ESSENTIAL GENES AND DEFICIENCIES IN THE *UNC-22 IV* REGION OF *CAENORHABDITIS ELEGANS*

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ABSTRACT

Five formaldehyde-induced deficiencies that uncover *unc-22 IV*, a gene affecting muscle structure in the nematode *Caenorhabditis elegans* were isolated and positioned. The largest deficiency, *sDf2*, extends in both directions from *unc-22* and is approximately 1.0–2.0 map units in length. The other four deficiencies, *sDf7*, *sDf8*, *sDf9* and *sDf10*, are all smaller than *sDf2* and are located within the region uncovered by this deficiency. Thirty-seven ethyl methanesulfonate-induced lethal and sterile mutations linked to *unc-22* were isolated and tested for complementation with *sDf2*. Nineteen lethal mutations failed to complement *sDf2*. Sixteen of these were further positioned by recombination mapping and also by deficiency mapping with *sDf7*, *sDf8*, *sDf9* and *sDf10*. These sixteen mutations define 11 new essential genes in this region. Eight of the genes lie in a 0.9-map unit interval to the left of *unc-22*, whereas the three remaining genes lie in a region of about 0.2 map units to the right of *unc-22*. We believe that two of the essential genes identified in this study, *let-56* and *let-52*, are the adjacent genes on either side of *unc-22*. The lethal mutations exhibit a wide range of terminal phenotypes: from first stage larva to sterile adult.

THE purpose of this study is to identify essential loci on either side of *unc-22 IV*, a gene affecting muscle structure in the nematode, *Caenorhabditis elegans* (BRENNER 1974; WATERSTON, THOMSON and BRENNER 1980; ZENGEL and EPSTEIN 1980; MOERMAN 1980). We are investigating the region around *unc-22* as part of an ongoing study to determine how muscle genes are regulated during development in *C. elegans*. Mutations in the *unc-22* gene cause a characteristic twitching pattern along the body wall musculature of this organism. Circumstantial evidence indicates that this twitch is not due to abnormal neuronal input to the muscle, but rather is a result of a disorder in the muscle cell itself (LEWIS *et al.* 1980; MOERMAN 1980). Also, the body wall musculature of *unc-22* mutants appears disorganized when viewed under the electron microscope (WATERSTON, THOMSON and BRENNER 1980; MOERMAN 1980).

A fine structure analysis of the *unc-22* gene itself is in progress in order to define the structural and regulatory elements of this gene (MOERMAN and BAILLIE 1979; T. ROGALSKI, unpublished results). The current estimate of the size of the *unc-22* locus is 1.5×10^{-2} map units (MOERMAN 1980). An important

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addition to the fine structure analysis would be the identification of the adjacent genes on either side of *unc-22*. This would define, more precisely, the extent of this locus. One of the objectives of this study is to identify these genes.

A second objective of this study is to determine how genes are organized in the genome of *C. elegans*. We are attempting to identify all of the genes in a small, defined region around *unc-22* and, eventually, would like to determine whether any of the other genes in this region also affect muscle structure or function. Since we expect most of the genes in this organism to be essential for development, we have concentrated on identifying lethal mutations. Our strategy for identifying essential loci near *unc-22* is analogous to one used in *D. melanogaster* for the saturation mapping of small regions of a genome (HOCHMAN 1971; JUDD, SHEN and KAUFMAN 1972; O'DONNELL *et al.* 1977; WOODRUFF and ASHBURNER 1979; HILLIKER *et al.* 1980). Lethal mutations and deficiencies were isolated and positioned in a small region surrounding *unc-22*, and lethal complementation groups were identified. The only other small genomic region in *C. elegans* that has been studied in this manner is the *unc-15 I* region (ROSE and BAILLIE 1980).

Although we have not identified all of the genes in this region, our study has identified 11 essential genes in a 1.1-map unit interval around *unc-22*. We believe that two of these genes are in fact the adjacent loci on either side of *unc-22*.

MATERIALS AND METHODS

The nomenclature follows the recommendations of HORVITZ *et al.* (1979).

