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Genetic organization of the *unc-22 IV* gene and the adjacent region in *Caenorhabditis elegans*

Teresa M. Rogalski* and David L. Baillie

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Summary. The genetic organization of the region immediately adjacent to the *unc-22 IV* gene in *Caenorhabditis elegans* has been studied. We have identified twenty essential genes in this interval of approximately 1.5-map units on Linkage Group *IV*. The mutations that define these genes were positioned by recombination mapping and complementation with several deficiencies. With few exceptions, the positions obtained by these two methods agreed. Eight of the twenty essential genes identified are represented by more than one allele. Three possible internal deletions of the *unc-22* gene have been located by intra-genic mapping. In addition, the right end point of a deficiency or an inversion affecting the adjacent genes *let-56* and *unc-22* has been positioned inside the *unc-22* gene.

Introduction

Our laboratory is investigating genomic organization and gene regulation in *Caenorhabditis elegans* by characterizing, first genetically and then at the molecular level, several small defined regions in the genome of this organism (Moerman and Baillie 1979; Rose and Baillie 1980; Rogalski et al. 1982; T. Rogalski and D. Baillie, unpublished results). This paper describes a genetic analysis of one of these regions: an interval of approximately 1.5 map units including *unc-22 IV*, a gene affecting muscle structure and function in *C. elegans* (Brenner 1974; Waterston et al. 1980; Zengel and Epstein 1980; Moerman 1980; Moerman et al. 1982). In this study we combine a fine-structure analysis of the *unc-22 IV* gene with the genetic characterization of a small, defined region around this muscle-specific gene. One of the goals of our analysis is to delimit the extents and relative positions of the structural and regulatory elements of the *unc-22* gene by intra-genic recombination mapping and by the identification of the adjacent genes on either side of *unc-22*. A second goal of the analysis is to determine the number of genes in the small region of linkage group *IV* defined by the deficiency *sDf2* (Moerman and Baillie 1981).

Previous genetic studies of the *unc-22* region of linkage group *IV* have included a fine-structure analysis of the *unc-22* gene (Moerman and Baillie 1979; Moerman 1980) and the identification of eleven essential genes near *unc-22* (Rogalski et al. 1982). This study extends both the fine-structure analysis of the *unc-22* gene and the characterization of the region defined by *sDf2*. We expand and refine the existing *unc-22* fine-structure map and position the right breakpoint of a chromosomal rearrangement inside the *unc-22* gene. We also identify and position nine additional essential genes and one nonessential gene in the interval around *unc-22*.

Materials and methods

Nematode strains and culture conditions. The nomenclature in this paper follows the uniform system adopted for *Caenorhabditis elegans* (Horvitz et al. 1979). The wild-type (N2) strain and strains carrying *unc-5(e152)IV*, *dpy-4(e1166)IV* and *dpy-11(e224)V* were originally obtained from the *Caenorhabditis elegans* var. Bristol, stock collection at the Medical Research Council, Cambridge, England. The *dpy-20(e1282)IV* strain was obtained from the Caenorhabditis Genetics Center at the University of Missouri, Columbia. The following mutations and deficiencies of the *unc-22* gene were isolated at Simon Fraser University: *s7*, *s8*, *s12*, *s18* and *s32* (Moerman and Baillie 1979), *s35*, *s36*, *sDf2*, *sDf7*, *sDf8*, *sDf9*, *sDf10* and *sDf19* (Moerman and Baillie 1981), and *s699* and *s700* (R. Rosenbluth, unpublished results). All of the *unc-22* mutations and deficiencies have a recessive Unc-22 (twitcher) phenotype except *sDf19*, which has a dominant twitcher phenotype. The lethal mutations and deficiencies, with the exception of *sDf19*, were maintained as described in Rogalski et al. (1982). In the case of *sDf19*, the heterozygous strain was maintained by selecting Unc-22 (twitcher) progeny each generation. Nematodes were cultured on NGM Agar streaked with *E. coli* OP50 as described by Brenner (1974).

