Mol Gen Genet (1990) 221:459–465

MGG

Genetic and fine structure analysis of *unc-26(IV)* and adjacent regions in *Caenorhabditis elegans*

David L. Charest*, Denise V. Clark, Michael E. Green, and David L. Baillie**
Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. Canada, V5A 1S6

Summary. The genetic organization of *unc-26(IV)* and adjacent regions was studied in *Caenorhabditis elegans*. We constructed a fine structure genetic map of *unc-26(IV)*, a gene that affects locomotion and pharyngeal muscle movement but not muscle structure. Eleven alleles were positioned relative to each other recombinationally and were classified according to phenotypic severity. The *unc-26* gene spans at least 0.026 map units, which is exceptionally large for a *C. elegans* gene. All but one allele, *e205*, are amorphic alleles. Interestingly, *e205* is hypomorphic but also suppressible by the amber suppressor *sup-7*. Nineteen lethal mutations in the *unc-26* region were isolated and characterized. The *unc-26* region is subdivided into four zones by five deficiency breakpoints. These mutations fall into 15 complementation groups. The stages of development affected by these mutations were determined.

Key words: *Caenorhabditis elegans* – *unc-26* – Intragenic mapping – Lethal mutations

Introduction

The proper development and function of the neuromuscular system involves the complex expression and regulation of a large number of genes. In the nematode *Caenorhabditis elegans*, there are 5 classes of interneurones, 7 classes of motorneurones, and 95 body muscles (White et al. 1976). There are well over 100 genes affecting behaviour in *C. elegans*. Mutations in genes that affect proper locomotion are termed uncoordinated (*unc*).

Polarized light microscopy, electron microscopy and biochemical analysis have demonstrated that mutants in more than 20 genes have defects in muscle structure (Waterston et al. 1980). Several mutants with altered cell lineages in motoneurones cause noticeable changes in movement (see White et al. 1982 for example). However, the great majority of the *unc* genes have no known physiological or biochemical function.

* *Present address:* Biomedical Research Centre, 2222 Health Sciences Mall, University of British Columbia, Vancouver, B.C. Canada V6T 1W5
** *Present address:* University of British Columbia, Faculty of Medicine, 2194 Health Sciences Mall, Vancouver, B.C. Canada V6T 1W5
Offprint requests to: D.L. Baillie

The suitability of *C. elegans* for genetic fine structure analysis has been demonstrated with the *unc-22(IV)* muscle gene (Moerman and Baillie 1979; Rogalski and Baillie 1985). Fine structure maps have also been generated for other muscle genes including *unc-15(I)* and *unc-13(I)* (Rose and Baillie 1980), *unc-54(I)* (Waterston et al. 1982) and *unc-60(V)* (McKim et al. 1988).

The first goal of this study was to investigate the genetic organization of the *unc-26(IV)* gene, which was first identified by Brenner (1974). The gene plays a definite role in locomotion since mutant alleles cause paralysis. Polarized light microscopy has shown that the mutant *unc-26(e205)* displays no morphological alterations in muscle structure (Waterston et al. 1980). We describe a phenotypic characterization and genetic fine structure analysis of 11 *unc-26* alleles. One of these alleles is the *mut-4(st700)*-induced allele *s1710*. This allele may provide the means to isolate the coding sequences of *unc-26*, as mutations isolated in a *mut-4* background most often are the result of an insertion of the transposable element Tc1 (Mori et al. 1988).

The second goal of this study was to characterize the essential genes in the regions adjacent to *unc-26* in order to determine the limits of *unc-26* on the genetic map. Additionally, a study of adjacent genes may reveal that genes in this region are expressed coordinately and/or are functionally related. We describe the mapping of 19 lethal mutations relative to *unc-26* using three-factor and deficiency mapping. The mutations fall into 15 previously unidentified complementation groups with lethal mutant phenotypes.

Materials and methods

General. All nematode strains were propagated and matings performed according to the protocol outlined by Brenner (1974). The animals were cultured on petri plates of nematode growth medium streaked with *Escherichia coli* strain OP50. All recombination experiments were carried out at a constant 20° C after the recommendations of Rose and Baillie (1979). The nomenclature in this paper follows that recommended by Horvitz et al. (1979).

Nematode strains. The mutant strains used in this study were derived from the wild-type *C. elegans* (var. Bristol) strain known as N2. N2 and the following mutant strains were obtained from the stock collection at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England or the *Caenorhabditis elegans* Genetics

