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Genetic analysis and complementation by germ-line transformation of lethal mutations in the *unc-22 IV* region of *Caenorhabditis elegans*

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Summary. The subject of this study is the organization of essential genes in the 2 map-unit *unc-22 IV* region of the *Caenorhabditis elegans* genome. With the goal of achieving mutational saturation of essential genes in this region, 6491 chromosomes mutagenized with ethyl methanesulfonate (EMS) were screened for the presence of lethal mutations in the *unc-22* region. The genetic analysis of 21 lethal mutations in the *unc-22* region resulted in the identification of 6 new essential genes, making a total of 36 characterized to date. A minimum of 49 essential genes are estimated to lie in this region. A set of seven formaldehyde-induced deficiencies of *unc-22* and surrounding loci were isolated to facilitate the positioning of essential genes on the genetic and physical maps. In order to study essential genes at the molecular level, our approach was to rescue lethal mutations by the injection of genomic DNA in the form of cosmid clones into the germ-line of balanced heterozygotes carrying a lethal mutation. The cosmid clones containing *let-56* and *let-653* were identified by this method.

Key words: *Caenorhabditis elegans* – Lethal mutations – Genome organization – Transformation

Introduction

Essential genes, or genes that are required for complete development and reproduction, have been genetically characterized in several regions of the *Caenorhabditis elegans* genome. Studies have been conducted in regions on linkage group I (LGI) (Rose and Baillie 1980; Howell et al. 1987; Howell and Rose 1990), LGII (Sigurdson et al. 1984), LGV (Rosenbluth et al. 1988), LGX (Meneely and Herman 1979, 1981) and LGIV around *ama-1* (Rogalski and Ridle 1988), as well as in the *unc-22* gene cluster (Rogalski et al. 1982; Rogalski and Baillie 1985; Clark et al. 1988), which is the subject of this study. Studies on the *unc-22* region have resulted in the identification of 30 essential genes in the 2 map-unit interval defined by the deficiency *sDf2*, which comprises about 1% of the genetic map. From these studies, it was estimated that 48 essential genes occupied the *unc-22* region, based on a Poisson calculation of the distribution of the lethal mutations. Therefore, the region had not been saturated with mutations in all essential genes.

Identification of all essential genes, together with the positioning of deficiency breakpoints on the genetic map, is valuable for understanding the molecular organization of the *unc-22* region. Mutants of the *unc-22* gene have a characteristic uncoordinated phenotype with "twitching" of the body wall musculature. The *unc-22* gene has been cloned (Moerman et al. 1986) and sequence analysis has shown that it codes for a muscle component that has a myosin light chain kinase domain and also has regions that share homology with the immunoglobulin superfamily (Benian et al. 1989). The *let-60* gene, which is involved in cell fate determination in the hermaphrodite vulva (Beitel et al. 1990; Han et al. 1990), was shown to be a *ras* homolog (Han and Sternberg 1990). Further analysis of other essential genes in the *unc-22* region is facilitated by the identification of restriction fragment length differences (RFLDs), which have been genetically mapped and cloned in the 2.5 map unit interval surrounding *unc-22*, between *unc-43* and *unc-31* (Baillie et al. 1985). The addition of overlapping cosmid clones (Coulson et al. 1986) and yeast artificial chromosome clones (Coulson et al. 1988) to the *C. elegans* physical map has resulted in identification of a total of about 1100 kb of DNA mapping in the 2 map unit interval that includes *unc-22*.

In this paper, we describe the identification of additional essential genes together with the correlation of the genetic and physical maps. We focus on the 0.2 map unit interval between *dpy-20* and *unc-22*. The placement of *dpy-20* on the physical map (Clark 1990) anchored

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a contiguous array of cosmid clones in this interval. These cosmids represent about 200 kb of DNA. The technique of germ-line transformation of cosmid clones was used to identify those clones that contain the coding regions for two of the four essential genes identified in the *dpy-20–unc-22* interval, *let-56* and *let-653*.

Materials and methods

Nematode strains and culture conditions. Nematodes were maintained on petri plates containing nematode growth medium (NGM) streaked with *Escherichia coli* OP50 (Brenner 1974). The genetic nomenclature follows the recommendations of Horvitz et al. (1979). The wild-type strain N2 (var. Bristol), and strains carrying the following mutations were obtained from the stock collection at the Medical Research Council, Cambridge, England or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia: LGIV: *dpy-4(e1166), dpy-13(e184); dpy-20(e1282), eDf19, him-8(e1489), mDf7* (Rogalski and Riddle 1988), *mec-3(e1338), unc-5(e152), unc-26(e345), unc-30(e191), unc-31(e169), unc-43(e266)*. The balancer *nT1(IV;V)* (Ferguson and Horvitz 1985) was from the strain CB3608, which has the genotype *dpy-20(e1282) dpy-26(n199)/nT1(IV); +/nT1(V)*. A strain carrying *nT1* linked to the lethal mutation *m435* was supplied by the Caenorhabditis Genetics Center. The strain carrying *nDf27(IV)* and the strain *lin-3(n378) unc-22(e66)* were obtained from the Laboratory of R. Horvitz, M.I.T.. The remaining LGIV mutations were isolated at Simon Fraser University: *unc-22(s7)* (Moerman and Baillie 1979), *sDf2, sDf7, sDf8, sDf9, sDf10* (Moerman and Baillie 1981), *sDf21* and *sDf22* (G. Wild and D. Baillie, unpublished results). Those lethal mutations induced by ethyl methanesulfonate (EMS) in the *sDf2* region that were isolated previously at Simon Fraser University and were used in this study are listed in Table 1.

