GENETIC ORGANIZATION OF THE REGION AROUND *UNC-15* (*I*), A GENE AFFECTING PARAMYOSIN IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

In the nematode *Caenorhabditis elegans* mutants in the gene *unc-15* (*I*) affect the muscle protein paramyosin (WATERSTON, FISHPOOL and BRENNER 1977). We have characterized 20 ethyl methanesulfonate-induced mutations in essential genes closely linked to *unc-15*. These lethals defined 16 new complementation groups. In the 0.65 map-unit interval around *unc-15* defined by *dpy-14* and *unc-56*, seven newly identified genes have been mapped relative to five existing genes. At present, the average distance between genes in this region is approximately 0.05 map units. Two genes, *unc-15* and *unc-13*, are only 0.025 map units apart. Partial fine-structure maps of alleles of these two genes have been constructed. This analysis of *unc-15* and genes adjacent to it is the first in a series of genetic and biochemical studies directed towards understanding the control of *unc-15* expression.

ONE approach to understanding the regulation of gene activity during tissue differentiation is the detailed study of a region around a known gene. In *D. melanogaster*, the elegant studies of CHOVNICK and his colleagues (see CHOVNICK, GELBART and MCCARRON 1977 for review) have demonstrated the value of this approach. We have taken a similar approach in *Caenorhabditis elegans* by studying the region around *unc-15* (*I*); *unc-15* mutants are paralyzed and have altered paramyosin. Paramyosin is a structural protein that forms the core of the thick muscle filament in invertebrates. While other models are not ruled out, evidence is consistent with the hypothesis that *unc-15* contains the coding sequence for a paramyosin subunit (WATERSTON, FISHPOOL and BRENNER 1977). *unc-15* is situated on chromosome *I*, closely linked to other genes that produce uncoordinated phenotypes. For example, another gene producing paralyzed mutants, *unc-87*, maps close to *unc-15* (MACLEOD and WATERSON, personal communication). This observation has led us to speculate that other genes closely linked to *unc-15* may also code for muscle-related proteins. Our ultimate goal is to recover mutations in all loci around *unc-15* and analyze the organization of the *unc-15* region. We hope that such an approach will provide insight into the genetic regulation of muscle differentiation.

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In *C. elegans*, unconditional lethals have been induced and characterized on LG *II* (HERMAN 1978) and the tip of the *X* chromosome (MENEELY and HERMAN 1979). We describe here the recovery and characterization of ethyl methane-sulfonate-(EMS) and formaldehyde-induced mutations around *unc-15*. This is the first attempt in *C. elegans* to identify all the lethal sites in a small region. To further characterize the organization of this region, we have constructed fine-structure maps for *unc-15* and its closest neighbor, *unc-13*.

MATERIALS AND METHODS

Our wild-type strain of *Caenorhabditis elegans* var. Bristol was obtained originally from S. BRENNER (MRC, Cambridge, England). Other mutant strains used in this study came from the laboratory indicated by the italicized letter prefixing the mutation number as described by HORVITZ *et al.* (1979). We followed the procedure for strain maintenance and genetic outcrossing described previously by ROSE and BAILLIE (1979).

Induction of lethals and deficiencies: A heterozygous strain, *dpy-14(e188)* + *unc-13(e51)*/+ *unc-15(e73)* +, was constructed. This strain was maintained by picking wild-type-appearing heterozygotes each generation and allowing them to self-cross. In order to induce lethal mutations, this strain was treated with 0.025 M EMS for 4 hr, modified from BRENNER (1974). Treated hermaphrodites were placed 1 per plate. F_1 individuals were placed on separate plates to self-cross. F_2 progeny were screened for the absence of dumpy- uncoordinated worms, indicating the presence of a lethal closely linked to *dpy-14* and *unc-13*. In this way, 1,600 chromosomes were tested. Lethals induced by this method were maintained by transferring the heterozygotes each generation.

