc-54*. Young adult hermaphrodites of genotype *unc-54(e1092) +/+* Let(*r202*) were mutagenized with EMS. Wild-type F_1 progeny were picked individually and transferred to Petri dishes. The offspring of these F_1 clones were scored for the presence of *e1092* paralyzed homozygotes. Those segregating no or few paralyzed progeny were retained. Complementation tests confirmed these strains to be heterozygous for *unc-54(e1092)*. The new genes defined in

Abbreviations: EMS, ethyl methanesulfonate; DEO, 1,2,7,8-diepoxyoctane.

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this study are *let-201(e1716)*, *let-202(e1720)*, *let-203(e1717)*, *let-204(e1719)*, *let-205(e1722)*, *let-206(e1721)*, *let-207(e1723)*, and *let-208(e1718)*.

Independence of *unc-54* Mutations. After mutagenesis, populations of *unc-54(e1152)* +/+ Let(*r202*) were immediately distributed to Petri dishes. No more than one *unc-54* mutant was ultimately retained from each dish. This ensures the mutational independence of each isolate.

Complementation Tests. (*i*) *let-201–let-208*. Males of genotype *Df(unc-54)* +/+ Let(*r202*) were mated with hermaphrodite strains heterozygous for a recessive-lethal mutation coupled to *unc-54(e1092)* [e.g., *let-201(e1716) unc-54(e1092)* +/++ Let(*r202*)]. The offspring from these matings were scored for the presence and frequency of paralyzed (Unc-54) males. Complementation between a deletion and recessive-lethal mutation is indicated when such animals make up ⅓ of the viable outcrossed progeny.

(*ii*) *let-49*. Males of genotype *lev-11(x12) let-49(st44)*/+ + were crossed with deletion heterozygotes [*Df(unc-54)* +/+ Let(*r202*)]. The F_1 progeny issuing from these crosses were scored for the presence of approximately ¼ lethal zygotes having the phenotype of *let-49*. Animals homozygous for the recessive lethal mutation *let-49(st44)* have a conspicuous terminal phenotype that occurs during third stage larval growth. The presence of nearly 50% male progeny indicated a successful mating. In no case is the *Df/Df* or Let(*r202*)/Let(*r202*) terminal phenotype similar to the *let-49* terminal phenotype; the results of these tests were unambiguous.

(*iii*) *let-50*. Males of genotype *unc-54(e1300) let-50(st33)*/+ + were crossed with deletion heterozygotes [*Df(unc-54)* +/+ Let(*r202*)]. The presence of approximately ¼ paralyzed (Unc-54) male progeny indicated that the deletion in question complements *let-50(st33)*.

(*iv*) *lev-11*. *lev-11* mutants are resistant to the cholinergic agonists levamisole and tetramisole (10). They also display a characteristic twitching phenotype that is exacerbated by the presence of the drug. Males of genotype *Df(unc-54)* +/+ Let(*r202*) were mated with *lev-11(x12)* hermaphrodites. Male cross-progeny were transferred to plates containing 1 mM tetramisole and scored for the characteristic twitching phenotype of *lev-11*.

(*v*) *lev-10*. *lev-10* mutants are resistant to the cholinergic agonists levamisole and tetramisole, but only after a period of recovery after application of the drug (10). Males homozygous for *lev-10(x17)* were mated with deletion heretozygotes [*Df(unc-54)* +/+ Let(*r202*)]. Male cross-progeny were transferred to Petri dishes containing 1 mM tetramisole for 12 hr. Noncomplementation was indicated when ½ of these males were drug resistant; complementation was indicated when all males were drug sensitive. The drug-resistance phenotype of *x17* hemizygotes (*Df/lev-10*) was considerably weaker than that of *x17/x17* homozygotes, but a failure to complement was unambiguous.

(*vi*) *unc-59* and *unc-75*. Males heterozygous for *unc-59(e261)*, *unc-59(e1005)*, and *unc-75(e950)* were mated with deletion heterozygotes [*Df(unc-54)* +/+ Let(*r202*)]. F_1 male progeny were scored for the appropriate Unc phenotype.