Isolation and mapping of unc-22-linked lethal and sterile mutations. Several *unc-22*-linked lethal and sterile mutations were isolated using essentially the same protocol described in Rogalski et al. (1982). However, in this study only 0.025 M ethyl methanesulfonate (EMS) was used, and the F1 hermaphrodites were incubated at 20° C instead of 26° C.

The procedures used to position the visible, lethal and sterile mutations and to identify lethal complementation groups are the same as those described in Rogalski et al. (1982).

Complementation tests between the lethal mutations and the dominant mutation *sDf19* were performed as fol-

* *Present address:* Division of Biological Sciences, University of Missouri, Columbia, MO 65211 USA

Offprint requests to: D.L. Baillie

lows. Hermaphrodites with the genotype, *sDf19(IV)/+; dpy-11(e224)V,* were mated to *let-a(sx) unc-22(s7)/++* males and the outcrossed Unc-22 hermaphrodites were picked and allowed to reproduce in order to determine their genotypes. If none of the F1 hermaphrodites from a particular cross carried the *let-a unc-22* chromosome, then the lethal mutation was considered to be included in the deficiency.

Mapping alleles within the unc-22 gene. The procedure used for the fine-structure mapping of the *unc-22* gene was modified from that of Moerman and Baillie (1979). Thirty to 100 young adult hermaphrodites of the genotype *unc-5 unc-22(sx) dpy-4/+ unc-22(sy)+* were placed singly on large (100 mm) petri plates and transferred to fresh plates every 6 to 12 h until there were approximately 200 plates. The plates were incubated at 15° C to 20° C until the F2 generation was mature (6 days at 20° C) and then they were screened for individuals that did not twitch (either wild-type, Dpy, Dpy Unc-5 or Unc-5). All nonUnc-22 recombinants were picked and their genotype confirmed. Since the hermaphrodites used in the fine-structure mapping experiments were heterozygous for the flanking markers, *unc-5* and *dpy-4*, the relative positions of the two *unc-22* alleles being mapped could be determined. If *sy* is to the left of *sx*, then the recombinant *unc-22(+)* chromosome will carry the *unc-5* marker, and recombinant individuals will be phenotypically wild-type (*unc-5 + +/+ sy +*) or Unc-5 (*unc-5++/unc-5 sx dpy-4*). Alternatively, if *sy* is to the right of *sx* then the *dpy-4* marker will be carried on the recombinant chromosome and the nonUnc-22 recombinants will be either wild-type (*++dpy-4/+sy +*) or Dpy-4 (*++dpy-4/unc-5 sx dpy-4*).

The map distance between two *unc-22* alleles was calculated using the formula $d = 2(W) \times 100/T$ from Moerman and Baillie (1979) where d = map distance; W = nonUnc-22 recombinants and T = total F2 progeny. The total number of F2 progeny was estimated in the following manner. First, the total number of F1 individuals was estimated by counting all of the F1 progeny on a sample (20%) of the plates. This number was then halved to give the number of F1 heterozygotes, since the required recombination event can only occur in these individuals. Next, the total number of progeny produced by an *unc-5 unc-22(sx) dpy-4/+ unc-22(sy)+* heterozygote was determined by completely counting all of the progeny of 10 such hermaphrodites. However, not all of the progeny of an F1 heterozygote are screened in these experiments, since screening is done at low magnifications (6× and 12×) and only recombinant individuals which were at the L3 or later stages of development would be observed. Approximately one-half of the F2 progeny would be at the appropriate developmental stages at the time the plates were screened. Therefore, the total number of F2 progeny that were screened was estimated as the number of F1 heterozygotes multiplied by one-half the total progeny of a heterozygous hermaphrodite.