Center at the University of Missouri, Columbia: LGIII, *dpy-18(e364)*; LGIV, *dpy-4(e1166)*, *dpy-20(e1282)*, *unc-26(e176, e205, e314,* is: *e345, e429, e446, e568, e1048, e1196 and m2)* [note that the alleles *e176* and *e345* were initially assigned to *unc-48* in Brenner (1974)], *unc-31(e169)*; *nT1(IV;V)* (Ferguson and Horvitz 1985). The strain carrying *nDf27* was obtained from the laboratory of R. Horvitz, M.I.T. The strain RW7037 [*mut-4(st700)I;unc-22(st136)IV*] was obtained from I. Mori, D. Moerman and R. Waterston. The following LGIV mutations were isolated at Simon Fraser University: *unc-22(s7, s12)* (Moerman and Baillie 1979), *sDf2* (Moerman and Baillie 1981), *sDf21* and *sDf22* (G. Wild and D.L. Baillie, unpublished results; Clark et al. 1988), *sDf60* (Denise Clark, unpublished results). The 19 lethal mutations were isolated in this and a previous study (Clark et al. 1988) and are listed in Table 1. All lethal mutations, including deficiencies, were balanced using the translocation *nT1(IV;V)*, which suppresses recombination over LGIV (right) and LGV (left) and is associated with a recessive mutation which results in a vulvaless (Vul) phenotype (Ferguson and Horvitz 1985). The recombination-suppressed region includes the 21 map unit (m.u.) interval from *lin-1* to *dpy-4* on LGIV (Clark et al. 1988).

It is important to note that *unc-22* mutations are used as markers throughout this study. *unc-22(s7 and s12)* are conditionally dominant mutations where, in a 1% nicotine solution (Sigma), wild-type individuals are paralyzed while *unc-22/+* individuals have a "twitcher" phenotype. Homozygous *unc-22* individuals are unconditional twitchers (Moerman and Baillie 1979).

Isolation of EMS-induced recessive lethal mutations. Thirteen lethal mutations were isolated as described in Clark et al. (1988). This is indicated as Screen I in Table 1. Briefly, *unc-22(s7)unc-31(e169)/nT1(IV);+/nT1(V)* hermaphrodites were exposed to 0.012 M ethylmethane sulfonate (EMS) for 4 h and then allowed to self fertilize. All wild-type F_1 individuals were allowed to self fertilize on separate plates and the F_2 generation was screened for the absence of gravid Unc-22 Unc-31 hermaphrodites.

Six lethal mutations were isolated in the following way (Screen II): hermaphrodites of the genotype *unc-22(s7)unc-31(e169)* were exposed to EMS as above and mated to wild-type males. Wild-type fourth larval stage (L4) F_1 hermaphrodites were allowed to self fertilize and the F_2 generation was screened as above. Since the lethal mutation was not balanced, and the goal of the screen was to isolate lethal mutations tightly linked to *unc-22*, only F_1 lines that segregated three or fewer Unc-22 Unc-31 recombinant progeny were retained. The lethal mutations were subsequently balanced over *nT1* by crossing *unc-22 unc-31 [let-x]/+++* hermaphrodites to *unc-31/nT1(IV);+/nT1(V)* males and selecting wild-type F_1 hermaphrodites that segregate *nT1* homozygotes with the Vul phenotype and that twitch in 1% nicotine.

Complementation tests. Hermaphrodites of the genotype *unc-22(s7)unc-31(e169)[let-(sx)]/nT1(IV);+/nT1(V)* were crossed to N2 males and wild-type F_1 males that twitch in 1% nicotine were selected [*unc-22(s7)unc-31(e169)[let-(sx)]/+++*]. These males were then crossed to *Dfx/nT1(IV);+/nT1(V)* or *unc-22(s7)unc-31(e169)[let-(sy)]/nT1(IV);+/nT1(V)* hermaphrodites (*Dfx* is *nDf27*, *sDf2*, *sDf21*, *sDf22*, or *sDf60* and *let-(sx or sy)* is listed in Table 1). The male and hermaphrodite progeny were scored. The presence of fertile Unc-22 Unc-31 hermaphrodites or, in the case of *sDf21* or *sDf22*, Unc-31 hermaphrodites indicated complementation.

Lethal mutations mapping in the *unc-26* region were tested for complementation with *unc-26(e345)*. *unc-22(s7)unc-31(e169)[let-(sx)]/nT1(IV);+/nT1(V)* hermaphrodites were mated to N2 males and F_1 twitching males were selected in nicotine and crossed to *unc-26(e345)dpy-4(e1166)* hermaphrodites. The progeny of this cross were screened for paralyzed males. The presence of wild-type males and hermaphrodites that twitched in nicotine confirmed that the *unc-22 unc-31 [+let-(sx)] +/++ [unc-26(e345) +] dpy-4* heterozygotes were indeed viable and that the lethal mutation and *unc-26(e345)* complement each other.

Right-left positioning of lethal mutations relative to unc-26. Lethal mutations that mapped in zone 13 (see Results) were positioned relative to *unc-26(e345)*. The progeny of self-fertilized *unc-22(s7)unc-31(e169)[+ let-x] +/++ [unc-26(e345) +] dpy-4(e1166)* hermaphrodites were screened for Dpy-4 recombinant individuals (see Table 1). If the map order was *unc-22 – unc-31 – let-x – unc-26 – dpy-4*, then Dpy-4 recombinants segregated only Dpy-4 and Dpy-4 Unc-26 viable progeny. If the map order was *unc-22 – unc-31 – unc-26 – let-x – dpy-4*, then there were two classes of Dpy-4 recombinants. One class segregated only Dpy-4 and Dpy-4 Unc-26; the other class segregated, in addition, Unc-22 Unc-31 Dpy-4. In situations where recombinants did not segregate the appropriate progeny classes, the lethal mutation was assumed to be tightly linked to, or to the left of, *unc-26*.