RESULTS AND DISCUSSION

Nature of *unc-54* Dominance. Most mutations affecting *unc-54* are recessive; heterozygotes are indistinguishable phenotypically from wild type. Several alleles of *unc-54* are dominant; heterozygotes are paralyzed. MacLeod *et al.* (4) observed a striking correlation between the presence of *unc-54* myosin heavy chains *in vivo* and dominance of a mutation. Generally, recessive alleles of *unc-54* are null alleles (i.e., no product is stable *in vivo*). Strains harboring dominant mutations, however, accumulate normal amounts of a mutant *unc-54* myosin heavy chain that is defective for assembly. The *unc-54* allele *e1152* is one such dominant mutation, and we have used it extensively in this study. Mutants homozygous for *e1152* are severely paralyzed. Their body-wall muscle is disorganized (11), a typical feature of *unc-54* mutants. Animals heterozygous for *e1152* are also paralyzed, although much less severely than homozygotes. (*e1152* is, therefore, properly described as an *incompletely* dominant mutation; we shall refer to it simply as being dominant.) Fig. 1 is polarized-light micrographs of *e1152* homozygotes, *e1152*/+ heterozygotes, and control animals. The obliquely striated pattern of wild-type muscle (Fig. 1*A*) reflects its highly ordered sarcomeric structure. The muscle of *unc-54* mutants is severely disrupted (Fig. 1 *B* and *D*). The mutation *e1092* is a recessive allele of *unc-54*. As a heterozygote (Fig. 1*C*) it is essentially indistinguishable from wild type. The dominant heterozygote *e1152*/+, however, is defective (Fig. 1*E*). The inhibitory interactions between *e1152* mutant myosin and the process of sarcomere assembly in heterozygotes are unknown, but these interactions provide a selection for additional *unc-54* mutations.

Selection for *unc-54* Mutants. We reasoned that elimination of the *e1152* mutant myosin produced in heterozygotes would eliminate dominance of this mutation. Among a population of *e1152*/+ paralyzed heterozygotes, animals inheriting new recessive *unc-54* mutations within the *e1152*-containing gene copy will be phenotypically wild type. Such animals are highly motile and can be directly selected. In order for this selection to work, a balanced-lethal system is required, such that *e1152* heterozygotes can be exclusively or preferentially cultured. *Materials and Methods* describes construction of an x-ray-induced balancer chromosome designed for this purpose. This chromosome, designated Let(*r202*), contains a wild-type copy of *unc-54* and a recessive lethal mutation (*r202*). Animals homozygous for Let(*r202*) are inviable, dying in early larval stages. We have not fully characterized this chromosome genetically, but its balancing properties are adequate to maintain large popula-

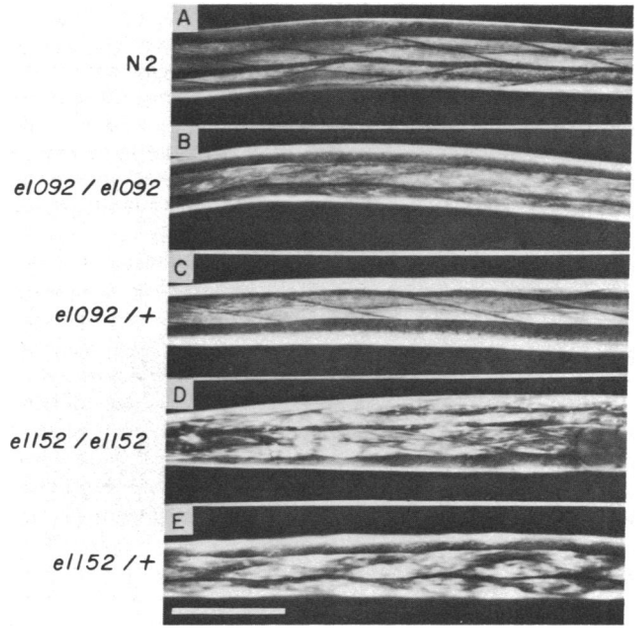

FIG. 1. Micrographs demonstrating *e1152* dominance. Individual animals were anesthetized with 0.5% 1-phenoxy-2-propanol, mounted in M9 buffer for microscopy, and viewed with a Zeiss Universal microscope using polarized light optics. All animals are adult males except *e1152/e1152* (*D*), which is an adult hermaphrodite. The muscle of *e1152*/+ heterozygotes (*E*) is disrupted, whereas that of a recessive heterozygote (*C*) is essentially indistinguishable from that of the wild-type strain N2 (*A*). (Bar represents 100 μm.)

tions of *e1152* heterozygotes.