Throughout this work, the *unc-22(s7)* mutation was used as a marker (Moerman and Baillie 1979). It is a conditionally semidominant mutation where the *unc-22(s7)/+* individual has a "twitcher" phenotype in a 1% nicotine solution (Sigma) and is otherwise phenotypically wild-type. Individuals that are homozygous wild-type are contracted and paralyzed in nicotine, whereas *unc-22(s7)* homozygotes are non-conditional twitchers.

Isolation of EMS-induced lethal mutations tightly linked to unc-22(IV). Hermaphrodites of the genotype (*unc-22(s7) unc-31(e169)* were exposed to 0.012 M EMS for 4 h. They were then allowed to recover for 1 h and were mated to wild-type (N2) males. The wild-type fourth larval stage (L4) F_1 hermaphrodites were isolated and allowed to self-fertilize. The F_2 generation was screened for the absence of gravid Unc-22 Unc-31 hermaphrodites. Because closely linked lethal mutations were being selected, any individual F_1 giving more than three Unc-22 Unc-31 or Unc-22 recombinant progeny was discarded. This screen provided a set of lethal mutations that were at most about 3 map units from *unc-22* (assuming 150 progeny were scored for each F_1 heterozygous for a lethal mutation). The strains carrying the lethal mutations in heterozygotes with a wild-type LGIV were maintained until strains containing the balancer *nT1* were constructed. The unbalanced strains were maintained by selecting three phenotypically wild-type individuals that twitched in nicotine [*(let-x) unc-22 unc-31/+ + +*] and in each generation selecting a line that did not segregate more than the expected number of Unc-22 Unc-31 progeny. The lethal mutations were balanced over *nT1* in one of the following two ways. First, hermaphrodites of the genotype *(let-x) unc-22 unc-31/+ + +* were mated with *unc-31/nT1(IV);+/nT1(V)* males. Wild-type L4 hermaphrodite progeny were selected in 1% nicotine as progenitors of the required strain, namely *(let-x) unc-22 unc-31/nT1(IV);+/nT1(V)*. These hermaphrodites, when self-fertilized, only segregated vulvaless (Vul) progeny and wild-type progeny that twitched in nicotine. The Vul phenotype is a characteristic of *nT1(IV;V)* homozygotes (Ferguson and Horvitz 1985). The second way of balancing the lethal mutations was to mate *(let-x) unc-22 unc-31/+ + +* hermaphrodites to N2 males and select wild-type F_1 males that twitch in nicotine. These males were mated to *dpy-20 dpy-26/nT1(IV);+/nT1(V)* hermaphrodites and wild-type L4 hermaphrodite progeny were selected in nicotine as twitchers. The correct strain [*(let-x) unc-22 unc-31/nT1(IV);+/nT1(V)*] was identified as the one that did not segregate Dpy progeny and did segregate Vul progeny.

Isolation of formaldehyde-induced deficiencies. Hermaphrodites of the genotype *unc-31(e169)* were exposed to 0.1% formaldehyde as recommended by Johnsen and Baillie (1988). Of these hermaphrodites, 5–10 were allowed to lay eggs on each 60 mm petri plate. The F_1 generation was screened in 1% nicotine for twitchers. Only one twitcher was selected from a plate if more than one was found. The self-progeny of each individual carrying a putative *unc-22* mutation were screened for Unc-22 Unc-31 individuals. Only the lines with no fertile Unc-22 Unc-31 animals were kept. These lines were the ones carrying putative deficiencies uncovering *unc-22* and a neighbouring essential gene or genes. Each strain carrying a putative deficiency was maintained by selecting three Unc-31 individuals that twitched in nicotine every generation until the deficiency was balanced. The deficiencies were balanced by mating *Df(unc-31)/+unc-31* hermaphrodites to *unc-31/nT1(IV);+/nT1(V)* males and selecting wild-type L4 hermaphrodite progeny that twitch in nicotine [*Df(unc-31)/nT1(IV);+/nT1(V)*] (parentheses around *unc-31* indicate that some deficiencies may delete this marker).