Two deficiencies of this region were recovered. *sDf5* was either spontaneous or induced with 0.05 M EMS, but *sDf6* was induced by treating wild-type male sperm with 0.07% formaldehyde as follows. Some 200 to 300 dumpy-uncoordinated (*dpy-5 unc-13/dpy-5 unc-13*) hermaphrodites were mated for 18 hr to wild-type males. The mated hermaphrodites were treated with formaldehyde in M-9 buffer for 4 hr. Treated worms were spotted on a plate for 30 min. Individual *dpy-5 unc-13/dpy-5 unc-13* worms were then placed on small (35 mm) petri plates. The F_1 progeny were counted and screened for the presence of exceptional dumpy or uncoordinated progeny. *sDf5* and *sDf6* are maintained heterozygously over *unc-15* (*e73*).

Mapping studies: *unc-37(e262)*, *unc-87(e1489)* and *mec-8(e398)* were mapped by generating a heterozygous strain that carried the gene to be mapped over either *dpy-5 dpy-14* or *dpy-14 unc-13*. These strains were maintained by selecting wild-type appearing hermaphrodites each generation. Recombinants that were homozygous for either *dpy-5*, *dpy-14* or *unc-13* were placed on individual plates and progeny tested. Mutations in *dpy-5* and *dpy-14* give rise to very different phenotypes that are easily distinguished (for example, *dpy-14* mutants are temperature sensitive, whereas *dpy-5* mutants are not). The results from these progeny tests were used to determine the position of the gene being mapped relative to *dpy-5*, *dpy-14* and *unc-13*. *unc-87* was further positioned by picking dumpy and uncoordinated recombinants from the heterozygous strain, *dpy-14* + *unc-37*/ + *unc-87* +.

Mutations in essential genes were induced in a *dpy-14 unc-13* chromosome, and maintained heterozygously over a *unc-15* chromosome. Thus, the position of the gene could be determined from the number of the dumpy, uncoordinated and dumpy-uncoordinated progeny present in the self-cross. In addition, the occurrence of uncoordinated recombinants that do not carry *unc-15* proves that the essential gene maps outside the *unc-15 unc-13* interval. Essential genes that mapped to the right of *unc-13* were further positioned by constructing the heterozygous strain, *dpy-14* + *unc-13 let-x* +/ + *unc-15* + + *unc-56*.

Complementation analysis: Male strains of each lethal mutation were constructed by crossing *dpy-14* + *unc-13*/ + *unc 15* + males (generated by *X*-chromosome nondisjunction) to each lethal. Two types of males are produced by this cross, one of which carries the lethal to be tested. Individual males were backcrossed to hermaphrodites from the same strain. Males and hermaphrodites from crosses that gave male progeny, but no dumpy-uncoordinated worms, were mated each generation to maintain a male strain. Te ensure that the dumpy and uncoordinated mutations are present, each strain was mated to *dpy-14* + *unc-13*/ + *unc-15* + males and the progeny examined for the presence of dumpy-uncoordinated males.

Mutant strains were mated *inter se* to determine if complementation occurred.

Mapping alleles within a gene: Intragenic mapping methods were modified from MOERMAN and BAILLIE (1979). Alleles of *unc-15* and *unc-13* were mapped in the following way: Triply-marked chromosomes were constructed that carried 1 allele of the gene to be mapped. A strain heterozygous for the 2 alleles was produced by mating heterozygous males (*e.g.*, *unc-15 (e1214)*/+) to the triple mutant (*e.g.*, *dpy-5(e61) unc-15(e73) unc-56(e403)*/ *dpy-5(e61) unc-15(e73) unc-56(e403)*). Paralyzed heterozygotes (Figure 1) were placed 1 per plate and allowed to self-cross. The number of progeny on a sample of plates was counted each generation, and the total number of screened chromosomes was estimated from these counts. All exceptional progeny (wild type, dumpy and uncoordinated) were progeny tested; only events showing exchange of flanking markers were used to calculate map distances between alleles (Figure 1).

