Genetic Organization of the *unc-60* Region in *Caenorhabditis elegans*

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ABSTRACT

We have investigated the chromosomal region around *unc-60 V*, a gene affecting muscle structure, in the nematode *Caenorhabditis elegans*. The region studied covers 3 map units and lies at the left end of linkage group (LG) V. Compared to the region around *dpy-11* (at the center of LGV), the *unc-60* region has relatively few visible genes per map unit. We found the same to be true for essential genes. By screening simultaneously for recessive lethals closely linked to either *dpy-11* or *unc-60*, we recovered ethyl methanesulfonate-induced mutations in 10 essential genes near *dpy-11* but in only two genes near *unc-60*. Four deficiency breakpoints were mapped to the *unc-60* region. Using recombination and deficiency mapping we established the following gene order: *let-336*, *unc-34*, *let-326*, *unc-60*, *emb-29*, *let-426*. Regarding *unc-60* itself, we compared the effect of ten alleles (including five isolated during this study) on hermaphrodite mobility and fecundity. We used intragenic mapping to position eight of these alleles. The results show that these alleles are not distributed uniformly within the gene, but map to two groups approximately 0.012 map unit apart.

THE genetic fine structure analysis of a chromosomal region provides a useful base from which the study of gene regulation and genome organization can be undertaken. In *Drosophila melanogaster* the genetic dissection within the regions of *rosy* (HILLIKER *et al.* 1980), *zeste-white* (JUDD, SHEN and KAUFMAN 1972), *Adh* (WOODRUFF and ASHBURNER 1979), and *bithorax* (reviewed by LAWRENCE and MORATA 1983) are elegant examples of this approach. In *Caenorhabditis elegans* the region around the muscle gene *unc-22 IV* has been studied intensively (MOERMAN and BAILLIE 1979; ROGALSKI, MOERMAN and BAILLIE 1982; ROGALSKI and BAILLIE 1985). Other studies include regions flanking two additional muscle genes, *unc-15 I* (ROSE and BAILLIE 1980) and *unc-54 I* (ANDERSON and BRENNER 1984) as well as a region on linkage group (LG) II between *dpy-10* and *rol-5* (SIGURDSON, SPANIER and HERMAN 1984), a region at the tip of the *X* chromosome (MENEELY and HERMAN 1979, 1981) and a region on LG I around *dpy-5* (HOWELL *et al.* 1987).

Our laboratory has undertaken the genetic analysis of a 20 map units (m.u.) region on LG V that is recombinationally balanced by the translocation *eT1(III;V)* (JOHNSEN, ROSENBLUTH and BAILLIE 1986). The present report is part of that effort and concerns a 3 m.u. subregion at the left end of LG V.

The large region balanced by *eT1(III;V)* constitutes the left half of LG V, extending from the center of the chromosome (the *dpy-11* region) to the left end (the *unc-60* region) (ROSENBLUTH and BAILLIE 1981). Mutant screens have shown that *dpy-11* lies in a gene cluster whereas *unc-60* is in a gene sparse region. For example, while screening both regions for visible mutations, BRENNER (1974) identified 10 genes in the center of LGV but only two genes, *unc-34* and *unc-60*, at the left end. Similarly, R. E. ROSENBLUTH, R. C. JOHNSEN and D. L. BAILLIE (unpublished results), by screening for EMS induced lethals all along the *eT1*-balanced region of LGV, recovered mutations in 16 genes near *dpy-11* but in only two genes near *unc-60*. Thus, only a few poorly mapped genes have been identified near *unc-60* (see RESULTS).

The focus of this paper is the relatively gene-sparse region surrounding *unc-60*. In order to understand the relationship between gene expression and genome organization in this region we have begun its detailed genetic analysis. Our goals are to characterize the region with respect to genes surrounding *unc-60* and to construct, using a set of recessive alleles, a fine structure map of *unc-60* itself.

Homozygous *unc-60* mutants have abnormal musculature. Their thin filament assembly is disorganized and they are often severely paralyzed (WATERSTON, THOMSON and BRENNER 1980). Intragenic fine structure analysis has been very informative in both prokaryotes and eukaryotes and an intragenic map of the *unc-60* gene should help to elucidate its size and organization. The short generation time and hermaphroditic mode of reproduction of *C. elegans* make it an attractive system for study at the intragenic level. Furthermore, since the *unc-60* homozygote is paralyzed, screening for the rare phenotypically wild-type individual arising from recombination between two

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alleles is not a difficult task. Required for intragenic mapping are a set of *unc-60* alleles and flanking recessive markers. Several *unc-60* alleles were already available (see RESULTS), but no easily identifiable marker had been mapped to the left of *unc-60*.

In this study we positioned *unc-34* as well as several essential genes and deficiencies with respect to *unc-60* and screened for new lethal mutations in the region. Two essential genes mapped closely to the right of *unc-60* while two other essential genes as well as *unc-34* mapped to the left. In addition, we characterized a set of *unc-60* alleles (both existing and newly isolated ones) and, using the new position of *unc-34*, carried out the first fine structure analysis of this gene. We found the recombinational size of *unc-60* to be 0.012 m.u.

MATERIALS AND METHODS

General: The nomenclature in this paper follows that of HORVITZ *et al.* (1979). Nematodes were grown and maintained on nematode growth medium streaked with *Escherichia coli* strain OP50 (BRENNER 1974). All recombination frequency experiments were carried out at 20° as per the results from ROSE and BAILLIE (1979). All mutations used were derived from the wild-type N2 strain var. Bristol. The N2 strain and the following mutations were originally obtained from either the MRC stock collection in Cambridge, England, or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia. For LGIII: *dpy-18(e364)*; for LGV: *unc-34(e315, e566, e951)*; *egl-2(n693)*, *unc-60(e677, e723, e890, m35)*; *emb-29(g52)*; *unc-46(e177)*; *dpy-11(e224)*; *unc-23(e25)*; *unc-42(e270)*; for LGX: *sup-7(st5)*. The *unc-60* allele *r398* was from P. ANDERSON. The suppressor *sup-12(st89)X* was from G. R. FRANCIS and R. H. WATERSTON. The deficiencies *mDf1(V)* and *mDf3(V)* (BROWN 1984) were from D. RIDDLE's laboratory. Deficiencies *nDf18(V)* and *nDf31(V)* were isolated in R. HORVITZ's laboratory and received from E.-C. PARK and C. TRENT, respectively. The following LGV lethal mutations and deficiencies were available from other studies in this laboratory: *let-326(s238)*; *let-334(s908)*; *let-336(s741)*; *let-337(s1018)*; *let-343(s1025)*; *let-405(s116)*; *let-424(s384)*; *rol-3(s422)*; and "*s*" numbered deficiencies (other than *sDf35*). All of these [except *let-405(s116)*] had been isolated as EMS or 1500R gamma irradiation induced LGV (left) lethals as described by ROSENBLUTH, CUDDEFORD and BAILLIE (1983). The translocation *eT1(III,V)* (abbreviated, *eT1*) is made up of *eT1(III)* segregating from LGIII and *eT1(V)* segregating from LGV. Genes from the left halves of LG's III and V (including *unc-60* and *dpy-11*) are carried on *eT1(III)*, whereas genes from the right halves are carried on *eT1(V)*. As a homozygote, *eT1* is viable and has an Unc-36 phenotype. As a heterozygote, it suppresses crossing over for LGIII(right) and LGV(left) genes. Only euploid progeny survive from *eT1* heterozygotes (ROSENBLUTH and BAILLIE 1981). The mutation *let-405(s116)* had been isolated as an EMS-induced lethal linked to the *dpy-11* region.