Complementation tests. All complementation tests were conducted at 20° C. The mutations isolated in screens for formaldehyde-induced deficiencies were subjected to a series of complementation tests with mutant alleles of *dpy-13, unc-5, unc-43, dpy-20, unc-30, unc-26,* and *dpy-4*. Once the positions of the two breakpoints had been

Table 1. Lethal alleles in the *unc-22* region used in this study

Zone[a]	Gene	Allele	Number of alleles	Phenotype of lethal arrest[c]		Reference[b]
				let-x/let-x	*let-x/sDf2*	
6	*let-52*	*s42*	1	Early larva	Early larva	1
5	*let-56*	*s1192*	8	Mid-larva	Mid-larva	3
1D	*let-59*	*s49*	5	Early larva	Early larva	1
3	*let-60*	*s1124*	3	Early larva	Egg to early larva[d]	3
1A	*let-61*	*s65*	1	Late larva	Late larva	1
1A	*let-63*	*s170*	2	Mid-larva	Mid-larva	1
1C	*let-64*	*s697*	3	Late larva (leaky)	Late larva	2
2	*let-65*	*s174*	5	Mid-larva	Mid-larva	1
8	*let-66*	*s176*	1	Early larva	Mid-larva	1
8	*let-67*	*s214*	1	Sterile	Mid-larva	1
9	*let-68*	*s1258*	5	Early larva	Early larva	3
1A	*let-69*	*s172*	3	Early larva	ND	1
1D	*let-70*	*s1132*	2	Early larva	Mid-larva	3
1C	*let-71*	*s692*	1	Sterile (leaky)	Sterile (leaky)	2
1A	*let-72*	*s52*	2	Late larva	ND	1
1D	*let-73*	*s685*	1	Sterile	Sterile	2
1D	*let-91*	*s678*	2	Mid-larva	Mid-larva	2
		s753		Late larva	Late larva	3
4A	*let-92*	*s504*	2	Early larva	Early larva	2
7	*let-93*	*s734*	1	Mid-larva	Mid-larva	3
1A	*let-96*	*s1112*	1	Mid-larva	Mid-larva	3
10	*let-97*	*s1121*	1	Early larva	Early larva	3
1D	*let-98*	*s1117*	1	Late larva	Late larva	3
10	*let-99*	*s1201*	1	Maternal effect lethal	Maternal effect lethal	3
1D	*let-100*	*s1160*	1	Early larva	Egg to early larva	3
1A	*let-307*	*s1171*	1	Mid-larva	Mid-larva	3
1A	*let-308*	*s1705*	1	Mid-larva[e]	Mid-larva	3
9	*let-309*	*s1115*	1	Late larva	Late larva	3
1D	*let-311*	*s1195*	1	Late larva	Late larva	3
1B	*let-312*	*s1234*	1	Late larva	Late larva	3
1A	*let-651*	*s1165*	2	Mid-larva	Mid-larva	3
		s1185		Early larva	Egg to early larva	3
1C	*lin-3*	*s751*	2[f]	Late larva	Early larva	3

[a] Refer to Fig. 1 for the location of each zone on the map

[b] For information on the isolation, mapping and characterization of lethal mutations from reference 1, see Rogalski et al. (1982) and Moerman (1980). For isolation and mapping of lethal mutations from reference 2, see Rogalski and Baillie (1985). Reference 3 is Clark et al. (1988)

[c] Lethal mutations described in references 1 and 2 are in *cis* with *unc-22(s7)*. All others are *in cis* with *unc-22(s7)* and *unc-31(e169)*. ND, not determined

[d] The egg to early phenotype of some *let-x/sDf2* individuals indicates that a more precise developmental arrest stage was not determined

[e] The phenotype of *let-308(s1705)* homozygotes and hemizygotes is with a background of *let-99(s1201)* in *cis*

[f] This is the number of lethal *lin-3* alleles isolated in saturation screens only

determined roughly, complementation tests with the appropriate lethal mutations were done (see below).

Positioning of new EMS-induced lethal mutations within *sDf2* was accomplished through complementation tests with a set of deficiencies whose breakpoints divide the *sDf2* region into 14 zones (see Fig. 1). Once a mutation had been localized to a zone, it was assigned to a complementation group through complementation tests with alleles of all genes within that zone. For complementation tests with alleles of previously identified genes defined by multiple alleles, usually only one representative allele was used (Table 1; see Results for further discussion). Representative alleles of all essential genes mapping to zone 1A were tested for allelism with *him-8(e1489)* and *mec-3(e1338)*. *let-60* mutations were tested for allelism with *dpy-20(e1282)*.

Preparation of cosmid and plasmid DNA for germ-line transformation. Cosmids were provided by A. Coulson and J. Sulston of the Medical Research Council, Cambridge, England. Recombinant DNA techniques employed in this study followed standard protocols as outlined by Maniatis et al. (1982).