Nematode strains and culture conditions: The wild-type (N2) strain and strains carrying *unc-5(e152) IV*, *unc-43(e266) IV*, *unc-31(e169) IV*, *unc-30(e318) IV* and *dpy-4(e1166) IV* were originally obtained from the Medical Research Council *C. elegans*, var. Bristol, stock collection at Cambridge (England). The mutation, *unc-22(s7)*, was isolated at Simon Fraser University (MOERMAN and BAILLIE 1979), as were the deficiencies *sDf2*, *sDf7*, *sDf8*, *sDf9* and *sDf10* (MOERMAN and BAILLIE 1981). The relative positions of the visible markers are shown in Figure 1. Lethal mutations and deficiencies were maintained in heterozygous strains of the genotypes *unc-22(s7) let-a(sx)/ + +* and *sDfy/+*, respectively. We are able to maintain these strains without losing the lethal or deficiency-carrying chromosome because worms heterozygous for a mutation or a deficiency in the *unc-22* locus can be distinguished from homozygous wild-type worms in a 1% nicotine solution (nicotine alkaloid, Sigma Chemical Co.). The heterozygotes twitch in the nicotine solution, whereas the homozygous wild types do not (MOERMAN and BAILLIE 1979, 1981). Culture conditions employed in this study have been described previously (BRENNER 1974; MOERMAN and BAILLIE 1979).

Isolation of unc-22-*linked lethal and sterile mutations:* Homozygous *unc-22(s7)* hermaphrodites were treated with either 0.025 or 0.05 M ethyl methanesulfonate (EMS) (BRENNER 1974) and then mated to wild-type males. Young, adult F_1 heterozygous hermaphrodites were placed on 35-mm Petri plates (one per plate) at 26° for 24 hr and then removed. Progeny were screened for fertile, adult Unc-22 (twitcher) worms. Fertility was established by observing internal eggs. A lethal mutation on the *unc-22(s7)* marked chromosome was identified by the absence of adult twitchers, and a putative lethal strain was established by selecting F_2 heterozygotes from the same plate (using nicotine). A sterile mutation was identified by the presence of sterile adult twitchers, and these mutations were treated as "lethals." The putative lethal strains were retested at 26°. To ensure that the lethal mutation was close to *unc-22*, only strains with a phenotypic ratio of wild type:Unc-22 of 100:1 or greater was retained. The confirmed lethals were then maintained at 13°. Since the isolation procedure had been carried out at 26°, it was possible to test the mutations for temperature sensitivity. Strains that segregated fertile, adult twitchers at 13° but not at 26° were identified as temperature sensitive lethals. These were maintained as *let-x unc-22* homozygotes at 13°.