The procedure for mapping the breakpoint of *sDf19* inside the *unc-22* gene was identical to that described above. Fifty *unc-5 unc-22(sx) dpy-4/+ sDf19 +* hermaphrodites were placed on large plates and transferred at 12 h intervals until there were 200 plates, and the F2 generation was screened for nonUnc-22 recombinants. The map distance from the deficiency breakpoint to the *unc-22* allele was calculated using the above formula except that the number of nonUnc-22 recombinants was multiplied by 4, since half of the recombinants would not be observed due to the dominant twitcher phenotype of the *sDf19/+* heterozygote.

Results

Essential genes around unc-22

In a previous study we isolated a set of lethal and sterile mutations linked to *unc-22(s7)* and tested these mutations for complementation with the deficiency *sDf2* (Rogalski et al. 1982). The sixteen mutations that were uncovered by *sDf2* were positioned by recombination mapping and deficiency mapping, and eleven essential genes were identified by complementation analysis. In this study we have isolated another set of *unc-22*-linked lethal and sterile mutations, positioned these mutations by recombination mapping and then determined which mutations were in the region defined by *sDf2*. The mutations that failed to complement this deficiency were further positioned by complementation with the *sDf7, sDf8, sDf9* and *sDf10* deficiencies and nine new lethal complementation groups were identified. In addition we also positioned, by recombination mapping, the lethal and sterile mutations from the previous study that complemented *sDf2*.

A total of 26 strains carrying a lethal or sterile mutation linked to *unc-22(s7)* was isolated in this study. The first step in mapping the lethal and sterile mutations was to determine whether each mutation was to the left or to the right of to *unc-22 IV*. The map distance from the lethal site to the *unc-22* gene was then determined by two-factor recombination mapping. Thirty-seven mutations were mapped in this manner, including 10 mutations (*s50, s52, s47, s51, s63, s166, s169, s212, s213* and *s215*) from our previous study (Rogalski et al. 1982) and one mutation (*s504*) which was isolated and positioned by D. Pilgrim (personal communication). Thirty-two of the lethal mutations mapped to the left of *unc-22*, whereas only five mapped to its right (data not shown). The recombination data obtained for the mutations uncovered by *sDf2* (see below) are summarized in Table 1.

The thirty-seven lethal and sterile mutations that were positioned by recombination mapping were tested for complementation with *sDf2*. Nineteen of these, 18 of which map to the left of *unc-22*, complemented *sDf2* and, therefore, lie outside of its region. The number of essential genes defined by these 19 mutations was not determined.

The remaining 18 lethal and sterile mutations failed to complement *sDf2*. The results of complementation tests with the deficiencies, *sDf7, sDf8, sDf9* and *sDf10*, localized 16 of these 18 mutations into six separate zones (see Fig. 1). Table 2 summarizes the results of these tests. Complementation tests were done between lethal mutations that occupied the same zone. These tests identified 12 essential genes. Further complementation tests were done with mutations representing the eleven previously identified essential genes (Rogalski et al. 1982), and the 20 genes defined by these tests are shown in the first column of Table 2. Figure 1 shows the positions of these genes. Sixteen lethals are in zone 1, and they define 12 loci, *let-69(s684), let-70(s689), let-71(s692), let-61(s65), let-72(s52, s695), let-59(s49, s172), let-62(s175), let-63(s170, s679), let-64(s171, s216), let-*

Table 1. Two-factor mapping results for *unc-22*-linked lethal and sterile mutations uncovered by the deficiency *sDf2 IV* in *Caenorhabditis elegans*