The cosmid and plasmid DNA used for germ-line transformation was prepared using the protocol supplied by J. Sulston (personal communication). DNA from overnight liquid cultures was extracted using the alkaline lysis technique described by Maniatis et al. (1982), ex-

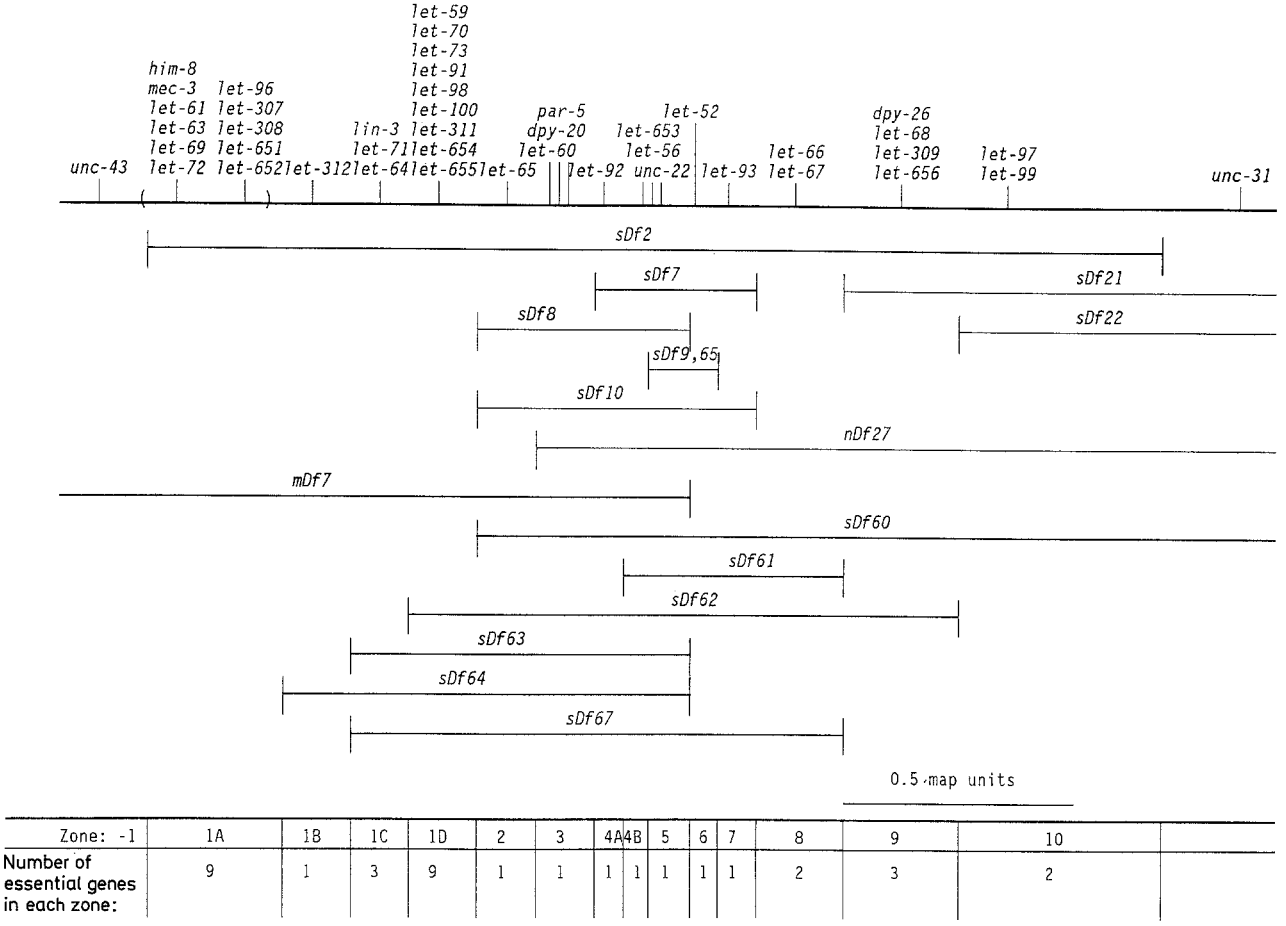

Fig. 1. Genetic map of the *unc-22* region which is defined by the extent of the deficiency *sDf2*. The zones and number of essential genes in each are indicated below the map. *Parentheses* on the genetic map line indicate loci that have not been positioned relative to each other. The *let-60* gene was positioned 0.01 map units to the left of *dpy-20* by M. Han (personal communication). The *dpy-26* gene was positioned into zone 9 by P. Meneely (personal communication). The *let-654* gene has been renamed *sem-3* because it has mutant alleles that affect sex muscle development in the hermaphrodite (M. Stern, personal communication)

cept that the phenol and chloroform extractions were omitted and no RNAase was used. Instead, an equal volume of 4.4 M LiCl was added to the plasmid or cosmid DNA solution to precipitate RNA. After centrifugation, the DNA was ethanol precipitated and the dried pellet was gently resuspended in 100 µl of 0.1 M potassium acetate (pH 7.4). The DNA was again precipitated with ethanol and the pellet was resuspended in 10 mM potassium phosphate pH 7.5. The appropriate amount of DNA was mixed with injection solution (Fire 1986) to a final concentration of 200–400 ng/µl.