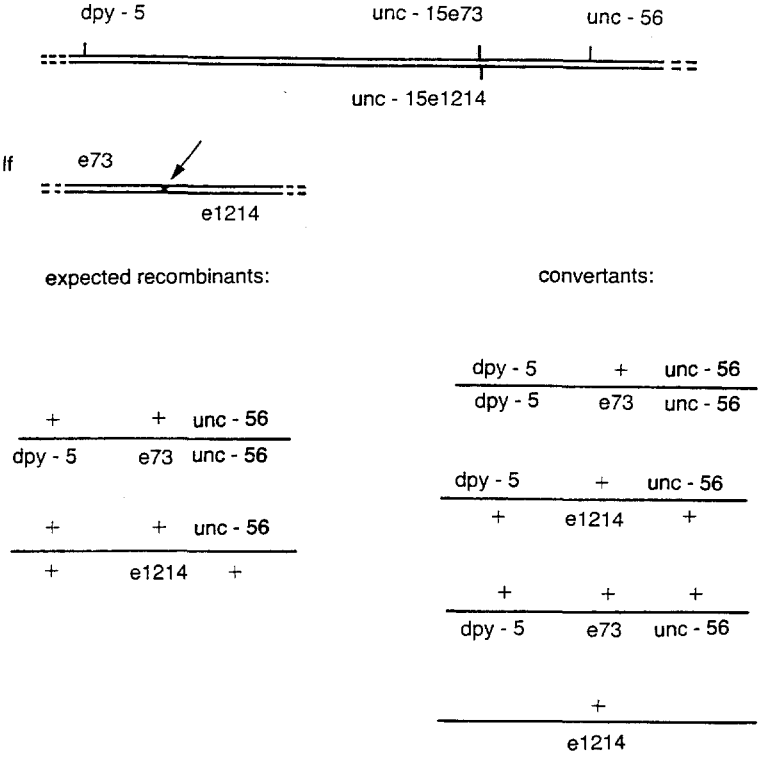

FIGURE 1.—Exceptional progeny expected when two alleles of the same gene are mapped. If hermaphrodites of the type shown are allowed to self-cross, intragenic recombination involving flanking markers and gene conversions will be rare. The positions of the two alleles were inferred from only those recombination events that also involved exchange of flanking markers.

RESULTS

Recovery of new mutations: From 1,600 treated heterozygotes, 32 recessive lethals and one recessive visible were recovered. Twenty of the recessive lethals were amenable to complementation analysis and recombination mapping; the other 12 strains were difficult ot analyze due either to low fecundity, ambiguity in mapping or segregation of a second-site mutation. One recessive dumpy, *s71*, was induced on the *unc-15* chromosome; *s71* defines a previously unidentified gene, *dpy-24(I)*, which maps to the right of *unc-56*.

Mapping of visible and lethal mutations: In order to determine the number of complementation groups formed by the 20 recessive lethal mutations recovered after EMS mutagenesis, male strains heterozygous for each lethal were constructed as described in MATERIALS AND METHODS. Males carrying one lethal were crossed to hermaphrodites carrying another lethal mutation, and the progeny were scored for the presence of dumpy-uncoordinated males. All pairwise complementation tests were done, and 16 complementation groups were identified.

Map positions for representative alleles of each of the lethal complementation groups were determined. Initially, nonvital loci in the region were mapped so that the essential loci could be positioned relative to known genes. *unc-37(e262)*, *unc-87(e1489)* and *mec-8(e398)* were positioned by three-factor crosses, as described in MATERIALS AND METHODS. Of these genes, only *mec-8* mapped between