Mutation	Gene	Position relative to *unc-22*	Number of progeny[a]		Map units from *unc-22*[b]
			Unc-22	Wild	
e1282	*dpy-20*	Not done	7	4186	0.25 (0.11–0.36)
s50	*let-56*	left	0	2342	–
s52	*let-72*	left	9	1423	0.94 (0.43–1.79)
s504	*let-92*	left	4	5632	0.11 (0.03–0.27)
s677	*let-92*	left	4	1980	0.30 (0.08–0.77)
s678	*let-91*	left	7	2279	0.46 (0.18–0.94)
s679	*let-63*	left	9	2112	0.64 (0.29–1.21)
s680	*let-68*	right	8	2250	0.53 (0.23–1.05)
s681		left	8	2360	0.51 (0.22–0.99)
s684	*let-69*	left	16	2011	1.19 (0.61–1.77)
s685	*let-73*	left	9	2148	0.63 (0.29–1.19)
s689	*let-70*	left	18	2521	1.07 (0.58–1.56)
s692	*let-71*	left	19	2478	1.15 (0.63–1.66)
s693	*let-68*	right	8	2301	0.52 (0.22–1.03)
s694	*let-65*	left	5	1785	0.42 (0.14–0.97)
s695	*let-72*	left	14	2261	0.92 (0.51–1.55)
s696	*let-68*	right	3	1524	0.29 (0.06–0.86)
s697	*let-74*	left	6	1542	0.58 (0.21–1.27)
s698		right	1	4185	0.04 (0.001–0.16)

[a] The hermaphrodites used for 2-Factor recombination mapping had the following genotype: *unc-22(s7) let-a(sx)*/+ +

[b] Map units = 100 p and $p = 1 - \sqrt{1 - 3U/U + W}$ where p = recombination frequency, U = number of Unc-22 progeny and W = number of Wild progeny. 95% confidence intervals are shown in the brackets and were calculated using the formula

$1.96/\sqrt{Npq}$ where N = W, p = U/W and q = 1 − p

or using the table of Stevens (1942)

73(s685), *let-74(s697)* and *let-91(s678)*. There are four lethals in zone 2 that define two genes, *let-65(s174, s254, s694)* and *let-60(s59)*. The two lethal mutations in zone 3, *s504* and *s677*, are alleles of *let-92*. Similarly, the four mutations in zone 4, *s46, s50, s168* and *s173*, are all alleles of *let-56*. This zone also contains the *unc-22* gene. Zone 5 contains *s42*, which defines the *let-52* locus, whereas zone 6 has five mutations that define three loci, *let-66(s176)*, *let-67(s214)* and *let-68(s680, s693, s696)*. Sixteen of the *let* loci map to the left of *unc-22*, and four map to the right of it. The lethal, sterile and visible mutations so far uncovered by *sDf2* represent 22 loci in a 1.5 map unit interval.

Two of the 18 lethal mutations uncovered by *sDf2* did not behave as simply as the other mutations. The *s681* mutation failed to complement mutations in two genes, *let-59* (*s49, s172*) and *let-62(s175)*, which map close to each other and may be adjacent genes. This mutation could be a small deletion or some other chromosomal rearrangement affecting these two genes. An alternative possibility is that *let-59* and *let-62* represent a complex locus with complementing alleles.

In the case of *s698*, the mapping experiments gave conflicting results. Recombination mapping placed *s698* 0.04 map unit to the right of *unc-22* (Table 1). These results suggest that *s698* is very close to *unc-22*. In contrast, *s698* complemented the four deficiencies, *sDf7, sDf8, sDf9*, and *sDf10*, as well as *let-66(s176)* and *let-67(s214)* but failed to complement the three alleles of *let-68*. Therefore, based on complementation results, this mutation lies in zone 6 and is an allele of *let-68*, which maps 0.5 map unit to the right of *unc-22*. An explanation for these conflicting data is that *s698* is an inversion with one breakpoint in the *let-68*