Germ-line transformation techniques. The technique used was essentially as described in Fire (1986). The *let-56* mutation-carrying strains used in transformation experiments were of the genotype *let-56(x) unc22(s7)* [*unc-31(e169)*];+/*nT1 let-?(m435)(IV;V)*. The *let-92* and *let-653* mutation-carrying strains were the same as above, except they had a nonlethal-bearing *nT1* translocation.

Results

Isolation of EMS-induced lethal mutations tightly linked to unc-22

The screens focused on isolating lethal mutations within roughly 3 map units of *unc-22*. A total of 79 mutations were isolated from 6491 chromosomes screened.

Isolation of formaldehyde-induced deficiencies

A total of 29 F_1 individuals that twitch in nicotine were isolated from approximately 558000 F_1s screened. Of these, eight gave no fertile Unc-22 Unc-31 progeny. Only strains containing the eight lethal mutations were retained and analyzed. The lethal phase for seven of these mutations was either embryonic or L1 larval. One of the strains carried a mutation in *unc-22* and a sterile mutation that did not map to the *sDf2* or the *mDf7* region. The other seven mutations (*sDf60, sDf61, sDf62, sDf63, sDf64, sDf65* and *sDf67*), deficiencies for *unc-22* and neighbouring essential genes, are shown on the ge-

netic map in Fig. 1. The right breakpoint of *sDf60* lies between *unc-30* and *let-325* (Edgley and Riddle 1987).

Complementation tests

To establish complementation groups, the 21 EMS-induced lethal mutations isolated in this study that mapped within *sDf2* were mapped to smaller intervals, or zones, using the deficiencies *mDf7, nDf27, sDf7, sDf8, sDf9, sDf10, sDf19, sDf21, sDf22, sDf60, sDf61, sDf62, sDf63, sDf64, sDf65* and *sDf67*. The *unc-22* region has now been subdivided into 14 zones defined by deficiency breakpoints (Fig. 1). To define complementation groups within a zone, representative alleles of previously characterized essential genes were used. In the cases where multiple alleles with different phenotypes were available, the representative allele used was the one that exhibited the earliest developmental arrest and would be most likely to be the null allele. The *inter se* complementation tests (data not shown), show that six new essential genes have been identified in the *sDf2* region in this study. Table 2 lists the 21 new mutations and their complementation groups, positions on the genetic map (zone), and effective lethal stages for homozygotes and hemizygotes (*let-x/sDf2*).

Clark et al. (1988) described one locus in zone 1 (Fig. 1), *let-91*, where the complementation data indicate that it could be a complex locus with complementing alleles. To test this interpretation further the left breakpoints of *sDf62, sDf63* and *sDf64*, formaldehyde-induced deficiencies generated in this study, were used to subdivide zone 1 into four subzones. The complementation data with regard to *let-91* are presented in Table 3. Zone 1A contains a new essential gene *let-651* with alleles *s1165* and *s1185* and zone 1D contains *let-91* with alleles *s753, s678* and *s1720* (Table 3). There are currently at least four complementation groups separating *let-651* and *let-91* (Fig. 1). However, one allele of each gene, *let-651(s1185)* and *let-91(s753)*, fail to complement, that is they produce a dominant synthetic lethal phenotype in the *trans*-heterozygote. The two genes map three deficiency breakpoints apart.

Table 2. Lethal mutations isolated in this study

Zone[a]	Gene	Allele	Phenotype of lethal arrest[b]	
			let-x/let-x	*let-x/sDf2*
1D	*let-59*	*s1087*	Egg to early larva	Egg to early larva
1A	*let-63*	*s1766*	Mid-late larva	Late larva
1C	*let-64*	*s1746*	ND	Sterile
2	*let-65*	*S1083*	Mid-larva	Sterile
		s1084, s1730	Mid-larva	Late larva
		s1777	Mid-larva	Mid-larva
8	*let-66*	*s1739*	Early larva	Early larva
9	*let-68*	*s1081*	Early larva	Early larva
1A	*let-69*	*s1085*	Early larva	Mid-larva
1D	*let-73*	*s1747*	Sterile	Sterile
1D	*let-91*	*s1720*	Mid-larva	Mid-larva
1A	*let-96*	*s1732*	Late larva	Mid-larva
9	*let-309*	*s1770*	Late larva	Late larva
1A	*let-652*	*s1086*	Mid-larva	Mid-larva
4B	*let-653*	*s1733*	Early larva	Early larva
1D	*let-654*	*s1734*	Early larva	Mid-larva
1D	*let-655*	*s1748*	Sterile	Sterile
9	*let-656*	*s1753, s1767*	Sterile	Sterile
1C	*lin-3*	*s1750*	Mid-larva	Mid-larva

[a] Refer to Fig. 1 for the location of each zone on the map
[b] Lethal arrest stages for hemizygotes were determined as previously described (Clark et al. 1988). Lethal arrest stages of homozygotes were only approximated