Lethal phases. The stage at which *unc-22(s7)unc-31(e169)*-linked lethal mutations arrest development was qualitatively determined by observing the phenotype of *unc-22(s7)unc-31(e169)[let-(sx)]/nDf27* or *sDf21* individuals at 20° C.

Isolation of a mut-4*-induced* unc-26 *allele.* The mutator strain RW7037 [*mut-4(st700)unc-22(st136)IV*] has an Unc-22 phenotype, and a wild-type background was necessary in order to screen for other Unc mutations. From previous work (Moerman and Waterston 1984), it was evident that it would be relatively simple to isolate a revertant of *unc-22(st136)* rather than recombinationally separating *mut-4(st700)* and *unc-22(st136)*. Additionally, simply reverting *unc-22(st136)* would not alter the genomic background of the mutator strain. Briefly, P_0 hermaphrodites were placed individually on plates and transferred every 24 h until four broods had been established. The F_1 generation was then screened for wild-type revertants. A single excision event allowed detection of a wild-type revertant in the first generation. A single wild-type individual was selected and its genotype determined by progeny testing. Once the reversion event had been demonstrated to be a germ-line excision of Tc1, a single wild-type individual was self-crossed and a homozygous wild-type strain was established. Thus the genotype of this strain is *mut-4(st700)IV*. Hermaphrodites of this strain were placed singly on petri plates and allowed to self-fertilize over 4 days. Each P_0 was transferred to a fresh plate every 24 h. This prevented overcrowding and improved the probability of finding a

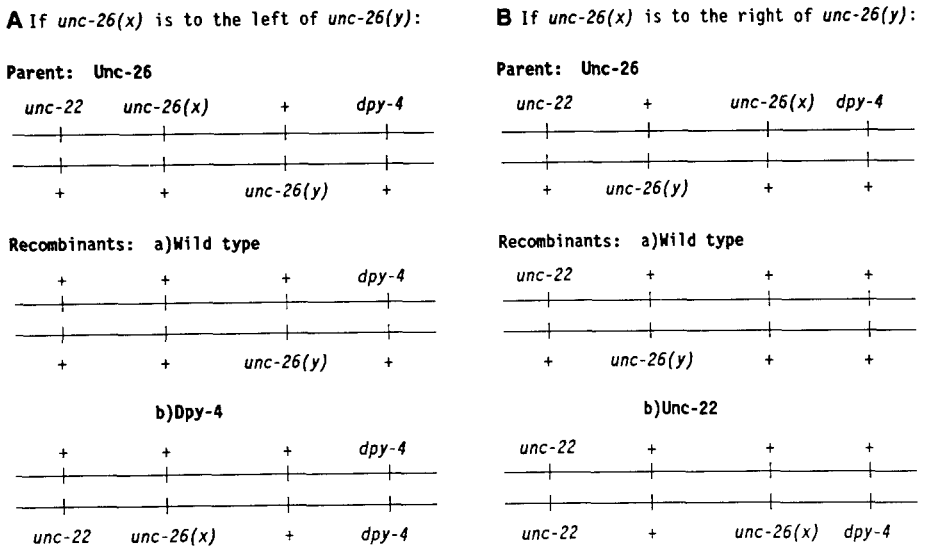

Fig. 1 A and B. The phenotypes and genotypes of hermaphrodites heteroallelic for *unc-26* and of the progeny that result from a single intragenic recombination event between alleles *x* and *y*. The genotypes are schematically shown with line drawings of LGIV. **A** and **B** outline the possibilities depending on the orientation of alleles *x* and *y*

rare mutant individual. After the F_2 generation had matured past the fourth larval stage, the plates were screened for morphological mutations. Putative *unc-26* mutations were complementation tested with the allele *e345* by crossing *unc-26(x)/+* males to *unc-26(e345)* hermaphrodites. The progeny were scored for the presence of wild-type and Unc-26 males.

Assessment of unc-26 *mutant phenotypes.* Phenotypic severity was measured qualitatively and was based on severity of paralysis. The phenotype of *unc-26(x)* hemizygotes was observed in hermaphrodites of the genotypes *unc-26/nDf27* and *unc-26/sDf21*. The hemizygous phenotype was compared with that of the homozygote in self progeny of *unc-26(x)/+* hermaphrodites. Because the homozygotes are observed after being outcrossed to N2 males, we presume that any difference between the hemizygous and homozygous phenotypes is due to the particular *unc-26* allele and not to a second mutation elsewhere in the genome.