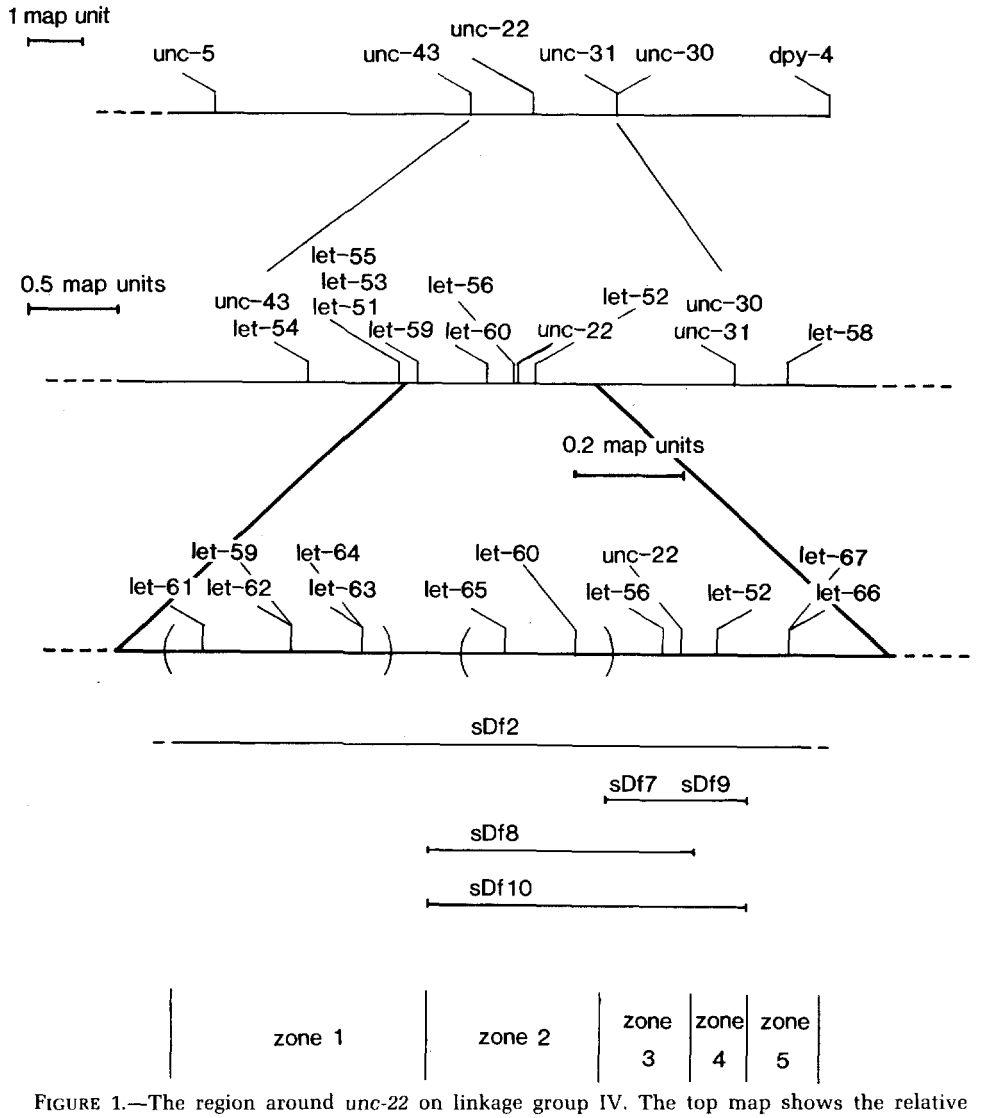

FIGURE 1.—The region around *unc-22* on linkage group IV. The top map shows the relative positions of visible genes in this region. The middle map is an expansion of the region between *unc-43* and *unc-30* showing the positions of nine essential genes relative to *unc-22*. The bottom map is an expansion of the region uncovered by *sDf2* showing the positions of 11 essential genes. The genes enclosed in brackets were not positioned relative to each other. The positions of the deficiencies are shown below the map. The five zones defined by the deficiency breakpoints are also shown.

Recombination mapping of lethal mutations: Lethal mutations were positioned relative to *unc-22* by the following protocol. The progeny of *unc-5(e152) + + dpy-4(e1166)/ + unc-22(s7) let-a(xc) +* hermaphrodites were screened for Unc-22 recombinants (either *unc-5 unc-22/ + unc-22* or *unc-22 dpy-4/ unc-22 +*), and these were progeny tested. If the Unc-22 recombinants segregated Unc-22 Dpy-4 progeny, then the lethal mutation must be to the right of *unc-22*, whereas if the Unc-22 recombinants segregated Unc-5 Unc-22 progeny, the lethal mutation must be to the left.

The map distance from *unc-22* to a lethal site was determined by measuring the recombination

frequency between *unc-22* and the lethal. Mapping was done at 20°, and all of the progeny were scored for recombinant events as described by ROSE and BAILLIE (1979). In the case of *s41*, *s43*, *s44*, *s45* and *s48*, five or ten *unc-22*(*s7*) *let-a*(*sx*)/ + + late larval hermaphrodites were placed on large (100 mm) Petri plates and transferred every 24 hr until no further progeny were produced. For the remaining lethals, 10, 20 or 30 late larval hermaphrodites were placed on small (35 mm) Petri plates, one per plate, and transferred every 6–18 hr. The F_1 phenotypes were scored, and the recombination frequency was calculated using the formula, $p = 1 - \sqrt{1 - (3U/U + W)}$, where p = recombination frequency, U = number of Unc-22 progeny and W = number of phenotypically wild-type progeny. In a similar manner, the recombination frequency from *unc-22* to several of the visible genes in the region was determined by placing 20 *unc-22 unc-x*/ + + late larval hermaphrodites on plates, one per plate, and transferring them every 6–18 hr. The F_1 progeny were scored, and the recombination frequency was calculated.