Table 3. Summary of relevant complementation data for *let-91* and *let-651*

	let-91			*let-651*	
	s678	*s753*	*s1720*	*s1165*	*s1185*
s753	−				
s1720	−	−			
s1165	+	+	+		
s1185	+	−[a]	+	−	
sDf62	−	−	−	+	+
sDf63	−	−	−	+	+
sDf64	−	−	−	+	+

[a] Indicates the allele-specific interaction between the two genes

Germ-line transformation rescue of lethal mutations

Figure 2 shows a detailed map of the *dpy-20–unc-22* interval. The deficiency breakpoints are placed according to their position on the genetic map. The cosmids used for germ-line transformation experiments are shown below the genetic map. The cosmids C13G4 and C35H3 were correlated with the genetic map by Tc1 tagging and cloning of *unc-22* (Moerman et al. 1986) and of *dpy-20* (Clark 1990), respectively.

The experiments conducted to attempt the rescue of lethal mutations are summarized in Table 4. Note that there was no control for incorporation of the cosmid into the germ-line unless rescue of a mutation was observed and thus our negative results are not compelling. Since the strains were heterozygous for the translocation *nT1*, many lethal mutation-bearing individuals had to be injected. Only 1/16 of the zygotes were homozygous for the lethal-bearing chromosome and euploid. Therefore, the probability of obtaining an F_1 that is a rescued mutant homozygote from a heterozygous parent carrying *nT1* was low. Stable rescue occurs when the transformed cosmid is incorporated into the germ-line and somatic cells and is transmitted to the next generation in a least a portion of gametes. Transient rescue occurs when the cosmid is only incorporated into somatic cells so that the mutant phenotype is rescued in the F_1 but not in the next generation.

The *let-56* allele *s173* was rescued by the cosmids C11F2 and ZK820. Three coding regions "p", "q" and "r" have been identified on C11F2 as regions that cross-hybridize with the *C. briggsae* genome and by hybridiza-

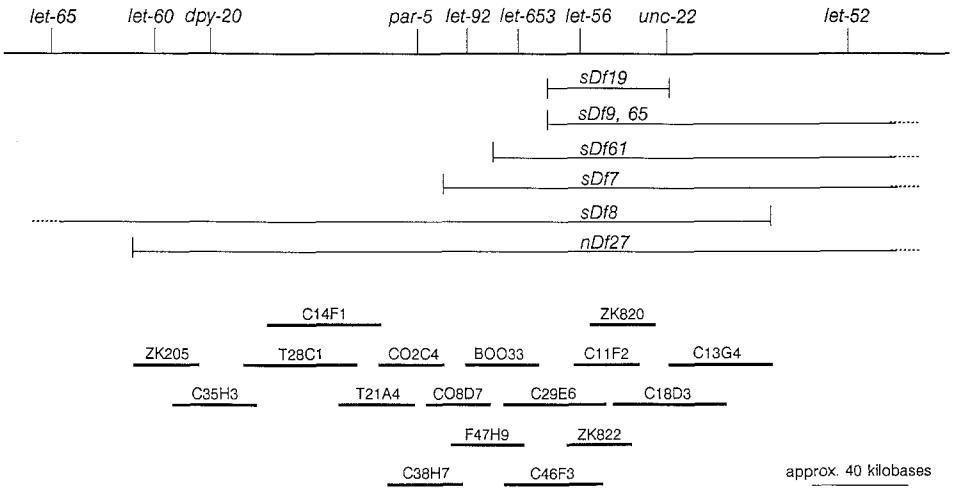

Fig. 2. Map of the *dpy-20–unc-22* region showing the genetically mapped deficiency breakpoints. Below the genetic map is a partial representation of the physical map, showing the cosmid clones used in germ-line transformation experiments

Table 4. Summary of lethal mutation rescue experiments

Gene	Allele	Strain	Cosmid or plasmid injected	Individuals injected	Rescued F_1s
let-56	*s46*	BC3488	C11F2	26	0
	s173	BC3491	C11F2	53	2 stable 1 transient
			ZK820	44	5 transient
			ZK822	11	0
			pCes122	40	0
			pCes124	33	0
			pCes125	22	0
	s1192	BC3492	ZK820	17	0
			C11F2	40	0
let-60	*s59*	BC2899	ZK205	17	0
let-92	*s504*	BC2903	T28C1	36	0
			C35H3, T28C1, T21A4	26	0
			C14F1, T21A4, C02C4	15	0
			T21A4, C38H7, F47H9	21	0
	s677	BC1899	T28C1	32	0
			C35H3, T28C1, T21A4	13	0
			C14F1, T21A4, C02C4	10	0
			T21A4, C38H7, F47H9	16	0
			T21A4, C02C4, C08D7	8	0
let-653	*s1733*	BC3261	C02C4	17	0
			C08D7	21	0
			B0033	6	0
			T21A4	18	0
			C08D7, B0033, C46F3	17	0
			T21A4, C02C4, C08D7	18	0
			F47H9, C29E6, ZK822	37	1 transient 1 stable
			T21A4, C38H7, F47H9	25	0
			F47H9	31	0
			C29E6	45	1 transient
			ZK822	34	0