Intragenic mapping of unc-26 *alleles.* The method used for mapping *unc-26* mutations was modified from Rogalski and Baillie (1985). Triply mutant hermaphrodites of the genotype *unc-22(s7)unc-26(x)dpy-4(e1166)* [or in one case *unc-22(s12)unc-26(e345)dpy-4(e1166)*] were mated to *unc-26(y)/+* males. Note that *x* and *y* represent two different alleles. Between 25 and 60 young F_1 Unc-26 hermaphrodites [*unc-22(s7 or s12)unc-26(x)dpy-4(e1166)/+ unc-26(y) +*] were set on 100 mm plates individually and allowed to self-fertilize through two generations. To avoid overcrowding, each P_0 was transferred to a fresh plate every 24 h. The plates were screened for non-Unc-26 exceptional individuals (either wild-type, Unc-22, or Dpy-4) after 7–8 days at 20° C. Only those individuals exhibiting an exchange of flanking makers were used in estimating the map distance separating the two alleles (see Table 2).

The map distance between two *unc-26* alleles was calculated using the formula of Moerman and Baillie (1979):

$$d = 2(W)\ 100/T,$$

where *d* is the distance in m.u., *W* is the number of exceptional individuals and *T* is the estimated number of chromosomes screened. The number of exceptionals was doubled since, in this situation, half of the recombinants were phenotypically Unc-22Unc-26, Unc-26, or Unc-26Dpy-4 and were thus not detectable. The total number of F_2 chromosomes screened, *T*, was estimated by doubling the number of F_2 progeny. The following scheme was used in determining the total number of F_2 progeny. First, the number of progeny produced by *unc-22 unc-26(x) dpy-4/+ unc-26(y) +* hermaphrodites was determined by counting the total progeny of five to ten individuals and calculating the mean brood size. The total F_1 progeny number was determined by multiplying the mean brood size by the number of P_0s. This number was further divided in half since one-half of the F_1s would be homozygous for either of the alleles and consequently would be unavailable for intragenic recombination. The final F_2 total was estimated by multiplying the total number of F_1 heterozygotes by the mean brood size. This number was further reduced by a half since, as was found by Rogalski and Baillie (1985), at the time of screening only approximately one-half of the F_2 progeny would have reached L3 or later developmental stages.

The recombinants were progeny tested in order to determine their genotypes and the relative positions of alleles *x* and *y*. The genotypes and phenotypes of the parents and possible recombinants are outlined in Fig. 1. These are the recombinants observed if there is exchange of only one flanking marker.

Results

The unc-26 *region*

The screen performed by Clark et al. (1988) resulted in 294 EMS-induced lethal mutations in the *nT1*-balanced region after screening 3398 F_1 chromosomes. Of the 169 lethal mutations that mapped to LGIV (right), 13 of these mutations fell outside the region defined by the deficiency *sDf2* but failed to complement *sDf21*, a deficiency that deletes *unc-26* (see Fig. 2). Therefore, the mutation frequency in this screen for EMS-induced mutations in essential genes in the *unc-26* region was 3.8×10^{-3}.

These 13 lethal mutations and 6 others that were iso-

lated as described in Materials and methods were mapped using the five deficiencies shown in Fig. 2. The deficiencies divide the region into four zones (11–14). These numbers were chosen because the *unc-26* region is continuous with the *unc-22* region to the left which has been divided into zones 1–10 by deficiency breakpoints (Clark et al. 1988). *Inter se* complementation tests between lethal mutations in each zone (data not shown) divided the lethal mutations into 15 complementation groups. None of the lethal mutations failed to complement *unc-26(e345)*.

An estimate of the total number of essential genes not yet identified, and therefore a total estimate of essential genes in the *unc-26* region, can be made using a Poisson calculation. Using the formula of Meneely and Herman (1979), we estimate that there are 16 genes not yet identified, and approximately 31 essential genes in the *unc-26* region. This is probably a minimum estimate because, in making the assumption that there is a Poisson distribution of lethal alleles, one is assuming that all essential genes are equally mutable.

The approximate stage at which each *unc-22 unc-31*-linked lethal mutation arrests development in the mutant hemizygote is indicated in Table 1. Note that the lethal phase represents the phenotype of *unc-22 unc-31 (let-x)/ sDf21 or nDf27* individuals, and that the eight essential genes to the right of *unc-26* have a sterile mutant phenotype, while four of the five to the left have an egg or early larval lethal phase. Although this difference is not statistically significant, this observation could reflect a functional organization of essential genes in the *unc-26* region with respect