The heterozygous strain *unc-54(e1152)* +/+ Let(*r202*) is paralyzed due to dominance of *e1152*. Virtually all viable progeny from this strain are paralyzed: ⅓ are *e1152* homozygotes and ⅔ are *e1152* +/+ (*r202*) heterozygotes. (*C. elegans* is a hermaphrodite; unless specified, all progeny discussed here are self-fertilization products.) Although *e1152* homozygotes are viable, they have very small brood sizes (average = 5 per generation). *e1152*/+ heterozygotes have substantially larger broods (average = 38 per generation). Thus, the strain *e1152* +/+ *r202* provides a balanced-lethal system that allows large populations of heterozygotes to be cultured. Wild-type progeny of this strain are observed at the low frequency of 7×10^{-5}. Such animals can be quickly identified among their paralyzed siblings. These wild-type offspring are recombinants; genetic tests indicate that they contain a fully wild-type linkage group I.

After mutagenesis of *unc-54(e1152)* +/+ Let(*r202*) heterozygotes with EMS, wild-type progeny arise at a frequency of 8×10^{-4}. Approximately 30% of these wild-type animals are heterozygous for newly induced *unc-54* mutations, and they segregate recessive homozygotes among their progeny. The remainder of F_1 wild-type animals (70%) are recombinants; they contain a fully wild-type linkage group I. (Deletion mutations will be considered below.) Using this technique, we have collected 30 *unc-54* mutations induced with EMS (*e1616* through *e1645*). Our method of isolation ensures that they are of independent origins. All are double mutants and contain the *e1152* allele in addition to a new *unc-54* allele. For 27 of the 30 strains isolated, the double mutant combination is fully recessive. Three mutations (*e1152e1618*, *e1152e1620*, and *e1152e1636*) retain very slight dominance.

Predominantly Null Alleles Are Selected. The *unc-54* mutations selected by this procedure should be null alleles (i.e., having no detectable *unc-54* product *in vivo*). We have examined the myosin isozymes found in 14 of the 30 EMS-induced mutations described above. These data are presented in Fig. 2. Except for *e1152e1618*, each mutant produces no detectable *unc-54* product; the pattern of myosin isozymes is identical to *e1092*, a recessive null allele of *unc-54*. The exceptional mutant *e1152e1618* exhibits a wild-type pattern of myosin isozymes and, as noted above, is one of three mutations that retains very slight dominance.

Deletion Mutations Affecting *unc-54*. In the experiments described above, we would not have recognized *unc-54* mutants that are inviable as homozygotes. For example, mutations that delete *unc-54* plus nearby essential genes will be recessive to wild-type genes and be lethal when homozygous. Animals heterozygous for such deletions will not segregate viable paralyzed progeny. To rigorously test for the presence of deletions, we have tested animals individually by using complementation analysis.

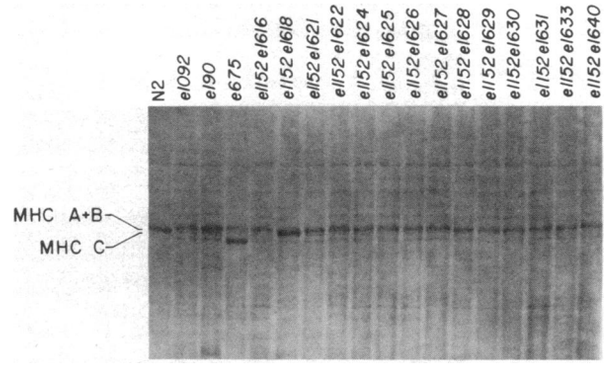

FIG. 2. Myosin heavy chain (MHC) patterns of *unc-54* mutants. Nematode samples from Petri dishes were collected and washed with water, resuspended and boiled in sample buffer, and centrifuged. Supernatants were electrophoresed in $NaDodSO_4$/polyacrylamide gels (7.5%) as described by Gibson (12). Myosin heavy chains A and B are only partially resolved by using this system. The control strains *e1092* and *e190* contain null alleles of *unc-54*. The control strain *e675* contains a small *unc-54* deletion that results in a myosin heavy chain that is of slightly lower molecular weight (13).

In two experiments, we picked 64 wild-type F_1 progeny after EMS mutagenesis of *unc-54(e1152)* +/+ Let(*r202*). Sixteen of these strains segregated recessive *unc-54* homozygotes among their progeny. The remaining 48 strains were crossed individually with males heterozygous for *unc-54(e1092)*. Offspring from these matings were scored for the presence of paralyzed (Unc-54) animals. None were observed. Thus, none of these 48 strains contained *unc-54* deletions extending to a lethal locus. Since 16 *unc-54* alleles giving viable homozygotes were produced in these experiments, we conclude that less than 7% of EMS-induced *unc-54* mutations are deletions resulting in inviable homozygotes.

