GENETIC ORGANIZATION IN *CAENORHABDITIS ELEGANS*: FINE-STRUCTURE ANALYSIS OF THE *unc-22* GENE

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ABSTRACT

Fine-structure analysis of the *unc-22* gene of *Caenorhabditis elegans* has revealed a number of sites that are separable by recombination. Eight new ethyl methanesulfonate-induced recessive mutations of the *unc-22* gene have been isolated. Using these new alleles, as well as *e66*, a number of separable sites have been identified and positioned relative to one another. The map distances obtained are found to be comparable to those associated with intragenic recombination in *Drosophila melanogaster*, indicating that genetic fine-structure analysis is feasible in *Caenorhabditis elegans*. Evidence of possible gene conversion is presented. A preliminary estimate of the *unc-22* gene size is 2.4×10^{-2} map units.

THE free-living nematode, *Caenorhabditis elegans*, has in recent years become a promising organism for the study of developmental processes. The cell lineage is almost entirely described (DEPPE *et al.* 1978; SULSTON and HORVITZ 1977), and it is amenable to genetic dissection (NIGON and DOUGHERTY 1950; BRENNER 1974; HERMAN 1978). The role of one gene in this organism, *unc-22* on linkage group IV (BRENNER 1974), is of particular interest to us since it is believed to play a role in muscle structure and function. Most mutations in this gene are recessive to wild type. When homozygous for *unc-22*, worms display a characteristic twitching pattern along the body-wall musculature; hence, homozygous *unc-22* worms are referred to as "twitchers". Compared to wild type, these worms are thinner, move more slowly and have a developmental time that is somewhat retarded (MOERMAN, unpublished observations). Electron microscope studies of twitchers reveal highly disorganized body-wall musculatures (POPHAM, BAILLIE and MOERMAN, unpublished observations). Our eventual goal is to determine how the *unc-22* gene is regulated during the development of *C. elegans*. We therefore would like to delineate the region of the genome containing the *unc-22* gene. Toward this end we have initially tried to obtain a fine-structure map of the *unc-22* gene.

Recombination analysis is one of the most powerful tools with which to probe the nature of the gene. *C. elegans* is quite suitable for this type of analysis. Its DNA content is known and an extensive map of the genome is available (SULSTON and BRENNER 1974; BRENNER 1974). This organism is easily cultured in

large numbers, and mutants are relatively easy to isolate. Methods for the selection and mapping of alleles of the *unc-22* gene are described here. This is the first extensive fine-structure map reported for *C. elegans*.

MATERIALS AND METHODS

Nematode strains, media and culture conditions: NG agar as described by BRENNER (1974) was used throughout this study. *E. coli* (OP-50) in nutrient broth (Difco) at 2–3 $\times$ 10^8 cells/ml were used for streaking plates. 100 mm Petri plates were used for the maintenance of the strains (at 16°) and for the isolation of mutations. *C. elegans* has two sexes: self-fertilizing hermaphrodites and males. The wild-type hermaphrodite stock, N-2-S, used in this study was derived from a single N-2 from the Cambridge stock collection. N-2-S male stocks were maintained by crossing males (*5AA,XO*) to N-2-S hermaphrodites (*5AA,XX*) each generation. The mutant strains utilized in this work are listed in Table 1.

Mutagenesis and protocol for isolating mutations in the unc-22 *gene*: The mutagen ethyl methanesulfonate (EMS) was used. N-2-S worms were washed off stock plates with M9 buffer (BRENNER 1974) and collected after centrifugation (200 $\times$ *g*). These worms were then suspended in either 0.05 M EMS (20 λ EMS into 4 ml. of M9 buffer), 0.025 M EMS or 0.0125 M EMS. After four hours at 20°, they were removed from the EMS with a pasteur pipette and spotted onto a petri plate. After approximately one hour, fourth-stage larvae and young adult worms were placed (20 per plate) on petri plates with lawns of OP-50. These worms were left to lay eggs for 24 hr and were then removed. Three days later, the F_1 progeny were screened for putative *unc-22* mutants.

To screen the F_1 progeny for putative mutations in the *unc-22* gene, we took advantage of the different behaviors exhibited by wild-type and twitcher worms in a 1% solution of nicotine alkaloid (Sigma). Worms when either homozygous or heterozygous for an allele of the *unc-22* gene twitch in a 1% solution of nicotine, whereas wild-type worms in this solution become rigid. Using nicotine, we could screen for twitchers in the F_1 generation. To insure that all new mutations were separate alleles, only one mutant was taken per plate. Prior to any further examination, these new mutants were outcrossed to wild-type males and allowed to resegregate from the F_1 progeny. A stock of the mutant self-fertilizing hermaphrodite was then established.