Isolation of recessive lethal mutations: The following screen recovered recessive lethal mutations in one of two places: tightly linked to *dpy-11* or *unc-60*. Heterozygous *unc-60(e677) dpy-11(e224)/sDf26* hermaphrodites were mutagenized essentially according to BRENNER (1974) except that the dose of EMS was 0.012 M. Individual wild-type F_1's were picked and any that gave very few or no fertile F_2 Dpy Unc-60 progeny carried the desired lethal mutation. These mutations were then balanced over *eT1* and put in pseudolinkage with *dpy-18* to allow complementation testing as described below.

Complementation tests: (*1*) *Lethal vs. visible marker* (*m*): Hermaphrodites *let-x/eT1* or *Dfx/eT1* were crossed to *m/+* males. Appearance of the visible marker among the F_1's indicated a failure to complement. (*2*) *Lethal vs. lethal*: These complementation tests required that the two lethal mutations be tightly *cis*-linked to a common visible marker. Many of the previously isolated lethals and deficiencies were not associated with either an *unc-60* or *dpy-11* marker. However, it was possible to take advantage of the characteristics of *eT1* (above), since the lethals were in the region balanced by *eT1*. From crosses between two *eT1* heterozygotes, only euploid progeny survive. Thus, surviving F_1's homozygous for normal LGIII must also be homozygous for normal LGV. Therefore all LGV lethals were balanced over *eT1* and their normal LGIII partners were marked, with *dpy-18(e364)*. Thus all lethals were in pseudolinkage with *dpy-18(III)*. Two *dpy-18/eT1(III)*; *let-x/eT1(V)* heterozygotes, each with a different lethal mutation or deficiency, were crossed. Complementation was indicated if mature Dpy (or Dpy Unc) progeny appeared among the F_1's.

Test for suppression of *unc-60* alleles: *unc-60* alleles were tested for suppression by the *sup-7(st5)X* amber suppressor as described by WATERSTON (1981). This was done by crossing *dpy-18(e364)/+*; *sup-7(st5)/0* males to the *unc-60("x")* homozygote. The cross progeny of genotype *dpy-18(e364)/+*; *unc-60("x")/+*; *sup-7(st5)/+* were selected and their self progeny examined for evidence of suppression of the *unc-60* allele. The *sup-7* marker was followed by the effect it had on the suppressible mutation *dpy-18(e364)*.

Intragenic mapping of *unc-60* alleles: The method used was modified from ROGALSKI and BAILLIE (1985). The P_0 heterozygote *unc-34(e566) unc-60(x) +/+unc-60(y) dpy-11(e224)* was constructed by mating *unc-34 unc-60(x)/++* males to *unc-60(y) dpy-11* homozygous hermaphrodites. Phenotypically Unc-60 (not Dpy Unc) L_4 hermaphrodite larvae were plated, one per plate, and allowed to self through the second (F_2) generation (7–8 days at 20°). In cases where large brood sizes prohibited efficient screening, the P_0 was transferred every 12–24 hr, depending on how large the broods were. After 7–8 days the plates were screened for exceptional, non-Unc-60 recombinants. These recombinants were progeny tested to determine their genotypes and consequently the relative positions of the *unc-60* alleles used. If allele "*x*" was to the left of allele "*y*" then the recombinants would appear wild-type and the recombinant chromosome would carry no mutant markers. Conversely, if "*x*" was to the right of "*y*," then recombinants would appear Dpy or Unc-34 and the recombinant chromosome would pick up both the *unc-34* and *dpy-11* markers. The above assumes no additional crossover events. *dpy-11* would be expected to cross off or onto approximately one out of five (0.18) recombinant chromosomes (assuming no interference).

The recombination distances were estimated from the formula of MOERMAN and BAILLIE (1979):

$$\text{m.u.} = 2(W)\ 100/T$$

where W is the number of recombinants and T the estimated number of chromosomes screened (MOERMAN and BAILLIE 1979). The W value was doubled because half the recombinants were phenotypically Unc-60 and went unnoticed. The number of screened chromosomes, T, was twice the number of screened F_2 heterozygotes. The latter number was obtained by counting the progeny brood size of ten *unc-34 unc-60(x) +/+ unc-60(y) dpy-11* hermaphrodites, taking the

average and dividing this value by two. The brood size was halved because half the F_1 progeny from the original heterozygote would be homozygous for a given allele and thus not available for intragenic recombination. Like ROGALSKI and BAILLIE's (1985) procedure, one-half the F_2 progeny were too young at the time of screening to be used when the brood sizes were large. Therefore, in such experiments, the F_2 estimate was further reduced by ½. The worms were screened twice over a 24-hr period.

RESULTS

Figure 1 shows the left half of LGV with the genes relevant to this study. Our study of the *unc-60* region consists of two parts: (1) the organization of the genes in the surrounding region (*i.e.*, to the left of the *sDf26* breakpoint) and (2) the analysis and mapping of *unc-60* alleles.

Region surrounding *unc-60*

Position of previously identified genes: In BRENNER's (1974) original screen he found only the gene *unc-34* near *unc-60*. Since then, these other genes have been identified: *egl-2* and *egl-8* (TRENT, TSUNG and HORVITZ 1983); *emb-29* (CASSADA *et al.* 1981); *ges-1* (MCGHEE and COTTRELL 1986); *let-336* (identified by *s741*) and *let-326* (ROSENBLUTH, CUDDEFORD and BAILLIE 1985). Also, one of the three parts of *mnDp26* maps here (HERMAN, MADL and KARI 1979). Only four of the above genes had been positioned with respect to *unc-60*. Data from TRENT, TSUNG and HORVITZ (1983, Table 4) suggest that the two *egl* genes lie to the left of *unc-60* while both *ges-1* (MCGHEE and COTTRELL 1986) and *unc-34* (BRENNER 1974) had been placed to its right. Since we found the mapped position of *unc-34* inconsistent with our own observations, we decided to map it and the three essential genes more precisely.