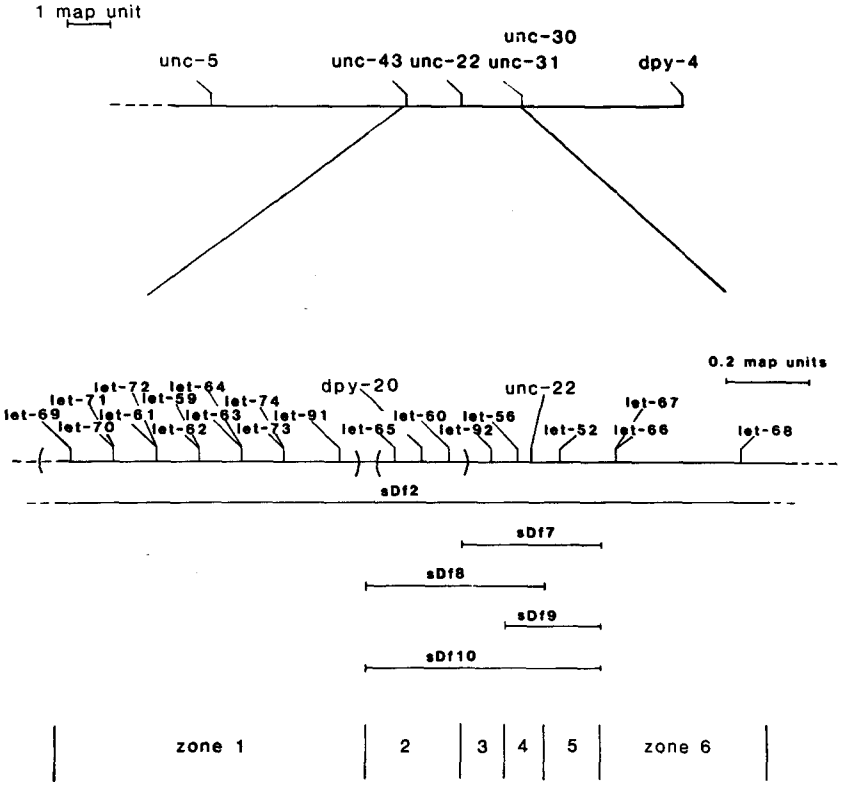

Fig. 1. Map of essential genes, non-essential genes and deficiencies in the region immediately adjacent to the *unc-22 IV* locus. The relative positions of nonessential genes in this region are shown on the top map. The bottom map is an expansion of the region between *unc-43* and *unc-31*. The positions of the deficiencies are shown below this map. The six zones defined by the deficiency endpoints are also shown. The genes in each zone have not been positioned relative to each other

Table 2. Complementation tests between mutations and deficiencies in the *unc-22 IV* region of *Caenorhabditis elegans*[a]

Genes	*Alleles*	*sDf7*	*sDf8*	*sDf9*	*sDf10*	*Zone*
let-69	*s684*	+	+	+	+	1
let-70	*s689*	+	+	+	+	1
let-71	*s692*	+	+	+	+	1
let-61	*s65*	+	+	+	+	1
let-72	*s52, s695*	+	+	+	+	1
let-59	*s49, s172*	+	+	+	+	1
let-62	*s175*	+	+	+	+	1
let-63	*s170, s679*	+	+	+	+	1
let-64	*s171, s216*	+	+	+	+	1
let-73	*s685*	+	+	+	+	1
let-74	*s697*	+	+	+	+	1
let-91	*s678*	+	+	+	+	1
let-65	*s174, s254, s694*	+	−	+	−	2
let-60	*s59*	+	−	+	−	2
dpy-20	*e1282*	+	−	+	−	2
let-92	*s504, s677*	−	−	+	−	3
let-56	*s46, s50, s168, s173*	−	−	−	−	4
unc-22	*s7*	−	−	−	−	4
let-52	*s42*	−	+	−	−	5
let-66	*s176*	+	+	+	+	6
let-67	*s214*	+	+	+	+	6
let-68	*s680, s693, s696*	+	+	+	+	6

[a] Complementation is indicated by + and noncomplementation is indicated by −

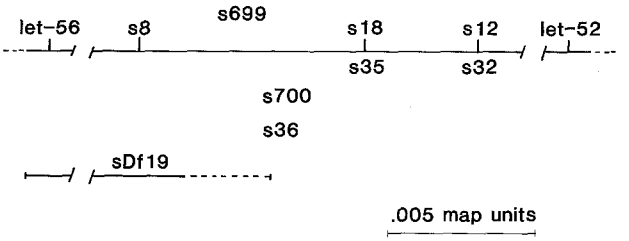

Fig. 2. A fine structure map of the *unc-22 IV* gene showing the positions of several newly mapped alleles and the inferred right breakpoint of *sDf19*. The positions of the *s8, s18* and *s12* alleles are from Moerman (1980). The mutations shown above the line have been positioned relative to each other