Identification of lethal complementation groups: The procedure for the complementation tests was modified from that used by BRENNER (1974) for visible mutations. Males of a lethal strain (*let-a*(*sx*) *unc-22*(*s7*)/ + +) were crossed with hermaphrodites of another lethal strain (*unc-22*(*s7*) *let-b*(*sy*)/ + +). The progeny of this cross were screened for males, to confirm that the hermaphrodites had outcrossed, and for the presence of adult male and hermaphrodite twitchers. When present, several hermaphrodite twitchers were put on a separate plate and examined for fertility. An absence of fertile, adult hermaphrodite twitcher progeny indicated that the two lethals failed to complement and were alleles of the same gene.

Deficiency mapping: The lethal-bearing strains were complemented with the deficiencies using the aforementioned procedure except that males heterozygous for the lethal mutation were mated to hermaphrodites carrying the deficiency, *sDfx*/ +. If no fertile, adult hermaphrodite twitchers were found, the *let* locus was considered to be included in the deficiency.

Complementation tests between visible mutations and deficiencies were done by crossing *sDfx*/ + hermaphrodites to the heterozygous mutant males. The progeny were screened for males with the mutant phenotype. The presence of these mutant males indicated that the deficiency uncovered the visible site.

Measurement of effective lethal phases: To construct growth curves for N2 and *unc-22*(*s7*), several hermaphrodites of each strain were put on a large (100 mm) plate at 20° for 2 hr and then removed. A sample of N2 and *unc-22* larvae from the plate were measured every 8 hr for 96 hr. The larvae were heat killed (by placing the tip of a hot soldering iron in the agar beside them) and then measured with an ocular micrometer at ×50 magnification. To determine the effective lethal phase of a lethal mutant, young twitcher larvae were removed from the lethal stock plate, placed on another plate for 3 days, and then measured. In some cases the *unc-22 let-a* mutant grew very slowly, and it was necessary to leave the worms longer than 3 days. Using the *unc-22* growth curve the final length of these doubly mutant worms allowed us to determine whether they were embryonic, early, mid or late larval lethals. We did not assign the larval lethals to a specific larval developmental stage because no record of their molts during development was made.

RESULTS

Of 3094 F_1 individuals whose progeny were examined, 49 were found to carry a lethal or sterile mutation linked to *unc-22*(*s7*). During the course of this study some of these original 49 mutant strains were discarded because of either poor fecundity or loose linkage to *unc-22*. Lethal mutations that were loosely linked to the *unc-22* locus could not be effectively maintained in our system. Three temperature-sensitive lethals were identified by our screen, *s54*, *s57* and *s72*. Data on these lethals are not reported in this paper.

Positioning nine lethal mutations by recombination mapping: Nine lethal mutations, *s41* to *s46*, *s48*, *s49* and *s59*, were initially positioned by recombination relative to the *unc-22* locus (Table 1, top). Seven of these mutations map to the left of *unc-22*, and two map to its right. This mapping data allowed five of the

TABLE 1

Left-right positioning and two-factor mapping results for lethal and visible genes in the unc-22 *region*

Gene (allele)	Position relative to *unc-22*	Phenotypes			Map units from *unc-22*[b]
		Twi[a]	Unc	Wild	
let-51(s41)	left	11		4456	0.4
let-52(s42)	right	3		6827	0.07
let-53(s43)	left	22		4582	0.7
let-54(s44)	left	32		3400	1.4
let-55(s45)	left	19		3361	0.6
let-56(s46)	left	1		5806	0.03
let-58(s48)[c]	right	5		519	1.5
let-59(s49)	left	13		3303	0.6
let-60(s59)	left	3		2097	0.2
unc-30(e318)	right	17	31	2777	1.3[d]
unc-31(e169)	right	17	26	2926	1.1[d]
unc-43(e266)	left	35	28	4225	1.1[d]
let-56(s168)	left	1		1919	0.07
let-56(s173)	left	1		5041	0.03
let-59(s172)	left	12		2257	0.8
let-61(s65)	left	13		2108	0.9
let-62(s175)	left	12		2394	0.75
let-63(s170)	left	8		1855	0.6
let-64(s171)	left	6		1360	0.65
let-64(s216)	left	9		2259	0.6
let-65(s174)	left	5		1793	0.4
let-65(s254)	left	3		1766	0.25
let-66(s176)	right	3		2088	0.2
let-67(s214)	right	7		4601	0.2

[a] Twi = Unc-22.
[b] Map units = 100 p.
[c] s48 has been lost.
[d] p was calculated using the number of Twi and Unc recombinants.

mutations to be assigned to separate genes. Pairwise complementation tests were done between the closely mapping mutations, *s43*, *s45* and *s41*. They all complement each other and, in addition, *s41* and *s49* complement each other. These nine mutations, therefore, define nine new loci in the *unc-22* region, *let-51* to *let-56*, *let-58*, *let-59* and *let-60* (Figure 1). Several visible loci in the region were also positioned (Table 1, middle; Figure 1).