Although recombination mapping had previously placed *unc-34* (BRENNER 1974) and *let-336(s741)* (ROSENBLUTH, CUDDEFORD and BAILLIE 1985) a considerable distance to the left of *dpy-11*, we found neither of these genes to be deleted by the overlapping deficiencies *sDf26* and *sDf28* (Figure 1A). Since *sDf28* deletes *unc-60*, this meant that both genes must lie to the left of *unc-60*. In the case of *unc-34* this was confirmed by three-factor mapping where 3/3 Unc-34 recombinants from *unc-34 unc-60(m35) +/++ dpy-11* picked up the *dpy-11* marker. Its distance from *unc-60* is 1.8 m.u. based on 9 Unc-34 recombinants, 804 wild-type and 235 Unc-60 progeny from *unc-34(e566) unc-60(e677)/++* hermaphrodites (the Unc-60 phenotype is epistatic to Unc-34).

In the case of *let-336(s741)*, placing it to the left of *unc-60* created an anomaly pointed out earlier (ROSENBLUTH, CUDDEFORD and BAILLIE 1985). In contrast to the deficiency data, two-factor distance mapping had placed *unc-46* closer to *s741* than to *unc-60*. Since then, another allele, *s957*, gave similar results. It had been suggested that *s741* might be associated with a crossover suppressor. To test this possibility we measured the recombination frequency between *unc-60* and *dpy-11* in an *s741* heterozygote. From + *unc-60(e677)* + *dpy-11(e224)/let-336(s741)* + *unc-46(e177)* + hermaphrodites there were 94 Dpy and 93 Unc-60 recombinants among 1164 adult progeny. Taking into account the presence of a *let* mutation and the fact that Unc-60 is epistatic to Unc-46, this gave an apparent map distance between *unc-60* and *dpy-11* of only 13.4 m.u. compared to the normal 17.9 m.u. (see Table 1). For a map distance of 17.9 the chi square value is 15.1 ($p < 0.01$). Thus, it is likely that *s741* is associated with a crossover suppressor. Therefore, *let-336* could be placed to the left of *unc-60* as well as *unc-34*. A breakpoint of *sDf34* (see below) then separated the two genes so that *unc-34* was shown to lie between *let-336* and *unc-60* (Figure 1A).

On the basis of deletion breakpoints (see below) *let-326(s238)* and *emb-29(g52)* were found to lie in the same zone as *unc-60*. The finding that *unc-34* is to the left of *unc-60* enabled us to position *let-326* more precisely. In 2 of 7 Unc-34 recombinants from *unc-34* + *unc-60* +/+ *let-326(s238)* + *unc-46* hermaphrodites, the *s238* mutation was not picked up. Thus, *let-326* mapped 0.5 m.u. [1.8(2/7)] to the left of *unc-60*. In addition, among 107 Unc-46 recombinants from + *unc-60* + *dpy-11/let-326* + *unc-46* + heterozygotes, 104 picked up *unc-60* while three did not. This result also placed *let-326* 0.4 m.u. [16(3/107)] to the left of *unc-60*. The mutation *emb-29(g52)* was subsequently mapped to the right of *unc-60* on the basis of its allelism to a new lethal mutation (described below). The gene order is therefore *let-336*, *unc-34*, *let-326*, *unc-60*, *emb-29*. We do not yet know the relative position of *ges-1* to *emb-29* nor where the two *egl* genes fit into the order.

Deficiencies in the *unc-60* region: Complementation testing genes in the *unc-60* region against LGV deficiencies showed that four break points now lie in the region (Figure 1A): the left-hand ones of *sDf28* and *sDf34* and the right-hand ones of *sDf32* and *sDf33*. Placing *sDf32* to the left of *unc-60* depended on the positions of *let-336* and *unc-34*. The failure of *sDf28* and *sDf32* to complement suggests the presence of at least one as yet unidentified essential gene to the left of *unc-60*.

New genes: In order to identify and position more genes in the region, we screened for lethal mutations tightly linked to *unc-60*. Basically, the protocol involved treating heterozygous *unc-60* hermaphrodites with EMS and screening the F_2 progeny for the absence of mature Unc-60s. To facilitate the subsequent positioning of recovered lethals, we linked *unc-60(e677)* to *dpy-11(e224)*. [Note: The closeness of *unc-34* would have made it a preferable marker but the