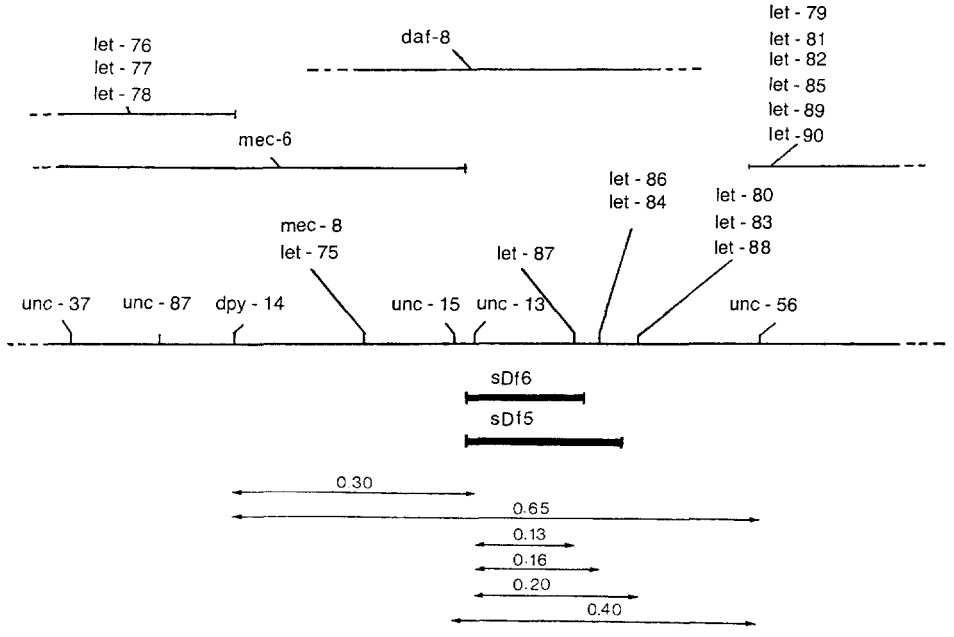

FIGURE 2.—The region around *unc-15* illustrating map positions for closely linked lethals, visibles and deficiencies. Two-factor recombination distances are represented by double-headed arrows. *mec-6* was positioned by M. CHALFIE and *daf-8* by D. RIDDLE.

dpy-14 and *unc-13* (Figure 2). The recombination distance between *dpy-14* and *unc-13* was determined as described previously (ROSE and BAILLIE 1979). Similar methods were used to measure the recombination distances between *dpy-14* and *unc-56* (0.7%) and between *unc-15* and *unc-56* (0.4%). *dpy-14*, *unc-15*, *unc-13* and *unc-56* were used to position the lethals. For example, *let-75* was positioned by analyzing the dumpy and uncoordinated recombinants from the heterozygous parent, *dpy-14(e188) let-75(s101)* + *unc-13(e51)*/+ + *unc-15 (e73)* +. Eight dumpy and six uncoordinated progeny were analyzed. Five of the uncoordinated recombinants did not carry *unc-15* (*dpy-14 let-75* + *unc-13*/ + + + *unc-13*), demonstrating that *let-75* is to the left of both *unc-15* and *unc-13*, but to the right of *dpy-14*. This places *let-75* close to *mec-8*.

Essential loci that mapped to the right of *unc-13* were further positioned by complementation with two deficiencies involving *unc-13*. Complementation tests showed that *sDf6* failed to complement *unc-13* and *let-87*; whereas, *sDf5* failed to complement *sDf6*, *unc-13*, *let-87*, *let-86* and *let-84* (Figure 2).

Phenotypes of visible and lethal mutations: Figure 2 illustrates 25 genes that are known to exist in the *unc-15* region. Mutants in some of these genes are known to be, or to interact with, muscle-defective mutants. For example, *unc-15* and *unc-87* mutants are paralyzed and have disorganized body-wall musculature; *unc-13* mutants are paralyzed when homozygous for *unc-22(s32)* (ANN ROSE, unpublished results). *dpy-14* mutants are first larval-stage lethals when homozygous for *unc-15* and *unc-54* (ANN ROSE, unpublished observations). Both *unc-15* and *unc-54* affect specific muscle proteins (see WATERSTON, THOMPSON and BRENNER 1980). *dpy-14* shows a similar pattern of synthetic lethality with *s106*. The strain *dpy-14* + *unc-13 let-87 (s106)*/ + *unc-15* + + segregates first larval-stage lethals. *unc-13* recombinants from this strain, however, segregate *unc-13 let-87* homozygotes that are semi-viable, although tiny and slow developing.