DEO Induces Large Deletions Frequently. DEO induces a high proportion of deletions in *Neurospora crassa* (14, 15). We have tested the properties of DEO in *C. elegans*. The strain *e1152* +/+ *r202* was mutagenized with DEO (2 mM for 3 hr). Wild-type progeny were present at a frequency of 4×10^{-4}. We picked 657 of these wild-type animals individually and allowed them to self-fertilize. Of these, 368 were either sterile or semi-sterile, yielding too few progeny to score reliably. Another 61 of the fertile strains proved to be heterozygous for recessive *unc-54* mutations giving viable homozygotes. Our method of isolation ensures that each of these mutations (*e1646* through *e1706*) resulted from an independent mutational event. We crossed 127 of the remaining fertile wild-type strains with *unc-54(e1092)*/+ heterozygous males. Thirteen of these matings yielded paralyzed (Unc-54) male offspring. Thirteen of the wild-type strains, therefore, were heterozygous for an allele of *unc-54* that is lethal when homozygous. Genetic analysis described below proves that these *unc-54* alleles are deletions.

We conclude that: (*i*) DEO is a potent mutagen for *C. elegans*; *unc-54* mutations are recovered at a frequency of 5×10^{-5} per gamete after DEO mutagenesis; and (*ii*) at least 18% of total DEO-induced *unc-54* mutations are large deletions. This latter figure is an underestimate for two reasons. First, in the experiments described above, roughly half (127/228) of the fertile F_1 wild-type strains was tested by complementation for the presence of deletions. If the untested F_1 wild-type strains contained deletions in the same proportion as the tested strains, then 27% of total DEO-induced mutations are large deletions. Second, deletions that remove the closely linked gene(s) affected by the balancer chromosome, Let(*r202*), would cause inviability and not be detected (see below).

These results suggested, but did not prove, that a substantial fraction of DEO-induced *unc-54* mutations are deletions. Formal genetic analysis requires that deletion mutations fail to complement or recombine with at least two mutations that are themselves separable by either complementation or recombination. Recessive lethality is an insufficient criterion for genetic deletion. It was necessary for us to more fully analyze the chromosomal region surounding *unc-54*.

Defining Essential Genes Near *unc-54*. *Materials and Methods* describes the isolation of a collection of EMS-induced recessive-lethal (*let*) mutations tightly linked to *unc-54*. These mutations are lethal when homozygous and recessive to wild type. They define a number of essential genes of unknown function near *unc-54*. We isolated each mutation coupled to the recessive *unc-54* allele *e1092*. These double mutant chromosomes are maintained heterozygous to the balancer chromosome Let(*r202*). For example, the genotype of strain CB2779 is *let-201(e1716) unc-54(e1092)* +/+ +

Table 1. Complementation between deletions and mutations near *unc-54*

	unc-75	*unc-59, let-203, let-202, let-201*	*let-204*	*lev-10*	*lev-11, let-205*	*let-207, let-206*	*let-49*	*unc-54*	*let-50*	*let-208*
eDf5	+	+	+	+	+	+	+	−	−	+
eDf11	+	+	+	+	+	+	−	−	+	+
eDf13, eDf14	+	+	+	+	+	+	−	−	−	−
eDf10	+	+	+	+	+	+	+	−	−	−
eDf15	+	+	+	+	+	−	−	−	+	+
eDf4	+	+	+	+	+	−	−	−	−	−
eDf16	+	+	+	+	−	−	−	−	+	+
eDf9	+	+	+	+	−	−	−	−	−	−
eDf7, eDf12	+	+	+	−	−	−	−	−	+	+
eDf6	+	+	−	−	−	−	−	−	−	−
eDf3	+	−	−	−	−	−	−	−	+	+

A + indicates complementation; a − indicates failure to complement.

Let(*r202*). This strain contains a recessive-lethal mutation (*e1716*) that defines the essential gene *let-201*; it is linked to the *unc-54* mutation *e1092* and held stably heterozygous with the balancer chromosome Let(*r202*). This strain is wild type, and only heterozygous progeny survive. Our method of isolating recessive-lethal mutations requires that the gene affected not be the same as the gene(s) affected by Let(*r202*). By screening 3130 mutagenized clones, we collected 29 independent recessive-lethal mutations linked to *unc-54*. Strains with two additional mutations, *let-49*(*st44*) and *let-50*(*st33*), were kindly supplied by R. H. Waterston.