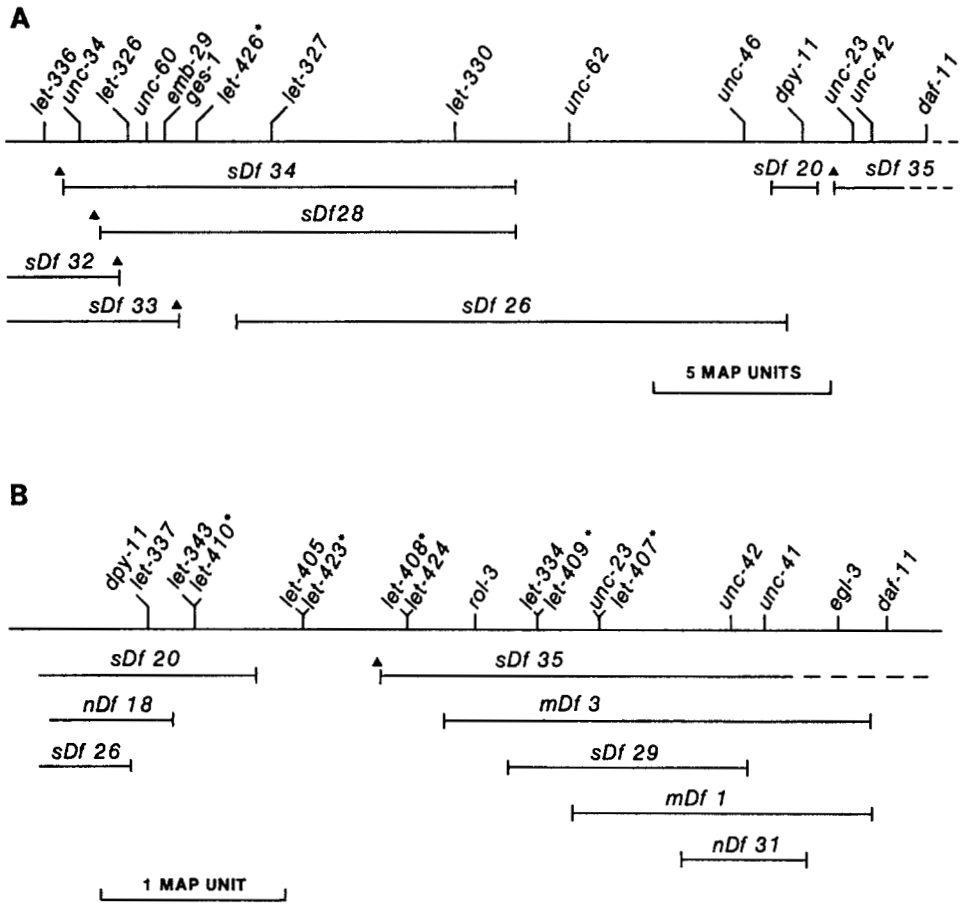

FIGURE 1.—Partial genetic map of *C. elegans* linkage group *V* (left). The region *let-336* to *daf-11* is shown in (A) and the region to the right of *sDf26* is in (B). The position of *-marked genes and ▲-marked deficiency breakpoints are based on this study. The right-hand breakpoints of *mDf1* and *mDf3* as well as both breakpoints of *nDf31* are based on data from C. TRENT (personal communication). The position of *ges-1* is based on MCGHEE and COTTRELL (1986 and personal communication). All other positions are based on data from either ROSENBLUTH, CUDDEFORD and BAILLIE (1985); and R. E. ROSENBLUTH, R. C. JOHNSEN and D. L. BAILLIE (unpublished results); or from SWANSON, EDGLEY and RIDDLE (1984).

fact that Unc-60 is epistatic to Unc-34 made *unc-34* less suitable.] To counteract the large map distance between *unc-60* and *dpy-11*, the *sDf26* deficiency was used in *trans*, both as a balancer and to select for mutations closely linked to either *unc-60* or *dpy-11* (see MATERIALS AND METHODS). From 1222 EMS treated chromosomes, 15 lethal mutations were recovered, or 1.3%. A sample of 434 F_1's that did produce F_2 Dpy Uncs, three Dpy Uncs per plate, were tested to see if they produced fertile F_3's. This procedure revealed a surprisingly large number of maternal effect mutations (13) for a 3.0% induction frequency. However, these mutations were not analyzed further in this study.

The lethal mutations were positioned on LGV by both recombination mapping and complementation testing. Recombinants from *unc-60* (*let-x*) *dpy-11*/+++ heterozygotes gave both two- and three-factor mapping data. Dpy Unc and Dpy recombinants indicated the lethal mapped to the left of *unc-60*. Unc-60 and Dpy recombinants indicated the lethal was between *unc-60* and *dpy-11* (but outside of *sDf26*). Dpy Unc and Unc-60 recombinants indicated the lethal was to the right of *dpy-11*. These data are summarized in Table 1 for eight of the lethals. Complementation testing (see MATERIALS AND METHODS) against deficiencies placed the lethals in specific zones and further testing against other nearby mutations assigned them to specific genes. Complementation testing of *unc-60(e677)* linked lethals was complicated by the fact that *e677/sDf28* and *e677/sDf34* were lethal (see below). However, it was possible to test them against *sDf33*. These results are summarized in Table 2 and Figure 1.

Of the 15 lethals, 2 (*s819* and *s826*) mapped to the *unc-60* region and 11 mapped near *dpy-11*. One of the remaining two, *s828*, was too leaky for further study. The other, *s820*, gave anomalous results. Recombinationally (Table 1), *s820* mapped between *unc-60* and *dpy-11* but 15 m.u. from each. It may be that the lethal phenotype is synthetic and requires homozygosity for both *unc-60* and *dpy-11* as well as *s820*.

Both of the lethals that mapped near *unc-60* are to its right. *s826* appeared to be between *sDf33* and *sDf26* and was assigned to *let-426*. The other, *s819*, failed to complement *sDf33* and mapped very close to *unc-60* (0.4 m.u. to the right). It was found to be allelic to the temperature sensitive *emb-29* allele, *g52* (CASSADA *et al.* 1981), and thus established the position of *emb-29*. We balanced *g52* over *eT1*, also placing it in pseudolinkage with *dpy-18*, and confirmed its position. As predicted, *g52* failed to complement *sDf33* and *sDf34*.

TABLE 1

Two- and three-factor mapping data for lethal mutations

Mutation	Self-progeny from *unc-60 (let) dpy-11/+++* ♂				Distance[a] from:		Gene order[b]
	Wild type	Recombinants			*unc-60*	*dpy-11*	
		Unc-60	Dpy11	Dpy11Unc60			
Control	2820	297	349	492	17.9[c]		
s817[d]	977		17			1.7	*unc-dpy-let*
s818	643	84		4	19.2	0.8	*unc-dpy-let*
s819	2244	7	237		0.4	15.5	*unc-let-dpy*
s820	1364	138	140		14.9	15.1	*unc-let-dpy*
s825	744	75			15.0		*unc-(let dpy)*
s826	1443	11	158		1.0	16.1	*unc-let-dpy*
s827	534	67		2	18.4	0.5	*unc-dpy-let*
s829	1225	135		6	17.6	0.7	*unc-dpy-let*

[a] Distance in map units = 100 p. For control $p = 1 - \sqrt{1 - (2R/total)}$, where R = Dpy + Unc. For experimental $p = 1 - \sqrt{1 - (3R/total)}$, where R = either (DpyUnc + Dpy) or (DpyUnc + Unc).

[b] Based on the type of recombinants (see RESULTS).

[c] Distance between the markers with no lethal.

[d] The strain containing the *s817* mutation lost the *unc-60* marker. This occurrence and the failure to keep *s817* balanced over *eT1* leads to the conclusion that *s817* is to the right of *dpy-11* and outside the *eT1* crossover suppressed region.