In our study, 17 of the 20 lethals arrested development in the first larval stage: *let-76(s80)*, *let-79(s81)*, *let-80(s96)*, *let-81(s88)*, *let-82(s85)*, *let-83(s97)*, *let-85(s142)*, *let-86(s141)*, *let-88(s132)*, *let-89(s133)* and *let-90(s140)*. Some of these may be synthetic lethals with *dpy-14*. Three of the lethals, *let-77(s90)*, *let-78(s82)* and *let-84(s91)*, arrest development at a later larval stage.

One of the lethals exhibits a range of phenotype. One allele of *let-87*, *s106*, is semi-viable in the absence of *dpy-14*, as discussed above. Crosses between *dpy-14* + *unc-13 let-87(s106)*/ + *unc-15* + + and + *unc-13 let-87(s87)*/ *unc-15* + + produce tiny, semi-lethal progeny as expected for noncomplementing alleles since the *s87* strain is wild type for *dpy-14*. Yet, among the self-cross progeny of the *s87* strain are first larval-stage lethals. This indicates that *s87* is not a synthetic lethal.

Mapping unc-15 *and* unc-13 *alleles:* The mapping of *unc-15* alleles is complicated by the fact that most alleles are complementing. WATERSTON, FISHPOOL and BRENNER (1977) have described the complementation pattern of *e73*, *e1214*, *e1215* and *e1402*. *e1214* produces no detectable paramyosin and fails to complement all other alleles. *e1215* complements *e73* and *e1402*. We have extended

TABLE 1

Fine structure mapping of unc-15 *alleles*

Parent (*x*/*y*)	Crossover	Convertants *x*	Convertants *y*	No. tested chromosomes
dpy-5 unc-15(e73) unc-56/+ *e1214* +	8†	0	0	480,000
dpy-5 unc-15(e1214) unc-56/+ *e1215* +	2*‡	0	0	250,000
dpy-5 unc-15(e1215) unc-56/+ *e1214* +	2*†	1*	4*	330,000
dpy-5 unc-15(su2000) unc-56/+ *e1214* +	4†	2	0	224,000
dpy-5 unc-15(su228) unc-56/+ *e1214* +	4†	0	0	88,000
dpy-5 unc-15(su2000) unc-56/+ *e73* +	2†	0	0	630,000
dpy-5 unc-15(e1215) unc-56/+ *su2000* +	2*†	2*	1*	275,000

* Clustered events recovered.
† *unc-56* carried by the recombinant chromosome.
‡ *dpy-5* carried by the recombinant chromosome.

this analysis: *su228* complements everything but *e1214*, and *su2000* fails to complement *e73*, *e1215* and *e1214*. We have mapped noncomplementing pairs of alleles as indicated in Table 1. From this table, it can be calculated that the maximum recombination frequency observed for *unc-15* is 4.5×10^{-3} map units across the *su228–e1214* interval.

The gene closest to *unc-15* is *unc-13*. We have scored 30,000 progeny from the *cis*-heterozygote, *unc-15(e73) unc-13(e51)*/ + + for *unc-13* recombinants; none were recovered. An upper 97.5% confidence limit for these data is 0.025 map units for the *unc-15* to *unc-13* distance. This agrees with the result of WATERSTON, FISHPOOL and BRENNER (1977), who calculated the distance to be $5/269 \times 2 = 0.037$ (0.01–0.08) map units.

