Genetic Organization of the unc-60 Region in Caenorhabditis elegans

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Manuscript received February 17, 1987
Revised copy accepted September 18, 1987

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 10 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), teste-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; Rożalski and Baillie 1985). Other studies include regions flanking two additional muscle genes, unc-15 I (Rose and Baillie 1986) 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
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°C 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(m693), unc-60(e777, e723, e890, m35); emb-29(g32), unc-46(e177); dpy-11(e224); unc-23(e25); unc-42(e270); for LGX: sup-7(u15). 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 mDj1(V) and mDj2(V) (Brown 1984) were used in D. Riddle's laboratory. Deficiencies nDj1(V) and nDj2(V) were isolated in R. Horvitz' 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-336(c238); let-334(s908); let-336(s741); let-337(s1018); let-343(s1025); let-405(s116); let-424(s384); rol-3(s422); and "u" deficiencies (other than nDj3). All of these [except let-405(s116)] had been isolated as EMS resistant mutants and transferred to a high density strain of CB1156. The tester strain OP50 was used for these experiments.

**Complementation tests:** (1) Lethal vs. visible marker (M): Hermaphrodites let-336/et1 or Dpy/et1 were crossed to m/+ males. Appearance of the visible marker among the F1'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 F1'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-11(900). Thus all lethals were in pseudolinkage with dpy-18(III). Two dpy-18(III)/u15; let-336(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 F1's.

**Test for suppression of unc-60 alleles:** unc-60 alleles were tested for suppression by the sup-7(u15)X amber suppressor as described by Waterston (1981). This was done by crossing dpy-18(s364)++; sup-7(u15)/0 males to the unc-60(x) homozygote. The cross progeny of genotype dpy-18(s364)/u15; unc-60(x)/u15; sup-7(u15)/+ 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(s364).

**Intragenic mapping of unc-60 alleles:** The method used was modified from Rogalski and Baillie (1985). The P0 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) L4 hermaphrodite larvae were plated, one per plate, and allowed to self through the second (P1) generation (7-8 days at 20°C). In cases where large brood sizes prohibited efficient screening, the P0 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):

\[ m.u. = 2(W)\ 100/T \]

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 F2 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 F1 progeny from the original heterozygote would be homozygous for a given allele and thus not available for intragenic recombination. Like ROGALSKI and BAILEY'S (1985) procedure, one-half the F2 progeny were too young at the time of screening to be used when the brood sizes were large. Therefore, in such experiments, the F2 estimate was further reduced by \( \frac{1}{2} \). The worms were screened twice over a 24-hour 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-326 (identified by *s741*) and let-326 (ROSENBLUTH, CUDDEFORD and BAILEY 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-326(s741) (ROSENBLUTH, CUDDEFORD and BAILEY 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 BAILEY 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 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 F2 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
The lethals 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.
and provided a new deficiency breakpoint between two deficiencies, separated from each other. The mutation s817 revealed one of the EMS-induced mutations to be s334 to the conclusion that s817 is to the right of dpy-11 and outside the eT1 crossover suppressed region.

### TABLE 1

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* = complementation, – = failure to complement, and ND = not tested.

### TABLE 2

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<td>g52 emb-29</td>
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</table>

* + = 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. JOHNSON 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.
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 F2 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 L1 and L2 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(eT1) 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 (F1 wild-types) for a frequency of 3.4 X 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 heterozygous.