TABLE 2

Complementation results[a]

Mutation Gene	Deficiency										
	sDf32	*sDf33*	*sDf34*	*sDf26*	*sDf20*	*nDf18*	*sDf35*	*mDf3*	*sDf29*	*mDf1*	*nDf31*
s815 let-410	+	+	ND	+	−	+	ND	+	+	ND	ND
s816 let-343	+	+	ND	+	−	+	ND	+	+	ND	ND
s818 let-423	+	ND	ND	+	+	ND	+	+	ND	+	ND
s819 emb-29	+	−	ND	+	ND	ND	ND	+	ND	ND	ND
s823 let-409	+	+	ND	+	+	ND	−	−	−	+	+
s825 let-337	ND	ND	ND	+	−	−	+	ND	ND	ND	ND
s826 let-426	+	+	ND	+	ND	ND	ND	+	ND	ND	ND
s827 let-408	+	+	ND	+	+	ND	−	+	+	+	+
s829 let-405	+	+	+	+	+	ND	+	+	+	+	+
s830 let-407	+	+	ND	+	ND	ND	ND	−	−	−	+
s833 rol-3	+	+	ND	+	+	ND	−	−	+	+	+
g52 emb-29	+	−	−	+	ND	ND	ND	ND	ND	ND	ND

[a] + = complementation, − = failure to complement, and ND = not tested.

The 11 mutations near *dpy-11* were also mapped, as our laboratory's interests cover the whole LGV (left) region. To do so we made use of data from a study to be published elsewhere (R. E. ROSENBLUTH, R. C. JOHNSEN and D. L. BAILLIE). In that study, the genes *let-337*, *let-343*, *let-405* (or *let-424*), *rol-3*, *let-334* and *unc-41* define deficiency breakpoints between *sDf26* and *unc-41* (except those of *sDf35* and *nDf31*) as shown in Figure 1B. Ten out of the 11 lethals mapped to the right of *dpy-11* and one, *s825*, was not separated from *dpy-11*. Complementation testing revealed one of the EMS-induced mutations to be a deficiency, *sDf35*, of the *let-424 unc-41* region. It provided a new deficiency breakpoint between *sDf20* and *mDf3* and positioned the genes *let-405* and *let-424* relative to each other. The mutation *s817* could not be kept balanced over *eT1* and was not studied further. Four mutations, *s825*, *s816*, *s829* and *s833*, were found to be alleles of *let-337*, *let-343*, *let-405* and *rol-3*, respectively. The remaining five mutations identified five new genes (*let-410*, *let-423*, *let-408*, *let-409* and *let-407*) as shown in Table 2 and Figure 1B.

Characterization of genes surrounding *unc-60*: Table 3 summarizes the mutations in the *unc-60* region excluding *unc-60* alleles. These include mutations of four non-essential genes (*unc-34*, *egl-8*, *egl-2* and *ges-1*). The alleles of *unc-34* were compared with respect to morphology, movement and fecundity; both as homozygotes and as hemizygotes over *sDf32*. No significant effects of hemizygosity were found nor did we observe any differences between the different alleles. Thus, none of the alleles showed evidence of being hypomorphs. The only known mutation of *egl-2* (*n693*), is a dominant one (TRENT, TSUNG and HORVITZ 1983). It was made heterozygous to the three deficiencies that span the region: *sDf32*, *sDf33*

TABLE 3

Characteristics of genes in the *unc-60* region

Gene	Allele	Phenotype	Average brood size[a] Homozygous	Average brood size[a] Hemizygous[b] (×4/3)
let-336	*s741*	Early larval lethal		
	s951			
unc-34	*e315*	Uncoordinated kinker and coiler both as homozygote and hemizygote[b]	222 (3)	249 (3)
	e566		234 (2)	201 (3)
	e951		256 (3)	177 (3)
	s138[c]		193 (8)	212 (7)
egl-8	*n488*	Variable Egl[d]		
egl-2	*n693dom*	Egl both as homozygote and when hemizygous to *sDf*'s *32*, *33*, and *34*		
let-326	*s238*	Mid-larval lethal		
unc-60		See Table 4		
emb-29	*b262ts*	Embryonic lethal at 25°; affects cell cycle[e]		
	g52ts	Embryonic lethal at 25°		
	s819	Embryonic lethal at 16–25°		
ges-1	*ca-1*	Gut esterase[f]		
let-426	*s826*	Mid-larval lethal		

[a] Number in parentheses = number of broods scored.
[b] Tested over *sDf32*.
[c] *s138* is a gamma ray induced *unc* mutation isolated and mapped to LGV by A. M. ROSE (personal communication). We found it to be an allele of *unc-34*.
[d] TRENT, TSUNG and HORVITZ (1983).
[e] HECHT *et al.* (1987).
[f] MCGHEE and COTTRELL (1986).

and *sDf34*. In all three cases, *n693/sDf* was Egl, indicating that if *egl-2* is deleted by any of these deficiencies, *n693* is probably not a hypermorph. The gene *ges-1* is also believed to be a nonessential gene (J. D. MCGHEE, personal communication). It codes for a gut esterase (MCGHEE and COTTRELL 1986).

The alleles of essential genes were characterized with respect to the stage at which they caused developmental arrest. Only *emb-29* has alleles arresting development before egg hatching. All three of its alleles arrest in embryogenesis. HECHT *et al.* (1987) have shown that the allele *b262* defines a cell division cycle function. The alleles of the other three essential genes arrest development after embryogenesis. We tested the *sDf32* homozygote to check if *sDf32* deletes an undetected essential gene that arrests at an earlier stage than the *let-336* alleles. *sDf32* also arrests in the early larval stage suggesting that there are no embryonic lethals deleted by this deficiency.

To test whether any genes exist in the *unc-60* region that give rise to an easily detectable haplo-insufficient phenotype, we compared morphology, movement and hermaphrodite fecundity among *dpy-18/+;x unc-46/+* heterozygotes (where *x* was either the wild-type allele or a deficiency). No obvious differences were observed using *sDf32*, *sDf33* and *sDf34*. Furthermore, males heterozygous for any of the deficiencies mated successfully. Thus, there are no genes in the region that have a dosage-dependent effect upon the phenotypic characteristics tested.

Alleles of *unc-60*

Alleles of *unc-60* came from two sources. An initial set of five alleles was available from other researchers. Four of these were isolated by BRENNER's (1974) method of looking for visible mutations among the F_2 progeny from 0.05 M EMS-treated N2 hermaphrodites. These were *e677*, *e723*, and *e890* (isolated by S. BRENNER), and *m35* (isolated by D. RIDDLE). All four alleles have similar paralyzed phenotypes. The mutant worms move well in the L_1 and L_2 larval stages but lose this ability with progressing age. The fifth allele, *r398*, was isolated as a suppressor of *unc-105* (P. ANDERSON, personal communication) in a general screen for muscle defective mutants according to the method of PARK and HORVITZ (1986). Allele *r398* is antidystrophic. It moves poorly as a late larva but is more mobile as it gets older. The second set of alleles were isolated in this study. Since neither of the above screening procedures had been specific for *unc-60*, a large number of other *unc* mutations were recovered. We therefore chose the method of precomplementation to isolate new *unc-60* alleles.