TABLE 1

Mutant strains

Linkage group	Gene name	Allele‡	Phenotype
IV	*dpy-4*	*e1166**	dumpy
	unc-5	*e152*	uncoordinated (slow)
	unc-22	*e66*	uncoordinated (twitches)
		s7†	uncoordinated (twitches)
		s8	uncoordinated (twitches)
		s12	uncoordinated (twitches)
		s13	uncoordinated (twitches)
		s14	uncoordinated (twitches)
		s16	uncoordinated (twitches)
		s17	uncoordinated (twitches)
		s18	uncoordinated (twitches)

* *e*, isolated at Cambridge, England.
† *s*, isolated at Simon Fraser, Canada.
‡ All *unc-22* alleles except *s7* and *s8* were isolated using 0.05 M EMS. *s7* was isolated using 0.025 M EMS and *s8* was isolated using 0.0125 M EMS.

Construction of triple mutants: Triple mutants of the following genotype, *unc-5(e152) unc-22(sx) dpy-4(e1166)*, were needed to position the twitcher alleles relative to one another. These were constructed using the following protocol. Homozygous twitcher hermaphrodites were outcrossed to N-2-S males. The F_1 male progeny were then mated to hermaphrodites that were homozygous for *dpy-4(e1166)*. From this cross young hermaphrodite progeny that twitched in a solution of 1% nicotine were picked and allowed to lay eggs. When the latter had grown, they were screened for dumpys that twitched in the 1% nicotine solution. Once found, these dumpys were placed on a plate and allowed to lay eggs, one-fourth of which should be homozygous dumpy and twitcher. The double dumpy twitchers were then outcrossed to N-2-S males. The F_1 male progeny this time were crossed to hermaphrodites homozygous for *unc-5(e152)* and 1% nicotine solution was added to the progeny. Again the young hermaphrodites that twitched were selected and allowed to lay eggs. These were allowed to mature and *unc-5* hermaphrodites that twitched in nicotine were placed on fresh plates. Approximately one-fourth of their progeny were triple mutants. Individuals of the genotype, *unc-5(e152) unc-22(sx) dpy-4(e1166)*, were picked and established as a stock.