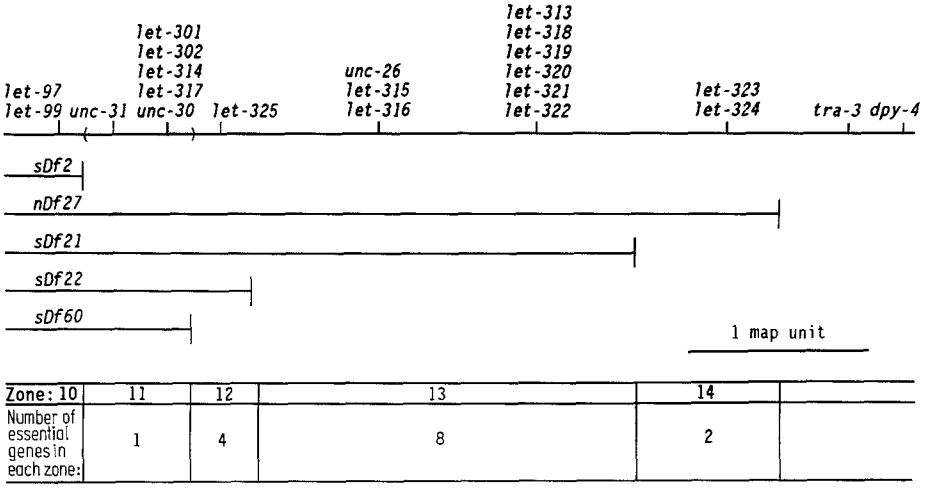

Fig. 2. The *unc-26* region. Essential genes were positioned by three-factor mapping and deficiency mapping. The deficiencies divide the region into zones 11–14, shown below the map along with the number of essential genes in each zone. The positions of *lev-1* and *ced-3* are not shown because their positions relative to the deficiency breakpoints shown are not known

Table 1. Mapping data for lethal mutations in the *unc-26* region

Gene	Allele	Screen[a]	Zone[b]	Dpy-4 recombinants[c]	Position relative to *unc-26*[d]	Lethal phase
let-301	*s1134*	I	11	ND	ND	Early larva
	s1735	II	11	ND	ND	Early larva
let-302	*s1159*	I	11	ND	ND	Embryo-early
let-313	*s1135*	I	13	9/14	Right	Sterile
let-314	*s1206*	I	11	ND	ND	Embryo-early
let-315	*s1101*	I	13	0/6	Left or close	Mid larva
let-316	*s1227*	I	13	0/22	Left or close	Early larva
let-317	*s1153*	I	11	ND	ND	Embryo-early
	s1182	I	11	ND	ND	Embryo-early
let-318	*s1218*	I	13	7/10	Right	Sterile
let-319	*s1233*	I	13	10/13	Right	Late larva
	s1754	II	13	ND	ND	Late larva
let-320	*s1248*	I	13	11/11	Right	Sterile
	s1757	II	13	ND	ND	Sterile
let-321	*s1228*	I	13	9/11	Right	Sterile
let-322	*s1238*	I	13	5/19	Right	Mid larva
let-323	*s1719*	II	14	ND	ND	Sterile
let-324	*s1727*	II	14	ND	ND	Early larva
let-325	*s1738*	II	12	ND	ND	Sterile

ND, not determined

[a] See Materials and methods

[b] Deficiency mapping results (see Fig. 2)

[c] This number indicates the fraction of Dpy-4 recombinants that segregated Dpy-4, Unc-26 Dpy-4 and Unc-22 Unc-31 Dpy-4 progeny classes

[d] Determined from three-factor mapping experiments

to the time at which the gene products are required in development. Isolation of more alleles in this region is necessary to strengthen or to refute this idea.

Isolation of a mut-4(st700)*-induced allele of* unc-26

The F_1 and F_2 progeny of 250 phenotypically wild-type hermaphrodites of the genotype *mut-4(st700)I* were screened. Two uncoordinated mutants were recovered and one of these, *s1710*, was found to be allelic to *unc-26(e345)*. Approximately 10^6 chromosomes were screened to obtain *unc-26(s1710)*.

unc-26 *mutant phenotypes*

The 11 mutants displayed variation in the severity of paralysis, as observed in homozygotes. The most severe or strongest allele was *e1048*, where the worm remains completely paralyzed at all stages of its life. The weak alleles displayed different paralyzed phenotypes during the early larval stages. The *e314* allele showed good mobility throughout its life cycle. *e205*, *e429*, and *e568* individuals exhibited limited movement in the first two larval stages but it progressively improved with age. As adults, *e205* and *e429* were capable of moving in sinusoidal patterns over short distances.

The nature of these mutations and their effects on the activity of the gene product was assessed by altering the copy number of the gene (Muller 1932). *unc-26* hemizygotes were constructed [*unc-26/sDf21 and nDf27*] for each of the 11 alleles and were examined for changes in the mutant phenotype. Only the allele *e205* showed a reduction in mobility, causing more paralysis when over either deficiency. This suggested that *e205* is a hypomorph. The remaining 10 alleles displayed no detectable differences in their phenotypes between homozygotes and hemizygotes.

The allele *e1048* has the most severe mutant phenotype. Therefore, to test the possibility that *e1048* is a null allele of *unc-26*, we compared the phenotypes of *e205/e1048* heterozygotes versus *e205/sDf21* heterozygotes. As judged by mobility, it appears that *e1048* causes the same reduction of mobility in *e205* heterozygotes as does *sDf21*, and therefore *e1048* behaves like a null allele.

Based on the above results, the 11 *unc-26* alleles were placed in an allelic series from the weakest allele to the strongest allele:

e205 < *e429* < *e314* < *e568* < *m2*, *e345*, *s1710*, *e1196*, *e446*, *e176* < *e1048*. The alleles *e429* and *e205* were ranked according to the movement pattern they displayed on the agar surface.