Table 3. Results of fine-structure mapping experiments between alleles of the *unc-22 IV* gene in *Caenorhabditis elegans*

Alleles tested[a]	Recombinants[b]	Total F2 progeny	Map distance	L/R position
s12/s32	0	350,000	−	−
s18/s35	0	150,000	−	−
s18/s36	0	270,000	−	−
s8/s699	2 Wild, 1 Dpy-4	340,000	0.0018	*s8/s699*
s18/s699	2 Unc-5, 1 Wild	510,000	0.0012	*s699/s18*
s8/s700	4 Dpy-4, 4 Wild	610,000	0.0026	*s8/s700*
s18/s700	0	780,000	−	−
s8/sDf19	0	180,000	−	−
s18/sDf19	2 Unc-5	270,000	0.003[c]	*sDf19/s18*
s12/sDf19	6 Unc-5	270,000	0.009[c]	*sDf19/s12*

[a] The mutation at the left was on the *unc-5(e152) unc-22(sx) dpy-4(e1166)* chromosome

[b] Only recombinants resulting from a single crossover are shown. The genotype of all the recombinants was determined. The wild recombinants listed in this Table segregated the appropriate markers

[c] The number of recombinants was multiplied by 4 because one-half of them will not be seen due to the dominant twitcher phenotype of the *sDf19* mutation

gene and the other breakpoint near *unc-22* in a nonessential region, or in another essential gene in zone 6 which has not yet been identified.

The visible mutation *dpy-20(e1282)* was also tested for complementation with *sDf2* and was found to lie inside the region uncovered by this deficiency. The map distance between *dpy-20* and *unc-22* was determined (Table 1), and this gene was then positioned into zone 2 by complementation mapping with *sDf7, sDf8, sDf9* and *sDf10* (Table 2). In addition to the twenty essential genes, there are at least two nonessential genes in the 1.5 map unit interval defined by *sDf2*.

Fine structure analysis of the unc-22 gene

Three of the *unc-22* alleles that were used in this study, *s8, s12,* and *s18*, had been positioned relative to each other in the initial fine-structure map of this gene (Moerman and Baillie 1979). The *s8* and *s12* mutations are located near the left and right boundaries of the map whereas *s18* lies near the center (see Fig. 2). These three alleles were used as reference points to position several other mutations in the *unc-22* gene.

Three intragenic mapping experiments were performed in order to refine the positions of 3 previously mapped *unc-22* alleles (Moerman 1980). The results of these mapping experiments are summarized in Table 3 and Fig. 2.

The EMS-induced *unc-22* allele, *s32*, is suppressed by the informational suppressors *sup-5 III* (Waterston and Brenner 1978) and *sup-7 X* (Waterston 1981) and is, therefore, in the structural element of the *unc-22* gene (Moerman 1980; D. Moerman, personal communication). Prior to this analysis, *s32* had been positioned only relative to *s8*, which is near the left boundary of the fine-structure map (Moerman 1980). We have positioned *s32* relative to *s12*, previously the right-most allele of the *unc-22* gene. Since no recombination occurred between these two alleles (Table 3), the site of the *s32* mutation must be very close to the *s12* site (Fig. 2).

Four formaldehyde-induced *unc-22* mutations had previously been positioned within the *unc-22* fine-structure map between *s8* and *s12*. We have mapped two of these mutations, *s35* and *s36*, against the *s18* allele of this gene. In both cases, no recombination occurred between the two alleles being tested (Table 3). These results suggest that both *s35* and *s36* map very close to *s18*. The new positions of these two mutations are shown in Fig. 2.