Positioning the deficiencies: The nine mapped *let* loci, as well as *unc-30* and *unc-31*, were then used to position five deficiencies (*sDf2*, *sDf7*, *sDf8*, *sDf9* and *sDf10*) that delete the *unc-22* locus. These deficiencies had been generated with formaldehyde in a previous study (MOERMAN and BAILLIE 1981), and *sDf2* was described at that time. The results of complementation tests between the deficiencies and the mapped loci are given in Table 2. The positions of the deficiencies are shown in Figure 1. It can be seen that five loci (*let-51*, *let-53*, *let-*

TABLE 2

Complementation tests between genes and deficiencies in the unc-22 *region*[a]

Genes	Alleles	Deficiencies					Zone
		sDf2	*sDf7*	*sDf8*	*sDf9*	*sDf10*	
let-54	*s44*	+	+	+	+	+	
let-53	*s43*	+	+	+	+	+	
let-55	*s45*	+	+	+	+	+	
let-51	*s41*	+	+	+	+	+	
let-61	*s65*	−	+	+	+	+	1
let-62	*s175*	−	+	+	+	+	1
let-59	*s49, s172*	−	+	+	+	+	1
let-63	*s170*	−	+	+	+	+	1
let-64	*s171, s216*	−	+	+	+	+	1
let-65	*s174, s254*	−	+	−	+	−	2
let-60	*s59*	−	+	−	+	−	2
let-56	*s46, s168, s173*	−	−	−	−	−	3
let-52	*s42*	−	−	+	−	−	4
let-66	*s176*	−	+	+	+	+	5
let-67	*s214*	−	+	+	+	+	5
let-58	*s48*	+	ND[b]	ND	ND	ND	
unc-22	*s7*	−	−	−	−	−	3
unc-30	*e318*	+	+	+	+	+	
unc-31	*e169*	+	+	+	+	+	

[a] Some of these data are from MOERMAN and BAILLIE (1981). Complementation is indicated by + and noncomplementation is indicated by −.

[b] ND = complementation tests were not done because this mutation was lost.

54, *let-55* and *let-58*) lie outside of *sDf2*, whereas four loci (*let-52*, *let-56*, *let-59* and *let-60*) are within the region uncovered by *sDf2*. The map in Figure 1 also shows that the *sDf2* region can be divided into several smaller regions by the breakpoints of the other four deficiencies.

Mutations outside the sDf2 *region:* In addition to the original nine lethal mutations, 28 mutations were tested for complementation with *sDf2*. Thirteen of this second set of lethals complemented *sDf2* and, therefore, lie outside its region. One of these, *s47*, complemented each of the five mutations originally mapped outside *sDf2* and was assigned to a new locus, *let-57*. Another mutation, *s53*, failed to complement *let-54*(*s44*). No further mapping was done with these or any of the other mutations outside *sDf2*. The majority of the strains that were lost or discarded during this study came from this group.

Mutations inside the sDf2 *region:* The remaining 15 lethal and sterile mutations failed to complement *sDf2*. Twelve of these were positioned relative to *unc-22* by recombination mapping (Table 1, bottom), and three could not be

maintained and were discarded. Therefore, we have identified a total of 19 lethal mutations in the region uncovered by *sDf2*, and 16 of these 19 lethals have been further positioned by recombination mapping. The results of complementation tests with the deficiencies, *sDf7*, *sDf8*, *sDf9* and *sDf10*, localized these 16 mutations into five separate zones. Table 2 summarizes the results of these tests. *Inter se* complementation tests were done between lethals that occupied the same zone, and the 11 genes identified by these tests are shown in the first column of Table 2. Figure 1 shows the positions of these genes. Seven lethals are in zone 1, and they define five loci, *let-61*(*s65*), *let-62*(*s175*), *let-59*(*s49*, *s172*), *let-63*(*s170*) and *let-64*(*s171*, *s216*). There are three lethals in zone 2 that define two genes, *let-65*(*s174*, *s254*) and *let-60*(*s59*). The three lethal mutations in zone 3, *s46*, *s168* and *s173*, are all alleles of *let-56*. The *unc-22* locus is also in this zone. Zone 4 contains *s42* which defines the *let-52* locus, whereas zone 5 has two mutations that define two loci, *let-66*(*s176*) and *let-67*(*s214*). Lethal complementation groups located in the same zone were not positioned with respect to each other. Eight of the *let* loci map to the left of *unc-22*, and three map to the right of it. The lethal, sterile and visible mutations so far uncovered by *sDf2* represent 12 loci in a 1.1-map unit interval.