Intragenic mapping of unc-26 *alleles*

For intragenic mapping 21 experiments were performed with 11 *unc-26* alleles (Table 2). The strategy involved screening for exceptional progeny in a background of paralyzed worms. The left-right position of any two alleles could be determined since one chromosome in the heteroallelic hermaphrodite contained the flanking markers *unc-22* and *dpy-4* (see Materials and methods). A total of 561 recombinants were recovered; 1 was sterile and 490 segregated progeny with phenotypes consistent with a single recombination event between the two *unc-26* alleles. The remaining 70 recombinants carried an *unc-26(+)* chromosome with no

Table 2. Summary of fine structure mapping data

Allele pair[a]	Total progeny/ heterozygote	Recombinants/ F_2s screened[b]	Map distance[c]	Allele order	Convertants or double recombinants
e345/e176	104	27/61000	0.044 (0.027–0.061)	*e176-e345*	2
e345/e205	150	38/146000	0.026 (0.018–0.034)	*e205-e345*	5
e345/e314	233	9/407000	0.002 (0.001–0.004)	*e314-e345*	0
e345/e429	147	38/184000	0.021 (0.014–0.028)	*e429-e345*	3
e345/e446	161	43/188000	0.023 (0.016–0.030)	*e446-e345*	4
e345/e568	152	52/173000	0.030 (0.022–0.038)	*e568-e345*	2
e345/e1048	126	33/119000	0.028 (0.019–0.037)	*e1048-e345*	1
e345/e1196	166	37/207000	0.018 (0.012–0.024)	*e1196-e345*	3
e345/m2	124	47/142000	0.033 (0.023–0.042)	*m2-e345*	3
e345/s1710	186	12/268000	0.005 (0.002–0.008)	*s1710-e345*	3
m2/e205	191	4/219000	0.002 (0.001–0.005)	*e205-m2*	3
m2/e345	172	37/207000	0.018 (0.012–0.024)	*m2-e345*	4
m2/e429	223	0/348000	(0.0–0.001)	–	0
m2/e446	197	15/218000	0.005 (0.002–0.008)	*m2-e446*	3
m2/e568	163	0/213000	(0.0–0.002)	–	0
m2/e1048	146	11/156000	0.007 (0.004–0.013)	*m2-e1048*	7
m2/e1196	168	4/212000	0.002 (0.001–0.005)	*m2-e1196*	5
m2/s1710	183	36/209000	0.017 (0.011–0.023)	*m2-s1710*	8
s1710/e314	171	7/409000	0.002 (0.001–0.004)	*e314-s1710*	5
s1710/e1048	155	40/180000	0.022 (0.015–0.029)	*e1048-s1710*	6
e429/e568	172	0/414000	(0.0–0.001)	–	3

[a] Alleles *x/y* where the genotype is *unc-22 unc-26(x) dpy-4/+ unc-26(y)+*

[b] The expected exceptional individuals with phenotypes Unc-22, Dpy-4 and wild type, resulting from a single exchange of a flanking marker

[c] The 95% confidence intervals are shown in parentheses and were calculated using the formula $1.96\ (Npq)^{1/2}$, where N = number of F_2s screened, p = number of exceptionals/N and $q = 1-p$ or using the table of Stevens (1942). Only those individuals exhibiting a single crossover event were used in calculating the map distances

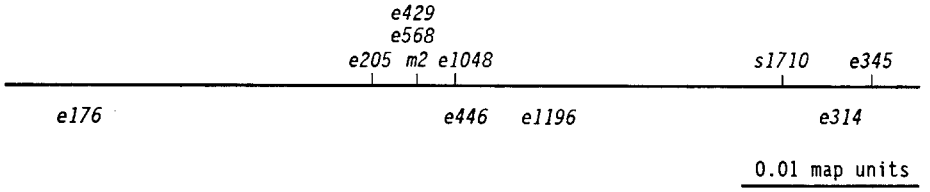

Fig. 3. Fine structure map of *unc-26*. Positions of alleles below the line with respect to each other and with those above the line are not definite (they are positioned here only by their recombinational distance from *e345*; see Table 2). Alleles above the line were positioned with respect to each other

flanking markers, an *unc-22 dpy-4* chromosome with an *unc-26(+)* allele or another unusual arrangement of flanking markers (data not shown). These 70 exceptional individuals could be the result of a double crossover or a gene conversion. The data here cannot formally distinguish between the two possibilities.

The *unc-26* intragenic map is shown in Fig. 3. The recombinational size of *unc-26* estimated from these data is at least 0.026 m.u. This distance is based on the recombination distance between the two alleles *e205* and *e345*. The additive distance from *e205* to *m2* to *e1048* to *s1710* to *e345* is 0.036 m.u., which is the same distance within the error limits. The *e345* to *e176* distance of 0.044 m.u. indicates that unc-26 may be larger than 0.026 m.u. However, the position of *e176* relative to *e205* was not determined.