tion to Northern blots of *C. elegans* total RNA (Prasad and Baillie 1989). The probes used for these hybridizations were *Pst*I fragments of C11F2. Because ZK820 does not share *Hin*dIII sites with the left end of C11F2 (J. Sulston and A. Coulson, personal communication), the coding region "p" is not likely to correspond to *let-56*. The 17 kb "q" and 8 kb "r" *Pst*I fragments of C11F2 were subcloned into pUC19. Plasmid pCes122 contains fragment "r" (S. Prasad, personal communication; Prasad and Baillie 1989). Fragment "q" (Prasad and Baillie 1989) was subcloned in both orientations in plasmids pCes124 and pCes125. No rescue of *let-56(s173)* was achieved with these subclones. This does not rule out the possibility that one of the *Pst*I fragments contains *let-56* sequences. It is possible that the complete gene is not contained in either fragment.

The only *let-653* allele, *s1733*, was initially rescued by the injection of three cosmids pooled together (F47H9, C29E6 and ZK822). Injection of each of these three cosmids into different individuals resulted in a transient rescue of *s1733* with C29E6. Since the only incidence of a fertile *s1733* was after injection of C29E6, and since a stable rescue of *s1733* was achieved with a group of three cosmids including C29E6, we conclude that C29E6 contains sequences sufficient for rescuing the *s1733* mutation. Referring to Fig. 2, this result indicates that the left-hand breakpoints of *sDf9*, *sDf19* and *sDf65* must map to either C29E6 or C11F2.

Copy number of cosmids in rescued lethal strains

Three strains with stably rescued lethal mutations were retained. Two of these strains (BC3608 and BC3624) were *let-56(s173)* homozygotes carrying C11F2. The other strain (BC3625) was a *let-653(s1733)* homozygote carrying the three cosmids F47H9, C29E6 and ZK822. Cosmids with a C prefix have the vector pJB8. This vector has some sequences in common with pUC19. The F and ZK cosmids have the Lorist 6 vector, which has lambda sequences. Genomic DNA from the above three transformant strains was extracted to test for the presence of cosmid vector sequences. The genomic DNA and cosmid DNA were digested with *Pst*I, the fragments were separated by electrophoresis and Southern blotted (see Materials and methods). Using pUC19 and lambda DNA as probes to Southern blots of genomic DNA extracted from these strains, it was apparent that all cosmids injected were present, or at least vector sequences were present in hundreds of copies per genome in all three strains (data not shown).

Discussion

The *unc-22 (IV)* region described in this paper is defined as the 2 map-unit interval spanned by the deficiency *sDf2*. In this study, 79 lethal mutations were isolated by screening for mutations tightly linked to *unc-22*. Twenty-one of these mapped to the *unc-22* region and were further characterized. To date, 84 lethal and sterile mutations have been isolated which fall into 36 complementation groups. A Poisson calculation, using the formula of Meneely and Herman (1979), results in a minimum estimate of 49 essential genes in the region. There are at least 28 essential genes and three non-essential genes (*dpy-20, him-8*, and *mec-3*) to the left of *unc-22* and eight essential genes and two maternal effect essential genes (*dpy-26* and *let-99*) to the right. A set of formaldehyde-induced deficiencies was also isolated. These deficiencies facilitated the ordering of essential genes with respect to each other. The *unc-22* region has now been divided into 14 zones that are defined by the breakpoints of 17 overlapping deficiencies including *sDf2*.

The technique used to establish complementation groups was to map mutations to a deficiency zone and then to perform complementation tests *inter se* between mutations in that zone. When an essential gene had multiple alleles, a representative allele with the most severe phenotype was chosen. There is a possibility that interacting mutations could have been missed by mapping lethal mutations in this way. The two types of interaction that could occur and not have been detected are interacting gene products and intragenic complementation. The first can be demonstrated by an interaction between two loci that map to different zones. The situation with *let-651* and *let-91* is a case in point. One allele of each locus, *let-651(s1185)* and *let-91(s753)*, failed to complement each other in a *trans*-heterozygote. Because this interaction is allele-specific and the loci are separated by three deficiency breakpoints, neither *s1185* nor *s753* can be a double mutant or a deficiency. Since *let-651(s1185)/sDf64* heterozygotes are viable, it follows that *s753* is not a null allele. We suggest that the two mutant gene products interact to give the dominant synthetic lethality. Whether or not the wild-type products also interact remains to be shown.

