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 multiallelic 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). Recesssive 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 many 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.

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(e31) / + + 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(e31)] are easily scored; they compose 15% (74/478) of this strain's progeny. Young adult hermaphrodites of genotype dpy-5(e61) unc-13(e31) / + + unc-54(e1152) were x-irradiated with 7000 rads (dose rate = 530 rads/min; 1 rad = 0.01 gray). After treatment, 2397 F1 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(e31). 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 F1 progeny were picked individually and transferred to Petri dishes. The offspring of these F1 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 hermaphro-
drites 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 1/4 of the viable outcrossed progeny.

(ii) let-49. Males of genotype lev-l1(x12) let-49(st44)/+ + were crossed with deletion heterozygotes Df[unc-54] +/+ Let(r202)]. The F1 progeny issuing from these crosses were scored for the presence of approximately 1/4 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 1/4 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 choliner-

genic 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-l1(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 choliner-

genic agonists levamisole and tetramisole, but only after a period of recovery after application of the drug (10). Males homozy-

gous for lev-10(x17) were mated with deletion hermaphro-
drites Df[unc-54] +/+ Let(r202)]. Male cross-progeny were trans-
f erred to Petri dishes containing 1 mM tetramisole for 12 hr. Noncomplementation was indicated when 1/2 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 comple-

tment 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)]. F1 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 as-

semblly. 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. 1A) reflects its highly ordered sarcomeric structure. The muscle of unc-54 mutants is severely disrupted (Fig. 1 B and D). The mutation e1152 is a recessive allele of unc-54. As a heterozygote (Fig. 1C) it is essentially indistinguishable from wild type. The dominant heterozygote e1152/+ , however, is defective (Fig. 1E). 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 popu-

lation of e1152/+ paralyzed heterozygotes, animals inher-

iting new recessive unc-54 mutations within the e1152-contain-

ing gene copy will be phenotypically wild type. Such ani-

mals are highly motile and can be directly selected. In order for this selection to work, a balanced-lethal system is re-

quired, such that e1152 heterozygotes can be exclusively or preferentially cultured. Materials and Methods describes construction of an X-ray-induced balancer chromosome de-

signated Let(r202). 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 hermaphroditic. The muscle of e1152/+ heterozygotes (E) is disrupted, whereas that of a re-

cessive 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; ⅔ are e1152 homozygotes and ⅓ are e1152 +/+ 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 × 10⁻³. 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 F₁ 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 isoforms 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 isoforms is identical to e1092, a recessive null allele of unc-54. The exceptional mutant e1152e1618 exhibits a wild-type pattern of myosin isoforms 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.

In two experiments, we picked 64 wild-type F₁ 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 × 10⁻⁷ 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 F₁ wild-type strains was tested by complementation for the presence of deletions. If the untested F₁ 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 surrounding 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

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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(st53), 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
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 deletion 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 F1 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. 4A. This nucleus contains a chromosomal fragment in addition to the normal 2n = 12 chromosomes. Such fragments are never observed in wild-type animals. Fig. 4B 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 × 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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