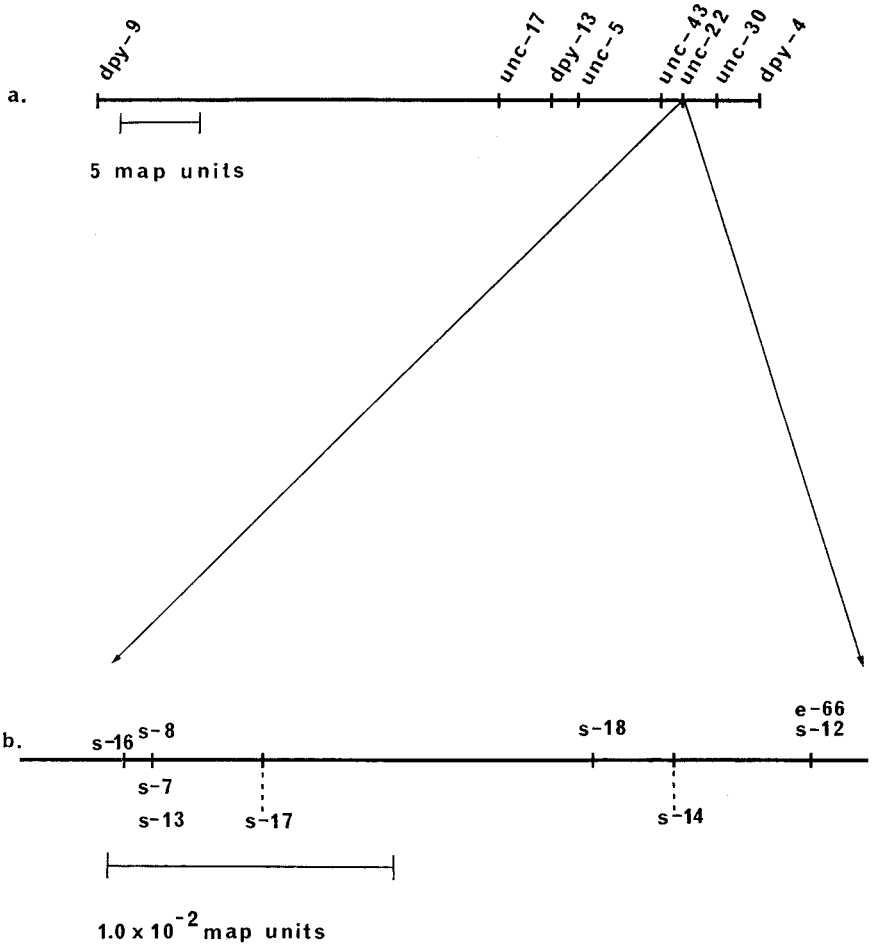

FIGURE 1a.—Recombination map of linkage group IV. Modified from BRENNER (1974) and RIDDLE (1978).

FIGURE 1b.—Fine-structure map of the *unc-22* gene.

Mapping of the mutations to linkage group IV and the unc-22 *locus*: In order to determine if a mutation was on linkage group IV, males of the putative genotype *unc-22(sx)* +/+ + were crossed to hermaphrodites that were + *dpy-4(e1166) IV*/+ *dpy-4(e1166)* IV. Young F_1 hermaphrodites that twitched in nicotine were picked. This insured that these hermaphodites would be heterozygous for both the twitcher mutation and the dumpy mutation. In a self-fertilizing hermaphrodite, if the twitcher mutation and the *dpy-4* gene were not linked, one would expect 1/16 of the F_2 progeny to be both dumpy and twitching. The *unc-22* gene, however, is about 5 map units from *dpy-4* (see Figure 1a). This means that mutations defective in *unc-22* should produce the double mutant segregants with a frequency of only 1/1600 in the F_2 generation.

Genetic complementation tests were done using the method described by BRENNER (1974). Heterozygous males of the genotype *unc-22(sx) IV*/+ were crossed to hermaphrodites of the genotype *unc-22(sy)* IV. The F_1 males were screened to determine if half of them were twitchers.

Mapping within the unc-22 *gene*: The intragenic mapping was done in a manner similar to a method described by R. WATERSTON (personal communication). Young adult hermaphrodites of the genotype + *unc-22(sy)* +/ *unc-5(e152) unc-22(sx) dpy-4(e1166)* were cultured, one per 100 mm plate, at 20° and allowed to lay eggs for 12 hr. They were then transferred to a new plate, again for 12 hr. This was done until there were six 12-hour broods for every heterozygous parent. The plates were then left until the F_2 generation was mature before they were scored.

An estimate of the total number of progeny screened was achieved by the following method. The progeny from a random sample of plates (approximately 10%) from each brood was counted. The mean number of progeny per plate was determined and this number was multiplied by the number of plates in the brood. The broods were then summed. Only worms that were in the third-larval stage or beyond were counted, since screening of the plates was done primarily at a low magnification and recombinants in earlier larval stages would have been missed. To score for recombinants, we looked for worms that were either wild type, dumpy, *unc-5*, or dumpy *unc-5* in appearance, that is, all nontwitcher phenotypes.

RESULTS AND DISCUSSION

Mapping the mutations to linkage group IV and the unc-22 *gene*: Using the technique described in the MATERIALS AND METHODS, eight new recessive twitcher mutations have been isolated (Table 1). All of these twitcher mutations map to linkage group IV. Complementation tests with all pair-wise combinations of *e66*, (the canonical allele of the *unc-22* locus), *s7*, *s8*, *s12*, *s13*, *s14*, *s17*, and *s18* have been done. All combinations gave twitcher males in the F_1. Therefore, all these mutations failed to complement one another. The allele *s16* has been tested only with *s8*. This pair also failed to complement. These two pieces of evidence, map location and complementation, confirm that these twitcher mutations are alleles of the *unc-22* locus.