The main problem with precomplementation is differentiating the new allele from the primary allele used to detect it. To overcome this difficulty, we first made an *eT1* chromosome that carried both a lethal and an *unc-60* mutation. An *eT1* translocation carrying the lethal mutation *s704* on one of its chromosomes already existed (R. E. ROSENBLUTH and D. L. BAILLIE, unpublished results). To put an *unc-60* mutation onto this *eT1* chromosome required mutagenesis since no recombination occurs between *eT1* and normal LGV in this region. To achieve this, hermaphrodites of genotype *let-?(s704) eT1/unc-60(m35) dpy-11* were mutagenized with 0.012 M EMS and screened for non-Dpy paralyzed worms in the first generation. This procedure produced two such hermaphrodites in a screen of 5800 chromosomes (F_1 wild-types) for a frequency of 3.4×10^{-4}. The new mutations (*s1310* and *s1331*) were made heterozygous over *dpy-11* and were confirmed to be *unc-60* alleles by complementation testing. One, *s1310*, was then used to detect new *unc-60* alleles on normal LGV chromosomes (see below). In addition, we recovered a homozygous *unc-60(s1310)eT1* free of *let-?(s704)* from an *eT1/let-?(s704) unc-60(s1310) eT1* heterozy-

TABLE 4

Summary of *unc-60* alleles

Allele	Ease of scoring[a]	Homozygous brood size[b]	Brood size[b] or viability[c] as hemizygote with:		
			sDf28	*sDf33*	*sDf34*
+			269 (3)	230 (3)	194 (4)
e677	1	24 (9)	0/10[c]	4/7[c] 4 (4)	0/10[c]
e890	1	42 (8)	0/10[c]	5 (7)	2/10[c] 2 (2)
e723	2	97 (7)	4 (8)	19 (8)	14 (7)
m35	2	118 (10)	8 (8)	18 (6)	14 (7)
r398	4–5	239 (10)	39 (8)	ND[d]	43 (7)
s1307	4–5	193 (8)	ND	ND	82 (10)
s1308	2	89 (10)	ND	ND	4 (5)
s1309	3	217 (8)	ND	ND	15 (7)
s1310[e]	1	27 (8)	4 (9)	4 (8)	5 (7)
s1331[e]	2	125 (4)	9 (4)	10 (4)	8 (4)

[a] 1 = severe allele (strong paralysis); 5 = weak allele (some movement).

[b] Number in parentheses is the number of broods scored.

[c] Fraction of *unc-60(x)/sDf(y)* larvae reaching maturity.

[d] ND = not tested.

[e] *s1310* and *s1331* are alleles on the *eT1* chromosome [homozygous *eT1* brood = 163 (ROSENBLUTH and BAILLIE 1981)].

gote. We do not know whether *s704* is on *eT1(III)* or on *eT1(V)*. It had therefore either segregated or recombined away from *unc-60(s1310)*. This was subsequently used (see below) to map the position of *unc-60* on the *eT1(III,V)* translocation and for intragenic experiments. The isolation of *unc-60* mutations on normal LGV's involved mutagenizing + *dpy-11/eT1 unc-60(s1310) let-?(s704)* hermaphrodites with 0.012 M EMS and screening the F_1's for rare Unc-60 worms. The latter were picked and allowed to segregate F_2's. Since the new *unc-60* alleles on LGV would remain stably linked to the *dpy-11* marker by virtue of *eT1*, they could be isolated as homozygotes in the form of Dpy Uncs. Note that the *eT1*-linked lethal, *s704*, was used to eliminate the Unc-60 (*s1310*) phenotype from being expressed and confusing the screening. From 18,800 chromosomes, four new mutations were recovered for a frequency of 2.1×10^{-4}. Thus, the average *unc-60* induction frequency for 0.012 M EMS was 1 of 4100 chromosomes. Three alleles from this screen were further analyzed. Allele *s1307* is antidystrophic like *r398*. Alleles *s1308* and *s1309* show the classic paralyzed phenotype.

In Table 4, the phenotypes of the *unc-60* alleles are compared with respect to mobility (column 2) and fecundity (column 3). The data allow the ten alleles to be ordered in series by severity. From the strongest to weakest allele they are *e677*, *e890*, *s1310*, *s1308*, *e723*, *m35*, *s1331*, *s1309*, *s1307* and *r398*. [The original *e723* strain (CB723) has half the brood size of an outcrossed strain (BC1961).] Each allele was then tested for fecundity and viability when heterozygous with the deficiency *sDf34* (column 6). All showed marked differences between the hemizygous and homozygous states; all exhibited more severe phenotypes over the deficiency. This suggests that all the alleles are hypomorphs; *i.e.*, the mutants produce a gene product of reduced activity, or less of a product with normal activity. Several of the alleles were also tested over *sDf28* and *sDf33* (columns 4 and 5). Their behavior was affected differently by the three deficiencies. In general, the *unc-60* alleles showed the most severe hemizygous phenotype with *sDf28* and the least with *sDf33*. This variability could be due to different cumulative effects produced by different sets of hemizygous genes. Both *e677* and *e890* were effectively mid-larval lethals when hemizygous with *sDf28* and *sDf34*. However, over *sDf33* the lethal phenotypes were leaky. A hemizygous lethality for *e677* had already been implied by HERMAN, KARI and HARTMAN (1982) for aneuploid segregants from a translocation. These results suggest that *unc-60* null alleles, such as amber mutations, may be recessive lethals.

Tests for suppression

Effect of *sup-7(st5)X*: Alleles suppressible by *sup-7* action (WATERSTON 1981; WILLS *et al.* 1983), must be within the coding element of the gene. Of the *unc-60* alleles tested (*s1307* and *r398* were not tested), none showed any sign of suppression in the presence of heterozygous or homozygous *sup-7*. This does not rule out the possibility that these are non-*sup-7* suppressible amber alleles or non-amber null mutations.