As well as positioning the lethal mutations, the complementation tests between the deficiencies and the lethals also refined the positions of the deficiency breakpoints. The results in Table 2 show that *sDf7*, *sDf8*, *sDf9* and *sDf10* are all smaller than *sDf2*. The two smallest deficiencies, *sDf7* and *sDf9*, are not more than 0.4 map units in length, whereas *sDf8* extends approximately 0.4–0.6 map units, and *sDf10* extends approximately 0.4–0.8 map units. There are at present three genes, *let-56*, *unc-22* and *let-52*, in the region uncovered by the smallest deficiencies.

Lethal phases: The various lethal homozygotes exhibited a wide range of terminal phenotypes; from the embryonic stage to the sterile adult (Table 3; Figure 2). *sDf2* homozygotes are embryonic lethals, but the remaining four deficiency strains develop to the first larval stage when homozygous. At present no clustering of genes affecting a particular developmental stage is apparent in this region.

The sterile category accounts for 14% (5 of 37) of our total lethals. In a study of the tip of the X chromosome, MENEELY and HERMAN (1981) also isolated adult steriles (9 of 54 isolates). Six of their adult steriles, representing five genes, are rescued by wild-type male sperm and, therefore, are presumably due to defects in either spermatogenesis or fertilization. In our case, only *s214* was rescued by wild-type male sperm. *s47*, *s171* and *s216* did not produce any offspring when crossed with wild-type males which indicates that the missing function cannot be supplied during embryogenesis. These genes either have a maternal role or they affect oogenesis.

DISCUSSION

The region of linkage group IV we are attempting to characterize is defined by the deficiency, *sDf2*. This region includes the *unc-22 IV* locus and extends approximately 0.9 map units to the left of *unc-22* and at least 0.2 map units and

TABLE 3

Lethal phases of mutations in the unc-22 *region*

Genotype (all *lets cis* with *unc-22(s7)*)	Terminal length (mm)	Effective lethal phase
Mutations inside *sDf2*		
let-61(s65) homozygote	0.76	Late larval
let-62(s175) homozygote		Multiple
let-59(s49) homozygote	0.28	Early larval
let-59(s172) homozygote		Early larval
let-63(s170) homozygote	0.57	Mid-larval
let-64(s171) homozygote		Sterile (leaky)
let-64(s216) homozygote	0.98	Sterile
let-65(s174) homozygote	0.6	Mid-larval
let-65(s254) homozygote	0.48	Mid-larval
let-60(s59) homozygote	0.44	Mid-larval (leaky)
let-56(s46) homozygote	0.78	Late larval
let-56(s46)/sDf2		Mid-larval
let-56(s173) homozygote	0.58	Mid-larval
let-56(s173)/sDf2		Early larval
let-56(s168) homozygote	0.65	Mid-larval
let-52(s42) homozygote	0.28	Early larval
let-52(s42)/sDf2		Early larval
let-66(s176) homozygote		Early larval
let-67(s214) homozygote	1.0	Sterile
let-? (s58) homozygote	0.6	Mid-larval
let-? (s167) homozygote		
let-? (s177) homozygote		Early larval
Mutations outside *sDf2*		
let-51(s41) homozygote		Embryonic
let-53(s43) homozygote	0.8	Late larval
let-54(s44) homozygote	0.3	L1–L2 molt
let-54(s53) homozygote	0.28	L1–L2 molt
let-55(s45) homozygote	0.32	Early larval
let-57(s47) homozygote	0.98	Sterile
let-58(s48) homozygote		
let-?(s50) homozygote	0.72	Late larval
let-?(s51) homozygote	0.56	Mid-larval