Mapping within the unc-22 *gene*: Table 2 shows the results of the fine-structure mapping procedure described in the MATERIALS AND METHODS. Eight new alleles and *e66* have been mapped within the *unc-22* locus. Six of eight tested alleles have been separated from *s8*. Also, *s18* and *s14* have been separated from *s12*. Since in all crosses the parent hermaphrodites were heterozygous for the flanking outside markers, the left/right position for any two twitcher alleles that exhibited recombination could be determined. That is, in the genotype + *unc-22(sy)* +/ *unc-5(e152) unc-22(sx) dpy-4(e1166)*, if *sy* were to the left of *sx*, then the recombinant chromosomes that could be scored would be detected as either wild-

TABLE 2

Recombination data between the various alleles of the unc-22 *gene*

Allele pairs tested†	Frequency of of *unc-22*+	Map distance (in map units)*	L/R	
s8/s7	0/84,000	—	—	
s8/s12	5/72,000	1.4×10^{-2}	*s8*	*s12*
s8/s13	0/64,000	—	—	
s8/s14	5/74,000	1.4×10^{-2}	*s8*	*s14*
s8/s17	1/58,000	3.4×10^{-3}	*s8*	*s17*
s8/s18	2/52,000	7.7×10^{-3}	*s8*	*s18*
s8/e66	8/70,000	2.3×10^{-2}	*s8*	*e66*
s18/s12	4/235,000	3.4×10^{-3}	*s18*	*s12*
e66/s12	0/224,000	—	—	
s14/s12	5/252,000	4.0×10^{-3}	*s14*	*s12*
s16/s8	1/262,000	7.6×10^{-4}	*s16*	*s8*

* Map distance (d) $= \frac{2(\textit{unc-22}+ \text{ recombinants}) \times 100}{\text{total offspring}}$.

† Mutation at left was in triple, *unc-5*, *unc-22*, *dpy-4*.

type or *unc-5* worms. If *sy* were to the right of *sx*, then the recombinant chromosomes would appear as either wild-type or *dpy-4* worms. To determine the genotype of the recombinant chromosomes, and to insure that we were observing recombinants, all exceptional individuals were progeny tested.

The results of these progeny tests showed that of the 36 putative recombinants recovered, two were sterile, 31 segregated the markers in a manner that was compatible with chromatid exchange and three were recovered without a flanking marker (Table 3). Only the 31 confirmed recombinants were used to determine the map distances in Table 2. The three exceptional individuals that lost their flanking markers could have resulted from outside marker exchange or gene conversion. Our data cannot distinguish between these two alternatives. Since gene conversion is well documented in fungal species (MITCHELL 1955; FOGEL and MORTIMER 1969), as well as in *Drosophila melanogaster* (SMITH, FINERTY and CHOVNICK 1970), we would expect it to occur in *Caenorhabditis elegans*. If these exceptional individuals are indeed convertants, then an estimated rate of gene conversion would be in the order of 10^{-5}. This is similar to the rate of gene conversion found for various alleles of the rosy locus in *Drosophila melanogaster* (CHOVNICK, BALLANTYNE and HOLM 1971).

The 31 confirmed recombinants give the left/right position of the various alleles. From this information, an intragenic map of the *unc-22* gene can be constructed (Figure 1b). On this map, the alleles that have been positioned unambiguously are shown above the line. These include, *s16*, *s8*, *s18* and *s12*, the allele *e66* is included above the line since it is closely linked to *s12*. Although the alleles, *s13* and *s7*, have not been separated from *s8*, they are listed below the line since they have not been positioned relative to either *s16* or *s17*. The allele *s17* has been separated from *s8*, but has not been positioned relative to any of the

TABLE 3

Progeny testing of putative recombinants

Allele pair tested	Total putative recombinants	Recombinant phenotype	Sterile*	Outside marker segregated
e152 s8 e1166/s12	5	dumpy		dpy
		dumpy		dpy
		wild		dpy
		wild		dpy
		wild		dpy
e152 s8 e1166/s14	5	dumpy		dpy
		dumpy		dpy
		dumpy		dpy
		dumpy		dpy
		wild		dpy
e152 s8 e1166/s17	1	wild		dpy
e152 s8 e1166/s18	2	wild		dpy
		wild		dpy
e152 s8 e1166/e66	8	wild		dpy
		wild		dpy
		dumpy		dpy
		wild		dpy
		dumpy		dpy
		dumpy		dpy
		wild		dpy
		wild		dpy
e152 s18 e1166/s12	6	wild		none
		wild		dpy
		wild		dpy
		wild		dpy
		wild		dpy
		wild	+	—
e152 s12 e1166/s14	7	uncoordinated		unc
		uncoordinated		unc
		wild		unc
		wild		unc
		wild		none
		uncoordinated		unc
		wild		none
e152 s8 e1166/s16	2	wild		unc
		uncoordinated	+	—

* Sterile recombinants were not included in the calculation of map distances.

other alleles. The position of *s17* is therefore shown with a dotted line. The same is true for *s14*, which is known to be between *s8* and *s12*, but has not been localized relative to *s18*. The distances shown between the various alleles should be taken as tentative since they are based on few recombinants.