Effect of *sup-12(st89)X*: The mutation *sup-12(st89)* is a recessive suppressor of the Unc-60 phenotype. It was isolated and characterized by G. R. FRANCIS and R. H. WATERSTON (personal communication), who found that different alleles of *unc-60* were suppressed to different extents. To compare the effect of *sup-12(st89)* on *e677*, *e723*, *e890*, *m35*, *s1308*, and *s1309*, we examined the mobilities of hermaphrodites that were homozygous for *sup-12(st89)* and one of these alleles (data not shown). In each case the paralysis was suppressed. The allele normally causing the severest paralysis, *e677*, showed the greatest mobility in the presence of *sup-12*. The suppression of the other five alleles varied, but we found no correlation between the degree of suppression and the severity of the original paralysis.

unc-60 is on *eT1(III)*

The induction of an *unc-60* allele (*s1310*) on an *eT1* chromosome made it possible to answer a question regarding the physical limits of the *eT1* translocation. Previous work (ROSENBLUTH and BAILLIE 1981) had concluded that *eT1(III, V)* was the product of two breaks; one on LGIII (between *sma-3* and *sma-2*) and one on LGV (between *dpy-11* and *unc-42*). While it was shown that *eT1(V)* (segregating from LGV) carried the whole of the right arm of LGIII, it was only

TABLE 5

Summary of fine structure mapping

Allele pair tested[a]	Total progeny/ heterozygote	Recombinants/ F_2 chromosomes	Map distance[b]	Allele order[c]	Conversion frequency
e677/m35	60	18/248,000	0.014 (0.007–0.022)	*e677-m35*	5/248,000
m35/e677	108	8/175,000	0.009 (0.003–0.017)	*e677-m35*	1/175,000
e677/e890	36	3/60,000	0.010 (0.003–0.015)	*e677-e890*	1/60,000
e677/e723	62	2/62,000	0.007 (–0.023)	*e677-e723*	0/62,000
e677/s1309	101	2/61,000	0.007 (–0.023)	*e677-s1309*	0/61,000
e677/s1308	50	0/80,000	(0.0–0.007)		0/80,000
e677/s1307	102	1/58,000	0.003 (–0.017)	*e677-s1307*	1/58,000
m35/e723	93	0/862,000	(0.0–0.0007)		0/862,000
m35/s1308	136	2/148,000	0.003 (–0.009)	*s1308-m35*	0/148,000
m35/e890	73	0/307,000	(0.0–0.002)		0/307,000
m35/s1307	222	0/67,000	(0.0–0.004)		0/67,000
m35/s1309	151	0/216,000	(0.0–0.003)		0/216,000
e723/e890	79	0/487,000	(0.0–0.001)		0/487,000
r398/e677	195	2/?[d]	(0.0–)	*e677-r398*	1/?[d]
r398/e890	168	0/77,000	(0.0–0.007)		0/77,000
m35/s1310[e]	37	0/78,000	(0.0–0.008)		0/78,000
s1308/s1310[e]	42	0/75,000	(0.0–0.008)		0/75,000

[a] Alleles *x/y* where parent was *unc-34 unc-60(x)+/+unc-60(y) dpy-11*.
[b] Confidence limits of 95% in parentheses (STEVENS 1942).
[c] Based on genotype of recombinant.
[d] The estimate of screened chromosomes was not determined. Only three-factor positioning was done.
[e] *s1310* is on the *eT1* chromosome.

assumed that *eT1(III)* (segregating from LGIII) carried the whole left arm of LGV. The possibility remained that *eT1* was the product of more than two breaks and only the medial portion of LGV was translocated. The following two tests show that the translocated portion of LGV extends to at least *unc-60*. First the F_1 progeny from *+/eT1(III); unc-60(m35)/unc-60(s1310) eT1(V)* hermaphrodites crossed to *+/eT1(III); unc-60(m35) dpy-11(e224)/eT1(V)* males will be Unc-60's, Unc-36's (*eT1* homozygotes) and wild types. The ratio of F_1 wild types segregating Dpy Unc F_2's to those segregating non-Dpy Unc F_2's depends on which chromsomes *s1310* segregates from. If a 1:1 ratio is observed, *s1310* is on *eT1(III)*, if the ratio is 0:1, then *s1310* is on *eT1(V)*. In fact, the data indicated that *s1310* is on *eT1(III)* (8 Dpy Unc to 4 Unc). Second, when hermaphrodites of genotype *+/unc-60(s1310) eT1(III); unc-60(e890)/eT1(V)* were crossed to *+/eT1* males there were Unc-60 male progeny among the F_1. Such males could have arisen only if the two *unc-60* alleles segregated into the same oocyte and again indicated that *s1310* is on *eT1(III)*. Thus, genetic evidence now shows that the translocated portion of LGV on *eT1(III)* extends to the left from a point between *dpy-11* and *unc-42* to at least *unc-60*.

Intragenic mapping of *unc-60* alleles

The demonstration that *unc-34* lies to the left of *unc-60* allowed us to position alleles of the *unc-60* locus relative to each other by recombination analysis using *unc-34* and *dpy-11* as flanking markers. The results are summarized in Table 5.

The greatest recombination frequency between alleles was observed in the *e677/m35* experiments. All the *unc-60* mutations on the normal LGV appear to be inseparable from one of two discrete sites; either from *e677* or, more often, from *m35*. Allele *s1308* may still be separable from *e677* since only 80,000 chromosomes have been screened, and *s1308* maps unusually close to *m35*. The other possibility is that *s1308* is a crossover suppressor. A similar situation applies to the allele *s1307*, that is tightly linked but separable from *e677*, but as yet inseparable from *m35*. There appears to be no correlation between an allele's position and its phenotypic effect. For example, the two severest alleles, *e677* and *e890*, map to opposite sites.

It is not known whether any or all of the alleles are in the coding element since none show *sup-7* suppression, temperature sensitivity or interallelic complementation. The gene size is putatively 0.012 m.u. This map distance is based on only those recombinants that appeared to be the result of a single crossover event in the first two crosses listed in Table 5. Considering all crosses, a total of 47 non-Unc-60 hermaphrodites were recovered. Thirty-eight of these could be explained by a single recombination event between alleles and were used to left-right position the alleles with respect to *unc-34* and each other. Nine exceptional recombinants suggested opposite orientations. The contradictions could be resolved by assuming that either gene conversion or a second crossover event between *unc-60* and one of the flanking

markers had occurred. For *unc-34*, the latter explanation was unlikely due to the small recombination distance between *unc-60* and this marker (1.8 m.u.). Unfortunately, recombination is a common event between *dpy-11* and *unc-60* (18%) and thus a second crossover event between *unc-60* and *dpy-11* cannot be ruled out. The frequency of conversion in the *e677/m35* experiments was 3 of 248,400 (1/83,000) (excluding *dpy-11* events) or 6 of 423,000 (1/71,000) assuming all anomalous events are gene conversions.

Using the *unc-60(s1310)* allele which, as already mentioned, is on the *eT1(III)* chromosome, we attempted to detect events indicating genetic exchange between the normal and *eT1* homologues at the intragenic level. *s1310* was placed heterozygous to *m35* and *s1308* chromosomes that also carried *dpy-11(e224)*. No non-Unc-60 recombinants were observed in 78,000 *m35/s1310* and 75,000 *s1308/s1310* chromosomes screened.