No other gene has been identified between *unc-15* and *unc-13*. Thus, it is possible that *unc-15* and *unc-13* are adjacent. If so, fine-structure analysis of the coding elements of these two genes would, by exclusion, define the intragenic interval. Toward this end, we began constructing an *unc-13* intragenic map. Four alleles have been mapped; two of those are suppressed by *sup-5* (WATERSTON and BRENNER 1978). The mapping data are presented in Table 2. From these data, we calculate recombination frequency of 2.4×10^{-3} map units across the *e51–s69* interval. A summary of the *unc-15 unc-13* mapping is illustrated in Figure 3.

TABLE 2

Fine-structure mapping of unc-13

Parent (*x*/*y*)	Crossover	Convertants *x*	Convertants *y*	No. tested chromosomes
dpy-5 unc-13(e51) unc-56/+ *e450* +	2†	0	1	500,000
dpy-5 unc-13(e51) unc-56/+ *s69* +	24†	1	0	1,000,000
dpy-5 unc-13(e51) unc-56/+ *e1091* +	1†	0	0	>1,000,000

† *unc-56* carried by the recombinant chromosome.

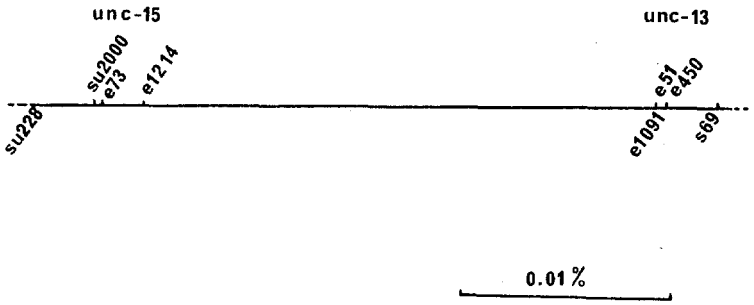

FIGURE 3.—Fine-structure map of *unc-15* and *unc-13*. Alleles shown above the line have been right-left positioned with regard to the adjacent alleles. Those shown below the line have been positioned relative to one other allele.

DISCUSSION

This paper describes a genetic analysis of the region around *unc-15* (*I*). Previously identified genes in this region were mapped, and new mutations were induced with EMS in order to identify essential genes around *unc-15*. We tested 1,600 *dpy-14 unc-13* chromosomes and recovered 32 lethal mutations.

Twenty of these were amenable to complementation analysis and recombination mapping. Of the 20 that were analyzed, 11 map within a 0.65 map unit region centered around *unc-15*. The frequency of induction of lethals in this region was 0.7% per map unit. BRENNER (1974) induced *X*-linked lethals, using 0.05 M EMS, at a frequency of 15% for the entire chromosome, which is 47.5 map units long, a frequency of 0.3% per map unit. MENEELY and HERMAN (1979) screened for lethal mutations over the duplication, *MnDp1*, which covers seven map units of the *X*-chromosome. In their study, a lethal induction frequency of 0.07% per map unit was observed. Our induction frequency (0.7% map unit) is within a factor of two of BRENNER's average frequency for the *X*-chromosome, but considerably higher than MENEELY and HERMAN's frequency for the tip of the *X*-chromosome. This difference may reflect a higher gene density around *unc-15* or a greater average mutability of the genes in this region.

In fact, the number of genes per map unit around *unc-15* is greater than the average number per map unit for the genome. For example, approximately three-quarters of the genes that BRENNER (1974) identified (118 genes across 320 map units) were situated in gene clusters like the one around *unc-15*, which we believe to be a consequence of differential recombination frequency. We have demonstrated a minimum of 12 genes across the *dpy-14–unc-56* interval, giving an average distance of 0.05% per site. Comparable results have been reported for regions of the *D. melanogaster* genome, 0.05% per band in the zeste-white region (JUDD, SHEN and KAUFMAN 1972) and 0.07% per band in the *Adh* region (WOODRUFF and ASHBURNER 1979).