The size of the unc-22 *gene*: A preliminary estimate of the *unc-22* gene size is 2.4×10^{-2} map units. This is based on the distance between the two outermost alleles, *s16* and *e66*. This distance was determined by adding the distance from

s16 to *s8* to the distance from *s8* to *e66*. Since this is the first recombination map for any gene in *C. elegans*, there are no other genes with which to compare in regard to size. *D. melanogaster*, however, offers a number of loci in which intragenic recombination has been observed. These loci are of two types; simple cistrons like rosy and maroon-like; and complex loci, examples of which are rudimentary and white. Rosy and maroon-like are both smaller than the *unc-22* locus, rosy being 5×10^{-3} map units (GELBART, MCCARRON and CHOVNICK 1976) and maroon-like, 1×10^{-3} map units (DUCK and CHOVNICK 1975). The complex loci, for example rudimentary at 7×10^{-2} map units (CARLSON 1971), are generally larger than the *unc-22* locus. The size of white, however, at 2.6×10^{-2} map units (JUDD 1964), is very close to our estimate for the size of the *unc-22* gene.

Is *unc-22* then a complex locus? We do not believe that it is. The similarity in recombination rates between the *unc-22* locus and the white locus may be misleading since these rates most probably do not reflect a similar amount of DNA. LEFEVRE (1971) has calculated that the white locus contains approximately 10 kb of DNA. In contrast, from the data of SULSTON and BRENNER (1974) on the amount of unique DNA in the worm, 6.7×10^7 base pairs, and the total map distances for the genome of 320 map units (BRENNER 1974; RIDDLE 1978), we have calculated that there is approximately 5 kb of DNA in the *unc-22* gene. This indicates that there is possibly a two-fold difference in the DNA content of these two loci. This difference in DNA content, as well as the allelism tests and the evidence of gene conversion, suggest that the *unc-22* locus is a single cistron, albeit a somewhat large one when compared to loci in *D. melanogaster*.

This can perhaps be explained by the following comparison. The average amount of DNA contained in 0.01 map units in *C. elegans* is 2.1 kb. This can be compared to 5.8 kb in 0.01 map units in *D. melanogaster*. This latter calculation is based on 1.6×10^8 base pairs as the total genome DNA represented as single copy and middle repetitive sequences (RASCH, BARR and RASCH 1971; MANNING, SCHMID and DAVIDSON 1975) and 275 map units as the total map length of the genome (LINDSLEY and GRELL 1968). This more than two-fold difference demonstrates that, on the average, recombination per nucleotide occurs more frequently in *C. elegans* than in *D. melanogaster*. We would expect then to see genes with a larger recombination size in *C. elegans*.

Applying the value of 2.1 kb of DNA per 0.01 map units also makes possible a rough estimate of the resolving power of our mapping procedure. The two closest alleles so far resolved are *s16* and *s8*. They are about 7.6×10^{-4} map units apart (Table 2). A first-order approximation of their physical separation is 160 nucleotides. It should be emphasized that 160 nucleotides is not the limit of resolution of this system. It is quite possible to extend the resolution to at least 40 nucleotides. This would involve the screening of 10^6 worms, a number well within the practical limits of this system.

Having constructed a preliminary fine-structure map of the *unc-22* gene, our approach now will be to concentrate on delineating the structural and regulatory

elements within this region. We may find, of course, that this entire region codes for the structural element, and that any *cis*-linked regulatory sites lie outside of this area, as was discovered at the rosy locus in *D. melanogaster* (CHOVNICK *et al.* 1976). We suspect that this may be the case for the following reason: if one takes the 2.4×10^{-2} map units as an average gene size and divides the total 320 map units by this number, one would estimate that there are 13,500 genes in this organism. This is a much higher number than BRENNER's (1974) estimate of 2,000 genes or BAILLIE's (unpublished results) of 4,000 genes, which are based on forward mutation rates. If, however, this whole region were structural and the regulatory sites were outside of it, then the number of genes would be considerably less. Further genetic analysis of this region may resolve this problem.

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