Discussion

In this study, we describe the genetic analysis of *unc-26(IV)* and essential genes in the surrounding region. We have defined the *unc-26* region as extending from the right breakpoint of *sDf2* to the right breakpoint of *nDf27* (see Fig. 2). This region contains at least 15 essential genes, identified in this study (see Table 1). Four other genes (*unc-31*, *unc-30*, *ced-3* and *lev-1*), in addition to *unc-26*, map in this region (Edgley and Riddle 1987). It remains to be determined if any of the non-lethal mutations in these four genes are allelic to the lethal mutations isolated in this study. We estimate that there are 31 essential genes in the region using the Poisson calculation of Meneely and Herman (1979). The region is divided into four zones by the breakpoints of overlapping deficiencies. Alleles of six essential genes in zone 13 were positioned to the right of *unc-26*. Alleles of all eight essential genes in zone 13 were found to complement *unc-26(e345)*.

Many *C. elegans* essential genes have been genetically characterized, particularly on LGI (Rose and Baillie 1980; Howell et al. 1987), LGIV (Rogalski et al. 1982; Rogalski and Baillie 1985; Clark et al. 1988), LGV (Rosenbluth et al. 1988; McKim et al. 1988) and LGX (Meneely and Herman 1979). Integration of these projects with the physical mapping project (Coulson et al. 1986) will serve to reveal the genomic organization of essential genes in *C. elegans* and allow us to investigate whether there is a functional organization of essential genes.

Eleven *unc-26* alleles were used to construct the fine-structure map shown in Fig. 3. The estimated size of the gene is a minimum of 0.026 m.u. based on the recombination distance between *e205* and *e345*. Several other genes in the *C. elegans* genome have been fine structure mapped: *unc-15(I)* (3×10^{-3} m.u.) and *unc-13(I)* (2.4×10^{-3} m.u.) (Rose and Baillie 1980), *tra-1(III)* (0.15 m.u.) (Hodgkin 1987), *ama-1(IV)* (1.1×10^{-2} m.u.) (Bullerjahn and Riddle 1988), *unc-22(IV)* (2.4×10^{-2} m.u.) (Moerman and Baillie 1979), *unc-54(I)* (2×10^{-2} m.u.) (Waterston et al. 1982) and *unc-60(V)* (1.2×10^{-2} m.u.) (McKim et al. 1988). Comparatively, *unc-26* appears recombinationally larger than the average *C. elegans* gene. Direct comparisons are misleading, however, since chromosomal regions display different recombination frequencies. Greenwald et al. (1987) have shown that the clustering of genes in the *lin-12* region on LGIII is due to recombinational suppression and not a nonrandom distribution of genes on the physical map. The amount of DNA per map unit (1×10^3 kb) is higher in this gene cluster than would be expected on the average (1×10^5 kb and 300 m.u. in the entire genome).

The essential genes in the *unc-26* region do not form as dense a cluster as do lethals in the *unc-22* region, which lies just to the left on the genetic map. In the screen for recessive lethal mutations in the *nT1*-balanced region (Clark et al. 1988), 17 essential genes were mutated in the 1.6 m.u. region around *unc-22* and 12 essential genes were mutated in the 3 m.u. region around *unc-26* (zones 11, 12 and 13). Since the *unc-26* region appears not to be a dense gene cluster, the apparent large recombinational size of *unc-26* may not reflect a large gene in terms of amount of DNA.

In most of the mapping experiments, recombinant chromosomes were recovered where there had not been an exchange of the flanking markers. These could be interpreted as double crossovers or gene conversion events. It is impossible for us to make a distinction between the two since only one of the products of a single meiosis is recovered. However, it has been found in fine structure mapping experiments of *unc-22* alleles that the ratios of recombinants to convertants of 87:15 obtained by Moerman and Baillie (1979) and 22:9 obtained by Rogalski and Baillie (1985) are greater than the expected double crossover frequency with no interference, suggesting that conversion events were recovered. Our ratio of 561:70 recombinants to convertants is consistent with this idea.

In an attempt to understand *unc-26* gene organization, the 11 alleles were evaluated for the range of mobility each displayed as homozygotes and hemizygotes (with the deficiencies *sDf21* and *nDf27*). The results of this analysis together with the fine structure mapping data reveal that the weaker alleles *e205*, *e429* and *e568* are positioned to the left end of the genetic map, whereas most of the stronger alleles map toward the right end. The three mutations *m2*, *e429*, and *e568* form a cluster. No recombinants were recovered in the mapping experiments using any pair of these three alleles.

One copy of the informational suppressor *sup-7(st5)X* (Waterston 1981) can suppress the *e205* allele (Leon Avery, personal communication). Therefore, *e205* must lie within

the coding region of *unc-26*. This result, together with the observation that *e205* is a hypomorph, indicates that a truncated gene product with reduced function is being synthesized in *unc-26(e205)* individuals. We therefore predict that the 3′ end of the gene is oriented to the left end of the genetic map where *e205* is situated. This prediction is also consistent with the observation that most of the stronger alleles map to the right end of the genetic map, which would be the 5′ end. It is not known whether any of the other *unc-26* alleles are amber mutations.

The fine structure mapping and characterization of *unc-26* alleles will be useful in the molecular characterization of the gene. The allele *s1710* was isolated in a mutator background and is therefore a candidate for isolating the Tc1-tagged sequence of *unc-26*. Our prediction of the direction of transcription and the molecular nature of the mutant alleles can then be investigated.