Two new gamma-ray-induced *unc-22* alleles were positioned within the *unc-22* fine-structure map relative to the *s8* and *s18* alleles (Table 3; Fig. 2). The *s699* mutation maps between *s8* and *s18* and is the first allele to be positioned in this region. The *s700* mutation maps to the right of *s8*; however, since this allele failed to recombine with *s18*, it is not positioned relative to this site.

The *sDf19* mutation was isolated as a dominant Unc-22 (twitcher) mutation that was also homozygous lethal (D. Moerman, unpublished results). Complementation tests were done with *sDf19* and mutations representing *let-51,*

let-59, let-60, let-92, let-56 and *let-52*. Only the *let-56(s46)* mutations failed to complement the lethal phenotype of *sDf19* indicating that this mutation affects another gene besides *unc-22*. Taken together, the fine-structure mapping experiments (Table 3) and complementation results indicate that *sDf19* is either a deletion that breaks inside the *unc-22* gene and extends into *let-56*, the adjacent gene to the left of *unc-22*, or an inversion with its breakpoints in these two genes. The right endpoint of *sDf19* was positioned inside the *unc-22* gene between *s8* and *s18* (Fig. 2). Since *sDf19* affects only *unc-22* and *let-56*, which may be adjacent genes, it is not possible to distinguish between these two possibilities at this time. The dominant twitcher phenotype of *sDf19* is presumably due to the breakpoint inside the *unc-22* gene.

A total of 37 nonUnc-22 hermaphrodites was found in the fine-structure mapping experiments. All of these exceptional individuals were allowed to produce self-cross progeny in order to determine their genotype. Twenty-two of these individuals were the result of a single recombination event between the two *unc-22* alleles being mapped (i.e. they showed the exchange of one of the flanking markers). Only these 22 recombinants were used to position alleles and to determine map distances (Table 3). Seven of the recombinant hermaphrodites carried *unc-22(+)* chromosomes without either flanking marker and three carried recombinant chromosomes with both *unc-5* and *dpy-4*. These ten individuals could have resulted from either a double crossover or gene conversion of one of the *unc-22* alleles. Two of the five remaining individuals were sterile, and the other three behaved anomalously.

Nine of the ten gene conversion or double crossover events occurred in the two mapping experiments involving *s700*. Six were found in the experiment with *s18* and three in the experiment positioning *s8* and *s700*. The remaining event occurred in the experiment that positioned *s699* relative to *s8*.

Discussion

The region around the *unc-22* gene on linkage group *IV* is at present the best genetically characterized small region in *C. elegans*. This interval, which is represented by the deficiency, *sDf2*, is approximately 1.5 map units in length and extends in both directions from the *unc-22* gene. A total of 32 lethal and sterile mutations defining 20 essential genes has been identified and positioned in the *sDf2* region (Table 2). Sixteen of the essential genes are in a 1.0 map unit interval to the left of *unc-22* whereas the remaining four genes are in an interval of approximately 0.5 map unit to its right. Only eight of the essential genes uncovered by *sDf2* are represented by more than one allele (*let-59, let-63, let-64, let-72* and *let-92* have two alleles each; *let-65* and *let-68* have three alleles each; *let-56* has four alleles). A Poisson analysis of the data indicates that this small region of the *C. elegans* genome is not saturated for essential genes. Using the equation of Meneely and Herman (1979) for estimating the total number of genes in a region, we estimate that there is a total of 32 essential genes uncovered by the *sDf2* deficiency. This region of Linkage Group *IV* is one of several regions of the *C. elegans* genome that have been extensively characterized (Meneely and Herman 1979, 1981; Rose and Baillie 1980; Sigurdson et al. 1984).

The extent of *sDf2* was determined from the recombination data obtained for the lethal and sterile mutations. These results indicate that the left endpoint of *sDf2* is between 0.8–1.2 map units from *unc-22*. The right endpoint of *sDf2* is less firmly positioned. This deficiency includes *let-68*, which maps 0.5 map unit from *unc-22* but does not include *unc-31*, which is approximately 1.1 map units from *unc-22* (Rogalski et al. 1982). Therefore, *sDf2* extends at least 0.5 map unit and possibly as far as 1.1 map units to the right of *unc-22*.