DISCUSSION

In this report we began a genetic dissection of the far left end of *LGV* in order ultimately to investigate the relationship between gene expression and genome organization in and around *unc-60(V)*. The region studied covers a minimum of 3 m.u. extending at least 1.8 m.u. to the left of *unc-60* and about 1.0 m.u. to the right.

The identifiable genes of *C. elegans* are not distributed evenly along the genetic map (SWANSON, EDGLEY and RIDDLE 1984; SIGURDSON, SPANIER and HERMAN 1984) and *unc-60* lies in a relatively gene sparse region. This fact was confirmed during our search for essential genes near *unc-60*. Screening simultaneously the *unc-60* and *dpy-11* regions for EMS-induced lethals, we recovered mutations in only two genes near *unc-60* and in ten genes within 1.7 m.u. of *dpy-11*. Only three other genes in the region, *egl-2*, *egl-8* (TRENT, TSUNG and HORVITZ 1983) and *ges-1* (MCGHEE and COTTRELL 1986), had previously been positioned relative to *unc-60*. In the present study we positioned a further five genes and four deficiency breakpoints: three genes and three breakpoints to the left of *unc-60* and two genes and one breakpoint to its right (Figure 1). The furthest gene to the left is either *let-336* or *egl-8*. Whether one of these is the "end-gene" for LGV is not known but it is intriguing to note that the two alleles of *let-336* affect recombination. The nearest identified genes to *unc-60* are now either *let-326* or *egl-2* on the left and *ges-1* or *emb-29* on the right. Whether any of these are the genes immediately flanking *unc-60* has not yet been determined. If they are, the gene density here would be about four genes per map unit and in striking contrast to the densities in such gene "clusters" as the *dpy-10 unc-4* region on LGII (at least 20 genes per map unit, SIGURDSON, SPANIER and HERMAN 1984), the *unc-43 unc-22* region on LG IV (at least 16 genes per map unit, ROGALSKI and BAILLIE 1985) and the *dpy-5 dpy-14* region on LGI (at least 18 genes per map unit, HOWELL *et al.* 1987).

Most *unc-60* mutants are paralyzed. Based on electron microscope studies, the wild-type *unc-60* function has been implicated to affect thin filament assembly (WATERSTON, THOMSON and BRENNER 1980). These authors demonstrated that in homozygous mutants, large accumulations of thin filaments aggregate at the ends of muscle cells near the dense bodies, while the thick filaments are still found around M lines in an irregularly structured A band.

For this study, five *unc-60* alleles were available from others, and six more were isolated by ourselves. Among the ten alleles that were studied, we found a wide range of phenotypic expression (Table 4). Interestingly, all ten expressed more severe mutant phenotypes as hemizygotes than as homozygotes, indicating that none are amorphs. The low viabilities of some hemizygotes suggest that an *unc-60* null allele may well be inviable. As yet, no *unc-60* lethal allele has been found. The nearest lethal mutations, *let-326(s238)* and *emb-29(s819)*, are each recombinationally separable from *unc-60(e677)* by about 0.4 m.u. and both *let-326(s238)* and *emb-29(g52)* complement *e677*.

It seems that the *unc-60* gene is a relatively large EMS mutational target. In two separate experiments, using 0.012 M EMS (one in an "*eT1*" background and one in a "normal" background), we recovered a total of six *unc-60* alleles among 24,600 screened chromosomes. At 0.012 M EMS, extensive analysis of lethals in the *unc-22* region has indicated that the average gene is hit once in 6000 chromosomes (D. V. CLARK and D. L. BAILLIE, unpublished results). Since *unc-60* is mutated with a frequency of one per 4100 chromosomes, it appears that *unc-60* is a larger than average gene.

One of our goals for this study was to construct an intragenic recombination map for *unc-60*. Eight alleles were found to span 0.012 m.u. We do not know whether the map spans the whole *unc-60* locus. Since no *sup-7* suppressible, temperature sensitive or complementing alleles have been detected, we are not able to discriminate between coding and linked regulatory regions. Therefore no definitive size estimate can be made for the *unc-60* product. A comparison with data from KARN, BRENNER and BARNETT (1983) suggests a large polypeptide. These workers found that *unc-54* uses 7,266 base pairs to code for a 210-kD *C. elegans* heavy myosin chain. It is relevant to point out here that at the gross phenotypic level we found hemizygous *unc-60(+)* individuals (*sDf34/+*) to be wild type in appearance. This is in contrast to observations of

unc-15 (coding for paramyosin) (WATERSTON, FISHPOOL, and BRENNER 1977). Either a decrease of 50% wild-type product is not enough to cause an effect on the gross phenotype; or, a 50% decrease has no effect because of the presence of another gene which encodes a product which can partially compensate for the missing wild-type unc-60 product; or, the gene is dosage regulated, such that a single copy produces twice the normal amount of product.

The *unc-60* alleles are not distributed evenly within the intragenic map. They appear to cluster around two sites, by *e677* and by *m35*. This is interesting and may represent the functionally important regions of the coding element or mutational hot spots. The gap between the sites potentially encodes a large intron or could specify a part of the gene product within which most mutational alterations lead to a lethal phenotype.

Apparent gene conversion events have been recovered in all *C. elegans* fine structure analyses. These events appear as double crossovers but at too high a frequency. ROGALSKI and BAILLIE (1985) found a conversion frequency of 6 in 786,039 (1/131,000) within *unc-22*, and MOERMAN *et al.* (1982) found a similar frequency with *unc-54*. In *unc-60*, among the *m35/e677* experiments, the frequency was 3 of 248,400 (1/82,800) for confirmed events and 6 of 423,000 (1/71,000) if all double crossover events are included. Thus, the gene conversion frequency observed for the *unc-60* locus is very similar to the frequency obtained for the other two muscle genes. Suprisingly, no conversion events were detected between the *m35* group of alleles. The nature of the mutations involved (HILLIKER and CHOVNICK 1981; CLARK *et al.* 1986) may have reduced the possibility of finding these events.

For the further analysis of the *unc-60* region, a number of experiments are already in progress. A large number of additional lethal mutations balanced by *eT1* have been recovered using EMS, formaldehyde, gamma radiation and UV mutagenesis (R. C. JOHNSEN, L. M. TURNER, H. I. STEWART and D. L. BAILLIE, unpublished results). We intend to analyze this region using an approach similar to that employed in the region flanking *unc-22(IV)*.

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