Acknowledgements. The authors would like to thank Raja Rosenbluth for helpful discussion during the course of this study and Kim McKim, Don Riddle, Ann Rose and the reviewers for their helpful comments on the manuscript. Additional thanks to Leon Avery for supplying the information regarding *sup-7* and *e205*. Additionally, we thank Margaret Rogalski for her technical assistance. This work was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada and the Muscular Dystrophy Association (MDA) of Canada grants to D.L.B. D.L.C. and M.E.G. were supported by NSERC of Canada undergraduate scholarships and D.V.C. by an MDA of Canada predoctoral fellowship.

References

Brenner S (1974) The genetics of *Caenorhabditis elegans*. Genetics 77:71–94

Bullerjahn AME, Riddle DL (1988) Fine-structure genetics of *ama-1*, an essential gene encoding the amanitin-binding subunit of RNA polymerase II in *C. elegans*. Genetics 120:423–434

Clark DV, Rogalski TM, Donati LM, Baillie DL (1988) The *unc-22(IV)* region of *Caenorhabditis elegans*: genetic analysis of lethal mutations. Genetics 119:345–353

Coulson A, Sulston J, Brenner S, Karn J (1986) Toward a physical map of the genome of the nematode *C. elegans*. Proc Natl Acad Sci USA 83:7821–7825

Edgley ML, Riddle DL (1987) *Caenorhabditis elegans*. In: Genetic maps 1987, vol 4. O'Brien SJ (ed) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Ferguson ED, Horvitz HR (1985) Identification and characterization of 22 genes that affect vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics 110:17–72

Greenwald I, Coulson A, Sulston J, Priess J (1987) Correlation of the physical and genetic maps in the *lin-12* region of *C. elegans*. Nucleic Acids Res 15:2295–2307

Hodgkin J (1987) A genetic analysis of the sex-determining gene *tra-1*, in the nematode *Caenorhabditis elegans*. Genes Dev 1:731–745

Horvitz HR, Brenner S, Hodgkin J, Herman RK (1979) A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol Gen Genet 175:128–133

Howell AM, Gilmour SG, Mancebo RA, Rose AM (1987) Genetic analysis of a large autosomal region in *Caenorhabditis elegans* by the use of a free duplication. Genet Res 49:207–213

McKim KS, Heschl MFP, Rosenbluth RE, Baillie DL (1988) Genetic organization of the *unc-60* region in *Caenorhabditis elegans*. Genetics 118:49–59

Meneely PM, Herman RK (1979) Lethals, steriles and deficiencies in a region of the X chromosome of *Caenorhabditis elegans*. Genetics 92:99–115

Moerman DG, Baillie DL (1979) Genetic organization in *Caenorhabditis elegans*: Fine structure analysis of the *unc-22* gene. Genetics 91:95–103

Moerman DG, Baillie DL (1981) Formaldehyde mutagenesis in the nematode *Caenorhabditis elegans*. Mutat Res 80:273–289

Moerman DG, Waterston RH (1984) Spontaneous unstable *unc-22* IV mutations in *C. elegans* var. Bergerac. Genetics 108:859–877

Mori I, Moerman DG, Waterston RH (1988) Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in *C. elegans*. Genetics 120:397–407

Muller HJ (1932) Further studies on the nature and causes of gene mutations. Proceedings of the Sixth International Congress of Genetics 1:213–255

Rogalski TM, Baillie DL (1985) Genetic organization of the *unc-22 IV* gene and the adjacent region in *Caenorhabditis elegans*. Mol Gen Genet 201:409–414

Rogalski TM, Moerman DG, Baillie DL (1982) Essential genes and deficiencies in the *unc-22 IV* region of *Caenorhabditis elegans*. Genetics 102:725–736

Rose AM, Baillie DL (1979) The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics 92:409–418

Rose AM, Baillie DL (1980) Genetic organization of the region around *unc-15I*, a gene affecting paramyosin in *Caenorhabditis elegans*. Genetics 196:639–648

Rosenbluth RE, Rogalski TM, Johnsen RC, Addison LM, Baillie DL (1988) Genomic organization in *Caenorhabditis elegans*: deficiency mapping on linkage group IV (left). Genet Res 52:105–118

Stevens WL (1942) Accuracy of mutation rates. J Genet 43:301–307

Waterston RH (1981) A second informational suppressor, *sup-7X*, in *Caenorhabditis elegans*. Genetics 97:307–325

Waterston RH, Thomson JN, Brenner S (1980) Mutants with altered muscle structure in *Caenorhabditis elegans*. Dev Biol 77:271–302

Waterston RH, Smith KC, Moerman DG (1982) Genetic fine-structure analysis of the myosin heavy chain gene, *unc-54*, of *Caenorhabditis elegans*. J Mol Biol 158:1–15

White JG, Southgate E, Thomson JN, Brenner S (1976) The structure of the ventral nerve cord of *C. elegans*. Philos Trans R Soc Lon 275B:327–348

White JG, Horvitz HR, Sulston JE (1982) Neurone differentiation in cell lineage mutants of *Caenorhabditis elegans*. Nature 297:584–593

Communicated by B.H. Judd

Received February 28, 1990