TABLE 3—Continued

Genotype (all lets *cis* with *unc-22(s7)*)	Terminal length (mm)	Effective lethal phase
let-?(s52) homozygote	0.76	Late larval
let-?(s62) homozygote		Sterile
let-?(s63) homozygote	0.55	Mid-larval
let-?(s64) homozygote	0.5	Mid-larval
let-?(s166) homozygote	0.33	Early larval
let-?(s169) homozygote	0.25	Early larval
let-?(s212) homozygote		Early larval
let-?(s213) homozygote		Early larval
let-?(s215) homozygote	0.26	Early larval

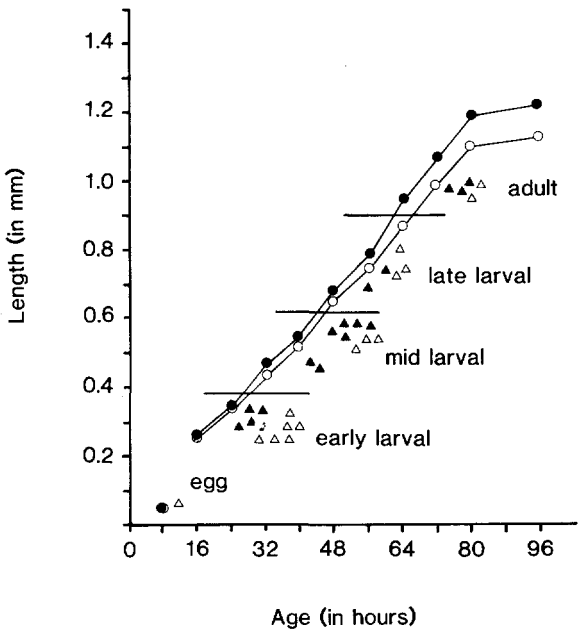

FIGURE 2.—Growth curves at 20° for N2 (●——●) and *unc-22(s7)* (○——○). The terminal lengths of *let-a(sx) unc-22(s7)* homozygotes are shown for 32 lethal mutations. ▲, Lethal mutations inside *sDf2*; △, lethal mutations outside *sDf2*.

no more than about 1.1 map units to the right of it. We have identified 19 lethal and sterile mutations that are uncovered by *sDf2*. Sixteen of these mutations were positioned by recombination mapping and also by complementation mapping with the deficiencies, *sDf7*, *sDf8*, *sDf9* and *sDf10*. These sixteen mutations identify 11 new essential genes in this region, *let-61*, *let-62*, *let-59*, *let-63*, *let-64*, *let-65*, *let-60*, *let-56*, *let-52*, *let-66* and *let-67*. Eight of these genes are in a 0.9-map unit region to the left of *unc-22*, whereas the remaining three genes are in a region of about 0.2 map units to the right of *unc-22*. Since only four of the esential loci uncovered by *sDf2* have more than one allele (*let-59*, *let-64* and *let-65* have two alleles each; *let-56* has three alleles), it is obvious that we have not identified all of the essential genes in this small region of the *C. elegans* genome.

The asymmetric distribution of the 11 lethal genes around *unc-22* is not surprising if the right endpoint of *sDf2* is only 0.2 map units from *unc-22*. However, the right endpoint of *sDf2* is not firmly positioned and could extend as far as 1.1 map units from *unc-22*. In that case the data may reflect a clustering of the lethal sites. BRENNER (1974) noted that the visibles tend to map in a cluster on the autosomal linkage groups and those of LG IV have been found to cluster on its right arm with the center of the cluster to the left of *unc-22* (HERMAN and HORVITZ 1980). HERMAN (1978) found that on LG II lethals cluster in the same region as the visibles. It will be necessary to define the right endpoint of *sDf2* more precisely and to position the lethals that map outside *sDf2* before we can determine whether the lethals in this region are also clustered.

In addition to the *unc-22 IV* region, two other genomic regions of *C. elegans* have been characterized. MENEELY and HERMAN (1979, 1981) found 24 essential and eight nonessential genes in a 7.5-map unit interval at the tip of the X chromosome, whereas ROSE and BAILLIE (1980) found seven essential and five nonessential genes in a 0.7-map unit interval around *unc-15 I*. Since none of these regions are completely characterized yet, it is necessary to estimate the total number of genes in each region before they can be compared. This has been done for the X chromosome region by MENEELY and HERMAN (1979, 1981). These authors calculated that there are a total of 45 essential genes in this region based on the fraction of mutated genes in their sample that are represented by more than one mutant. Using this same method we estimate that the total number of essential genes in the *unc-15 I* and *unc-22 IV* regions are 15 and 20, respectively. These data indicate a difference in gene density between the small, autosomal regions (28 and 19 genes/map unit, respectively) and the larger region of the X chromosome (7 genes/map unit). It will be interesting to see whether this difference is due to the regions being studied, to the chromosomes being studied or to a combination of both.