Genetic Fine Structure Surrounding *unc-54*. Our collection of deletions allows us to describe the genetic fine structure of the *unc-54* region on linkage group I. *Materials and Methods* describes experiments that test each deletion for its ability to complement other mutations near *unc-54*. The results of these tests are shown in Table 1 and diagrammed in Fig. 3.

Twenty of the 29 recessive-lethal point mutations that we isolated complement every deletion. These mutations map, therefore, outside the region deleted by even the largest of our deficiencies. They have not been studied further. Complementation data for the remaining 9 recessive lethal mutations (*e1716* through *e1724*) are included in Table 1.

The complementation data presented in Table 1 allow us to deduce a consistent deletion map for these mutations (see Fig. 3). This map represents the genetic fine structure of the chromosomal region surrounding *unc-54* and is derived solely from complementation data obtained in these experiments. These data prove that each of the *unc-54* mutations induced with DEO that give inviable homozygotes is a genetic deletion.

We have tested for complementation among the recessive lethals. With one exception, each of our recessive-lethal point mutations complements all other point mutations mapping within the same deletion interval. Thus, each of these mutations affects a different gene, and our collection of mutations defines only a portion of the genes in this region. The exceptional mutation, *e1724*, fails to complement *lev-11*(*x12*). Assuming that neither mutation is a deletion, then *lev-11* seems likely to be an essential gene for which certain alleles (such as *x12*) are levamisole resistant (see discussion in ref. 10).

Deletions often extend considerable distances leftward of *unc-54* (greater than 8 map units), but most of them have rightward endpoints very near *unc-54*. Seven of our deletions have one endpoint between *unc-54* and *let-208*. The frequency of recombination between *unc-54*(*e1092*) and *let-208*(*e1718*) is estimated to be less than 0.2% (data not shown). We have no point mutations rightward of *let-208* on linkage group I. Thus, we are unable to determine exactly how far *eDf4*, *eDf6*, *eDf9*, *eDf10*, *eDf13*, and *eDf14* extend in this direction.

We feel the most likely explanation for this clustering of deletion endpoints concerns the nature of the balancer chromosome Let(*r202*). This chromosome contains an x-ray-induced mutation, *r202*, that is lethal when homozygous. The

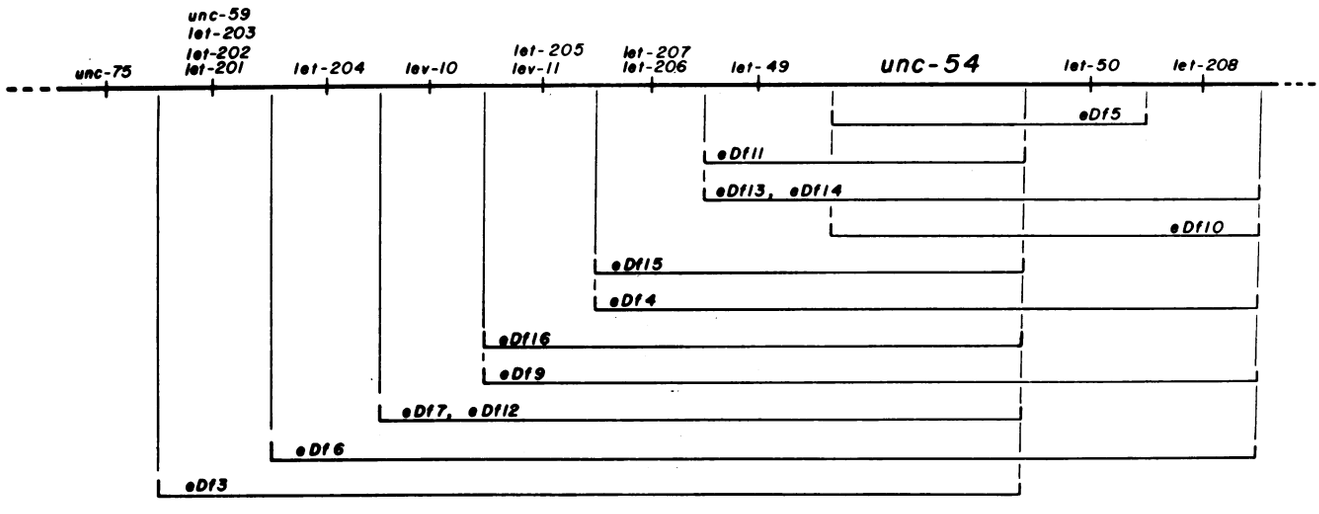

FIG. 3. Genetic fine structure surrounding *unc-54*.