The second type of interaction involves a single locus with complementing alleles. This can be explained as a gene with two functional domains. The *cha-1–unc-17* gene complex in *C. elegans* is an excellent example of such a phenomenon (Rand 1989). One would expect cases of allelic complementation to occur in genes that code for enzymatic proteins with multiple functional sites and structural proteins that form homomultimers, for example. In general, we tried to rule out the possibility of falsely identifying two loci when there is only one, by choosing the most severe phenotype (most likely caused by a null allele) for complementation tests when there were multiple alleles to choose from. However, using this approach, complementation between some alleles might be missed. This would become apparent during a more extended analysis of a particular essential gene. For example, in this study which focused on the *dpy-20–unc-22* interval, complementation tests *inter se* were performed between all eight alleles of *let-56* and it was found that they all failed to complement each other.

A systematic correlation of the genetic and physical maps in the *unc-22* region has been initiated by the identification of cosmids containing sequences needed to rescue mutations in two essential genes, *let-56* and *let-653*. The *dpy-20–unc-22* region was chosen to begin the correlation of the maps because it is the most completely characterized part of the *unc-22* region and a contiguous set of cosmids spanning this region is available. Molecular studies have provided information about the distribution and expression of coding regions in about 100 kb or one-half of the interval (Prasad and Baillie 1989). In addition, the coding region on fragment "q" (Prasad and Baillie 1989) has an open reading frame that has some amino acid sequence identity with the Na^+-H^+ antiport (S. Prasad, M. Marra, B. Kuchinka, D. Baillie, unpublished results). This coding region is a candidate for *let-56*. Six of eight coding regions identified and studied produce transcripts that are most abundant in the L2 larval stage (Prasad and Baillie 1989). This result is consistent with essential genes whose products are re-

quired at or before early larval stages, as judged by their lethal phases. The correlation between the developmental time of transcription and the lethal phase of mutations makes it tempting to speculate that the genes in this interval are expressed coordinately in the early larval stages. The *dpy-20* gene is also expressed most abundantly in L2 (Clark 1990).

The maternal effect gene *par-5* has been mapped to the *dpy-20–unc-22* interval genetically and molecularly. The *par-5* (*par*titioning) gene is somehow involved in the establishment or maintenance of cleavage patterns in the embryo (D. Shakes, personal communication). Mutations in this gene cause maternal effect lethality, resulting in embryos that have abnormal early cleavages and that fail to undergo morphogenesis. This gene has been tentatively placed on cosmid C38H7 by germ-line rescue (D. Shakes, personal communication). According to the cosmid map (Fig. 2; J. Sulston and A. Coulson, personal communication), C38H7 overlaps with C02C4 and C08D7. Prasad and Baillie (1989) found two transcribed regions on C02C4 and two on C08D7. One of these coding regions, "k", is expressed most abundantly in the adult hermaphrodite, which makes it a good candidate for the maternal effect *par-5* gene. Genetically, *par-5* maps in the same zone as *dpy-20*, which is zone 3. The *par-5* gene is approximately 0.05 map units to the right of *dpy-20* (Edgley and Riddle 1987). The *par-5* genetic and physical mapping data indicate that the *let-92* gene and the breakpoints of *sDf61* and *sDf7* must lie on or between the cosmids C38H7 and C29E6. Therefore, it should be straightforward to locate *let-92* by germ-line transformation experiments. Attempts were made to rescue *let-92* mutations using multiple cosmids from this region (Table 4). However, the experiments were not successful. One reason may be that the only cosmid containing a complete *let-92* gene carried a mutation. It also is possible that no single cosmid contained the whole gene. Alternatively, there may be lethality associated with multiple copies of *let-92*. For example, high copy number lethality may occur in the case of *let-60*, since Han and Sternberg (1990) were able to rescue loss-of-function mutations with the cosmid ZK205 at a concentration of 1–50 ng/μl, while we were not successful using the same cosmid at 200–400 ng/μl.

Extrapolating from the results of Prasad and Baillie (1989), there should be at least 14 coding regions in the *dpy-20–unc-22* region, if there is an average of two coding regions per cosmid. There are three identified essential genes in this interval, in addition to *par-5*. The disparity between the number of coding regions and the number of essential genes identified could be explained by the fact that some essential genes have not been identified, since the region is at most 73% saturated for mutations in essential genes. We may be underestimating the number of essential genes in the region owing to the high mutability of a subset of the essential genes relative to the others. In addition, there may be more non-essential genes than essential genes in this region. Non-essential genes could be members of a gene family or they could be single copy genes with a dispensible or redundant function. These genes would not be identified in the screens for lethal mutations. Indeed, one of the coding regions identified by Prasad and Baillie (1989) on cosmid C02C4 is possibly a member of a gene family, based upon the fact that a cDNA cross-hybridizes with about four other sequences in the genome.

To summarize, with the goal of a comprehensive study of a small region of the *C. elegans* genome, the isolation of lethal point mutations and deficiencies has resulted in identification of six essential loci to make a total of 36 now identified in the *unc-22* region. Interestingly, we have found two loci where one allele from each locus interacts with the other to produce dominant synthetic lethality, and thus there is a possibility that their wild-type products directly interact. In addition, we have demonstrated the correlation of the physical map with the genetic map using the approach of cosmid rescue of lethal phenotypes in the *unc-22* region of the *C. elegans* genome.

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