**TABLE 3**

**Characteristics of genes in the unc-60 region**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Phenotype</th>
<th>Homozygous</th>
<th>Average brood size*</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-336</td>
<td>s741</td>
<td>Early larval lethal</td>
<td>222 (3)</td>
<td>249 (3)</td>
</tr>
<tr>
<td></td>
<td>s951</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unc-34</td>
<td>e135</td>
<td>Uncoordinated</td>
<td>234 (2)</td>
<td>201 (3)</td>
</tr>
<tr>
<td></td>
<td>e566</td>
<td>both as homozygote and hemizygote</td>
<td>256 (3)</td>
<td>177 (3)</td>
</tr>
<tr>
<td></td>
<td>s138</td>
<td></td>
<td>193 (8)</td>
<td>212 (7)</td>
</tr>
<tr>
<td>egl-8</td>
<td>n488</td>
<td>Variable Egl#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>egl-2</td>
<td>n693dom</td>
<td>Egl both as homozygote and when hemizygous to sDf's</td>
<td></td>
<td></td>
</tr>
<tr>
<td>let-326</td>
<td>s238</td>
<td>Mid-larval lethal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unc-60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>emb-29</td>
<td>b262ts</td>
<td>Embryonic lethal at 25°; affects cell cycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ges-1</td>
<td>ca-1</td>
<td>Gut esterase/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>let-426</td>
<td>s26</td>
<td>Mid-larval lethal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number in parentheses = number of broods scored.

1 Tested over sDf32.

* 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.

4 TRENT, TSUNG and HORVITZ (1983).

H* HEGHT et al. (1987).

5 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. HEGHT 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 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).

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To test whether any genes exist in the unc-60 region.
TABLE 4
Summary of unc-60 alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Ease of scoring</th>
<th>Homozygous brood size</th>
<th>Brood size or viability as hemizygote with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>sDF28</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td></td>
<td>269 (3)</td>
</tr>
<tr>
<td>e677</td>
<td>1</td>
<td>24 (9)</td>
<td>0/10°</td>
</tr>
<tr>
<td>e890</td>
<td>1</td>
<td>42 (8)</td>
<td>0/10°</td>
</tr>
<tr>
<td>e273</td>
<td>2</td>
<td>97 (7)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>m35</td>
<td>2</td>
<td>118 (10)</td>
<td>8 (8)</td>
</tr>
<tr>
<td>r398</td>
<td>4-5</td>
<td>259 (10)</td>
<td>39 (8)</td>
</tr>
<tr>
<td>s1307</td>
<td>4-5</td>
<td>193 (8)</td>
<td>ND</td>
</tr>
<tr>
<td>s1308</td>
<td>2</td>
<td>89 (10)</td>
<td>ND</td>
</tr>
<tr>
<td>s1309</td>
<td>3</td>
<td>217 (8)</td>
<td>ND</td>
</tr>
<tr>
<td>s13l°</td>
<td>1</td>
<td>27 (8)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>s1331°</td>
<td>2</td>
<td>125 (4)</td>
<td>9 (4)</td>
</tr>
</tbody>
</table>

*1 = severe allele (strong paralysis); 5 = weak allele (some movement).

* Number in parentheses is the number of broods scored.

* Fraction of unc-60(ek)/sDF(y) larvae reaching maturity.

* ND = not tested.

* s1310 and s1331 are alleles on the eT1 chromosome [homozygous eT1 brood = 165 (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-2(s704) hermaphrodites with 0.012 M EMS and screening the F1’s for rare Unc-60 worms. The latter were picked and allowed to segregate F2’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 X 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 antidys trophy 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 (CB783) 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 weak. 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
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 over event between alleles and were used to left-right position of LGV. Such males could be cases only if the ratio is observed, s1310 is on eT1(III), if the ratio is 0:1, then s1310 is on eT1(V). In 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 were Unc-60 male progeny among the F1. 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.
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 between these two 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 between these two markers 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 1985) 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 Baille 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 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(s52) 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. Baille, 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 (1988) 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 the missing wild-type unc-60 product is not enough to cause an effect on the normal phenotype; or, the gene is involved in the intragenic map. They appear to cluster around two sites, by e677 and by m35. They 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 the missing wild-type unc-60 product is not enough to cause an effect on the normal phenotype; or, the gene is involved in 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.

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C. elegans unc-60 Region

Caenorhabditis elegans that alter contractility but not muscle structure. Cell 29: 775–781.


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Communicating editor: R. K. Herman