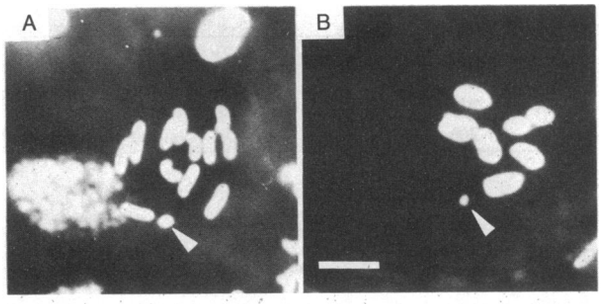

FIG. 4. Free duplications contained in CB2798. Chromosomes have been stained with Hoechst 33258 as described by Albertson *et al.* (16). An embryonic somatic karyotype (*A*) and a germ-line meiotic karyotype (*B*) are shown. In each case a small free duplication (arrowed) is present in excess of the normal $2n = 12$ chromosomes. (Bar represents 1 μm.)

balancing properties of Let(*r202*) may be due to the close proximity of *r202* to *unc-54*. Since deletion mutations are initially isolated heterozygous to Let(*r202*), this would act as a barrier beyond which deletions could not extend; such events would be lethal.

Duplications of *unc-54*. After EMS or DEO mutagenesis of *unc-54*(*e1152*) +/+ Let(*r202*), we consistently observed a class of F_1 progeny whose phenotype was intermediate between paralyzed and wild type. These animals might be classified "Slow" or "Semi-paralyzed." Among the progeny of such Slow animals are *e1152* +/+ *r202* paralyzed heterozygotes, *e1152/e1152* paralyzed homozygotes, and parental Slow phenotypes. For several isolates, segregation of this type has been carried through at least 20 generations. We have not thoroughly studied the genetic properties of these exceptional strains, but many of them exhibit cytological abnormalities that account for their behavior. These strains contain free extrachromosomal duplications. Chromosomal karyotypes of one example are shown in Fig. 4. An embryonic somatic karyotype is shown in Fig. 4*A*. This nucleus contains a chromosomal fragment in addition to the normal $2n = 12$ chromosomes. Such fragments are never observed in wild-type animals. Fig. 4*B* presents a germ-line meiotic karyotype from the same strain. The homologous chromosomes are paired as 6 bivalents, and an additional fragment is present. Independent isolates contain characteristic sizes of free duplications. The free duplication pictured in Fig. 4 is one of the smallest in the strains isolated.

We suggest the following explanation: Each of these free duplication chromosomes is derived from the balancer chromosome and contains a wild-type copy of the *unc-54* gene. Due to their aneuploid nature, these strains contain one copy of *e1152* but two copies of *unc-54*$^+$. We suggest that the inhibitory properties of the *e1152* mutant polypeptide can be reduced by increasing the level of wild-type gene expression. Segregation of the Slow phenotype results from meiotic segregation of the free duplication. The high instability of certain isolates suggests that mitotic segregation in the germ line may also occur.

These free duplications have not been extensively studied, and it is difficult for us to estimate precisely their frequency. After DEO mutagenesis of *e1152* +/+ *r202*, strains classified as Slow arise at a frequency of approximately $0.5–4 \times 10^{-4}$. One-fourth to one-half of these strains contain free duplications. The remainder are genetically stable and do not contain visible free duplications. Their phenotypic similarities to the duplication-containing strains, however, suggest that aneuploidy may also be the basis for their isolation. Free duplications similar to those reported here but involving another region of the nematode genome have been described by Herman *et al.* (17).

Conclusion. The selection for *unc-54* mutations described here is an application of the genetic principles discussed by Muller (8). Null alleles of *unc-54* can be selected by "reversion" of the paralyzed phenotype of dominant heterozygotes. This technique is applicable to many genes for which null mutations are recessive and for which dominant alleles are available. Muscle structural genes of *C. elegans* are well suited to this approach. Selective pressure coupled with the large populations of *C. elegans* make this procedure especially powerful. We find that roughly 25% of total *unc-54* mutations induced with the bifunctional alkylating agent DEO are large deletions, extending to at least one nearby essential gene. Molecular analysis of these deletions confirms this conclusion, and will be presented elsewhere.

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