The results obtained in our initial analysis (Rogalski et al. 1982) suggested that the essential genes in the *sDf2* region were clustered to the left of the *unc-22* gene. In order to determine whether this was the case, we positioned the lethal and sterile mutations that lie outside the region uncovered by *sDf2*. All but one of these mutations, including *s41, s43, s44,* and *s45* (Rogalski et al. 1982), map to the left of *unc-22* (data not shown). The single mutation, *s690*, that maps to the right of *unc-22* behaves anomalously and cannot be accurately positioned (T. Rogalski, unpublished results). Surprisingly, no lethal mutation, either inside or outside *sDf2*, has been found between *let-68* and *unc-31*, an interval of approximately 0.6 map unit. There are at present sixteen essential genes in the 1.0 map unit interval to the left of *unc-22* and only four essential genes in the 1.0 map unit interval to its right. These results support the previous suggestion that the essential genes around *unc-22* are clustered to the left of this gene.

The initial analysis of sixteen lethal mutations in the *sDf2* region identified *let-56* and *let-52* as the flanking genes to the left and right of *unc-22* (Rogalski et al. 1982). The data obtained in this analysis, which characterized a further 18 lethal mutations, still support this conclusion. The distance between these two genes, about 0.1 map unit, represents the maximum size of the *unc-22* gene. The existing *unc-22* fine-structure map occupies only a small portion of this interval.

The fine-structure map of the *unc-22* gene constructed by Moerman (1980) consisted of 15 *unc-22* alleles and the estimated size of this gene was between 0.01 and 0.02 map unit. We have positioned two new alleles, *s699* and *s700*, into this map. Both of these mutations were found to lie within the boundaries of the existing map.

As stated previously, one of the eventual goals of our analysis is to delimit the coding element of the *unc-22* gene. One of the *unc-22* alleles that we have positioned, *s32*, is in the coding region of the gene. The only other mutation that is believed to be in the coding region is the dominant allele, *m52* which has been positioned near *s18* (Moerman 1980). At this time the known coding region of the *unc-22* gene includes the right end of the fine-structure map (Fig. 2).

A comparison of the fine-structure mapping results of Moerman (1980) and the results obtained in this study suggests that the two gamma-ray-induced mutations, *s699* and *s700*, and the formaldehyde-induced mutation, *s36*, may be internal deficiencies of the *unc-22* gene. However, the differences in recombination distance observed between these two studies may not be significant since few recombinants were recovered and different methods were used to estimate total sample sizes. In order to determine whether any of these mutations are internal *unc-22* deficiencies it will be necessary to characterize them at the DNA level.

Another mutation affecting the *unc-22* gene that was positioned in this analysis was *sDf19*, which has a dominant Unc-22 (twitcher) phenotype and is also homozygous lethal.

Both the intra-genic recombination data (Table 3) and the results of the complementation analysis (see Results) indicate that this mutation is a deficiency or an inversion affecting the two adjacent genes *let-56* and *unc-22*.

The dominant Unc-22 phenotype of *sDf19* suggests that an aberrant *unc-22* protein is synthesized from the mutant gene. Possibly the normal *unc-22* promoter is intact and transcription occurs from this promoter. If the *sDf19* mutation is, in fact, a deletion, then the *unc-22* promoter would be located at the right end of the fine-structure map. Alternatively the *unc-22* promoter has been deleted and the remaining segment of the gene is coupled to another promoter and is transcribed from this site.

A molecular analysis of the region represented by *sDf2* is now in progress. Approximately 240,000 base pairs of DNA have been cloned in this interval of Linkage Group *IV* (Baillie et al. 1985). The *sDf2, sDf7, sDf8, sDf9* and *sDf10* deficiencies will be extremely useful in orienting the genetic map of this small region with respect to these DNA sequences.

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