We cannot formally exclude the possibility that regional gene clustering is due to increased mutagen sensitivity for these genes. We can, however, cite one example of a gene in this cluster that apparently gives fewer recovered mutants than does a gene outside the cluster. *unc-54* (*I*) maps to the tip of chromosome *I*. Two studies indicate that, under similar EMS-mutagenesis and screening conditions, fewer *unc-15* than *unc-54* mutations are recovered: one *unc-15* compared to five *unc-54* (BRENNER 1974) and one compared to 35 (ZENGEL and EPSTEIN 1980). Both genes mutate to give paralyzed mutants, but *unc-54* mutants are more readily recovered than *unc-15* mutants. This discrepancy could be a result of the mutability of the DNA, since mutational "hotspots" are well documented in prokaryotes. Or the discrepancy could be a result of the probability of recovering *unc-15* mutants. For example, the paramyosin-lacking *unc-15* allele, *e1214*, develops very slowly. Thus, reduced viability of paramyosin-null alleles could account for the reduced recovery rate. Alternatively, missense mutations affecting the long rod N-terminal portion of paramyosin might produce undetectable mutant phenotypes. Thus, the mutational target size would be considerably reduced. If the latter speculation is true, further fine-structure analysis of *unc-15* alleles would reveal a clustering of missense mutations within the *unc-15* map.

Previously, WATERSTON (unpublished results cited in WATERSTON, FISHPOOL and BRENNER 1977) detected recombinants between *e73* and *e1214* at a frequency of less than 0.01%. We have repeated this result, limiting consideration to those intragenic events for which flanking markers were recombinant. By counting all the progeny on a sample of plates, we could estimate the total number of chromosomes sampled. Using these methods, we found the *e73–e1214* distance to be 1.6×10^{-3} map units.

Only meiotic recombinant events were used to construct the map shown in Figure 3. One allele, *e1215*, could be positioned to the left of *su2000* by examining the phenotype of apparent recombinants. However, the fact that clusters of recombinants and convertants were recovered causes us to doubt that a meiotic event produced this result. For this reason, we have left *e1215* off the map.

We consider all the *unc-15* alleles shown in Figure 3 to be in the coding element: *su228*, *su2000* and *e73* because they are complementing alleles and *e1214* because it is suppressed by *sup-5*. The present map gives a minimum distance across the *unc-15* coding element of 4.5×10^{-3} map units. This minimum estimate may be falsely high since *unc-15* mutants produce few progeny ($\bar{x} = 31$ in our study). Only recombination events occurring in the first brood would be recovered. We have shown (ROSE and BAILLIE 1979) that recombination frequency in *C. elegans* is 50% higher in the first 12-hr brood ($\bar{x} = 54$ progeny) than the average recombination frequency calculated for all 250 progeny for a *dpy-5 unc-15*/+ + heterozygote. Therefore, the minimum distance across *su228–e1214* could be as small as 3×10^{-3} map unts.

The next closest gene to *unc-15* that has been identified is *unc-13*. Two of the alleles of *unc-13* that we mapped are suppressed by *sup*-5; thus, we believe that

at least a portion of the *unc-13* coding element is identified by the position of *e450* and *e1091*. The distance across *unc-13*, 2.4×10^{-3} map units, is comparable to the distance across *unc-15*, but smaller than the distance between *unc-15* and *unc-13*. Although these two genes have not been extensively mapped, nor the region around them extensively analyzed for mutable sites, we feel it worth commenting that the distance between *e1214* and *e51* is several times greater than the distance across either gene. Should this observation be substantiated by further investigation of the *unc-15–unc-13* intragenic region, it would agree with restriction map analysis of coding and noncoding regions. For example, the coding regions of the β-like globin genes (FLAVELL *et al.* 1978) and the ovalbumin-like genes (ROYAL *et al.* 1979) occupy only a small distance compared to the length of DNA between these genes. We would like to investigate the molecular arrangement of the DNA in this region by analysis of a *C. elegans* recombinant DNA clone that carries the *unc-15* gene.

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