From the range of terminal phenotypes displayed by the mutations uncovered by *sDf2* (Table 3, top; Figure 2) it appears that this region does not have a bias for genes specific to a particular developmental stage. Lethals blocking at various developmental stages from first stage larva to sterile adult were recovered. None of the 16 mutations found so far inside *sDf2* blocked during embryogenesis. The absence of embryonic lethals may be due to the strong maternal effects present in *C. elegans*. We did not expect to find any embryonic lethals in the region uncovered by *sDf7*, *sDf8*, *sDf9* or *sDf10*, since strains homozygous for these deficiencies survived embryogenesis. However, there may be genes essential for embryonic development that we have not yet identified in the regions uncovered by *sDf2*, as this deficiency blocks during embryogenesis when homozygous. Alternatively, the fact that *sDf2* homozygotes are embryonic lethals may be a result of the large size of this deficiency.

A primary objective of this study was to identify the genes adjacent to the *unc-22* locus. The size of the *unc-22* locus has been estimated to be 0.015 map units by fine structure recombination mapping (MOERMAN and BAILLIE 1979; MOERMAN 1980). The flanking genes, *let-56* and *let-52*, map very close to *unc-22*, approximately 0.03 and 0.07 map units, respectively. Therefore, the distances separating these two genes from *unc-22* are not appreciably larger than the

distance across the *unc-22* locus. Since *let-56* and *let-52* map so close to *unc-22*, they could possibly be lethal alleles of this gene. We have demonstrated that *let-52(s42)* is not an allele of *unc-22* because it can be separated from the *unc-22* locus by the deficiency, *sDf8* (see Table 2). From our genetic analysis we conclude that *let-56* is the adjacent gene to the left of *unc-22* and that *let-52* is the adjacent gene to the right of *unc-22*.

The mutant alleles of the two adjacent genes, *let-56* and *let-52*, were examined as hemizygotes and homozygotes in order to determine the effect of one or two copies of the mutant allele on the terminal lethal phenotype. Null mutants of essential genes over a deficiency are not expected to block earlier in development than homozygous null mutants, whereas weak missence alleles over a deficiency may block at the same or at an earlier developmental stage (see MENEELY and HERMAN 1979, 1981). Homozygotes of the genotype, *unc-22(s7) let-52(s42)*, die as early larvae, and *s7 s42/sDf2* animals also die as early larvae. This suggests that *s42* is possibly a null allele of *let-52*. The *let-56* strains give a more complicated pattern. The lethal homozygote, *s46 s7* is a late larval lethal, whereas homozygous *s173 s7* animals die in mid-larval development. However, as hemizygotes, *s46* and *s173* become mid-larval and early larval lethals, respectively. The simplest interpretation of these results is as follows: *s46* is a weak missence allele of *let-56*, and *s173* is possibly a more severe missence allele of the locus, but neither is a null allele. Presumably, a mutant that carried a null allele of this locus would die in an early larval stage. Therefore, the adjacent genes to either side of *unc-22* are, most likely, essential in early development. What functional relationship these two loci have with *unc-22*, if any, is unknown.

We plan to continue the characterization of the *unc-22 IV* region by isolating and positioning more deficiencies and lethal mutations. The lethal mutations will be tested for suppression by the informational suppressor, *sup-7* X, to identify null alleles of the *let* genes (WATERSTON 1981). In addition to the genetic analysis, this region will also be studied at the DNA level. It should be possible to clone the *unc-22* gene and its surrounding region using a restriction fragment difference between the Bristol and Bergerac strains of *C. elegans* that occurs near *unc-22* on LG IV (A. M. ROSE, D. L. BAILLIE, E. P. M. CANDIDO, K. A. BECKENBACH and D. NELSON, unpublished). We hope to eventually be able to compare the genetic map of this region with its DNA sequence.

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