Genetic Analysis of the *cha-1–unc-17* Gene Complex in Caenorhabditis

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ABSTRACT

In *C. elegans*, the gene *cha-1* is the structural gene for choline acetyltransferase, the enzyme which synthesizes acetylcholine. *cha-1* is a complex gene which includes the previously described *unc-17* locus; it has been hypothesized that a single protein is encoded which consists of several discrete structural domains. Mutations of the *cha-1–unc-17* locus can be assigned to one of four classes on the basis of phenotype and complementation properties. A fine-structure map of this region has now been obtained by recombinational mapping. It is a large locus, spanning at least 0.035 map unit. On the map, the mutations lie in four contiguous, nonoverlapping regions, corresponding exactly to the different classes as defined by complementation and phenotype. Several new *cha-1* mutations are described and mapped in the present study, including temperature-sensitive and lethal alleles.

FOR the past 10 years, most studies of the genetic regulation of neurotransmitter metabolism have been pursued using two model organisms, the fruitfly *Drosophila* and the nematode *Caenorhabditis elegans* (HALL 1982; RUSSELL 1981; RUSSELL and RAND 1986). The transmitter acetylcholine has been particularly well analyzed in both species. Such studies have included analysis of the enzymes of acetylcholine metabolism (RAND and RUSSELL 1985a; JOHNSON and RUSSELL 1983; KOLSON and RUSSELL 1985; SLEMMON *et al.* 1982; ZINGDE and KRISHNAN 1980) as well as characterization of mutations affecting these enzymes (RAND and RUSSELL 1984; JOHNSON *et al.* 1981, 1988; CULOTTI *et al.* 1981; HALL and KANKEL 1976; GREENSPAN 1980; GREENSPAN, FINN and HALL 1980).

In a previous communication (RAND and RUSSELL 1984), we described the isolation and properties of five mutations in Caenorhabditis causing a profound deficiency of choline acetyltransferase (ChAT; EC 2.3.1.6), the enzyme which synthesizes acetylcholine. We showed that all five mutations were allelic and that they defined a new gene, which we called *cha-1* (for choline acetyltransferase deficiency). We also presented biochemical and genetic evidence which strongly suggested that *cha-1* was the structural gene for ChAT (or a subunit of ChAT). Mapping experiments located *cha-1* on linkage group IV, within 0.02 map unit of the previously identified gene *unc-17*. This was of interest, because both *unc-17* and *cha-1* mutants displayed the same spectrum of phenotypes, including resistance to cholinesterase inhibitors (see RESULTS), except that *unc-17* mutants contained normal levels of ChAT activity.

Complementation testing of *cha-1* and *unc-17* mutations revealed an unusual pattern: in general, the two types of mutations behaved as two discrete complementation groups, but there were three anomalous alleles (out of a total of 16 tested), originally termed "overlap" alleles, which appeared to be members of both complementation groups. This complex pattern led us to propose a multiple-domain model of ChAT structure and function (RAND and RUSSELL 1984, 1985b; RUSSELL and RAND 1986). Briefly, this model suggested that (1) ChAT is a multifunctional polypeptide with two discrete functional domains, one domain containing the ChAT catalytic activity and the other domain involved in a different function (perhaps localization of the enzyme), and proper function of *both* domains is necessary for normal neural and neuromuscular transmission; (2) the *cha-1* locus and the closely linked *unc-17* locus are both parts of a single complex gene, *cha-1* mutations affecting primarily the catalytic domain of the protein, while *unc-17* mutations affect the other functional domain; and (3) the enzyme normally functions *in vivo* as a homodimer, and a dimer consisting of one *cha-1*-encoded molecule and one *unc-17*-encoded molecule would allow the proper localization of enough catalytic activity for normal function, thus explaining the complementation observed between most *cha-1* alleles and most *unc-17* mutations.

One way to test this model was through fine-structure genetic analysis of the entire region, and such experiments provide the basis of the present study. Also included is a description of several newly-identified *cha-1* and *unc-17* mutations, including temperature-sensitive and lethal *cha-1* alleles. A preliminary

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account of some of these results has appeared in abstract form (RAND 1985).

MATERIALS AND METHODS

Strains used: *Caenorhabditis elegans*, var. Bristol, strain N2 was used throughout this study as the wild-type strain (BRENNER 1974). Nematodes were grown at 20° on NGM agar petri dishes with the *Escherichia coli* strain OP50 as food source (BRENNER 1974). The isolation of the following *cha-1* and *unc-17* alleles has already been described (RAND and RUSSELL 1984): *b401, p503, p1152, p1154, p1156*, and *p1160*. Strains containing the following *unc-17* alleles were obtained from JONATHAN HODGKIN: *e113, e245, e283, e284, e327, e334, e335, e359, e464, e795* and *e876*. The *cn101* allele was isolated as a heat-shock paralytic mutation by HOSONO, KUNO and MIDSUKAMI (1985). The allele *p300* was isolated by CARL JOHNSON as being resistant to 1 mM aldicarb (an inhibitor of acetylcholinesterase). Strains containing *cn101* or *p300* were outcrossed four times before being used for genetic analysis. The alleles *p1182* and *p1186* were isolated by KRISTIN PETERSON following "heat-shock" mutagenesis of the Bergerac strain of *Caenorhabditis elegans* (EMMONS *et al.* 1983); they were identified as being resistant to 0.5 mM aldicarb (K. PETERSON and R. L. RUSSELL, unpublished data). These two mutations were then extensively crossed into an N2 genetic background. The *m324* allele was isolated by ROGALSKI and RIDDLE (1988) in a screen for EMS-induced lethal mutations linked to *dpy-13*. This lethal mutation was maintained as a balanced stock using the reciprocal translocation *nT1* (FERGUSON and HORVITZ 1985). The following marker mutations on linkage group IV were used: *lin-1(e1275)* (obtained from the Caenorhabditis Genetics Center), *osm-3(p802)*, and *dpy-13(e184)* (both obtained from RICHARD RUSSELL). Genetic nomenclature follows HORVITZ *et al.* (1979).

Enzyme assays: For complementation and gene dosage studies, ChAT activity was measured in individual nematodes essentially as described previously (RAND and RUSSELL 1984), except that the final concentration of neostigmine was 20 μM, and the incubation temperature was lowered to 5°.

Genetic analysis: All crosses were performed at 20° using standard methods (HERMAN and HORVITZ 1980). Crosses involving *cn101, p1182* or *p1186* were performed at 20°, and progeny were transferred to 25° for 30–120 min before being scored.

The complementation protocol used to test *cha-1* and *unc-17* alleles has already been described (RAND and RUSSELL 1984). This procedure was used in all cases except for *m324*. Complementation between *m324* and an allele *q* was assessed by mating heterozygous *q dpy-13(e184)/+ +* males with *m324 dpy-13(e184)* IV/+ + *nT1* hermaphrodites, and scoring all the Dpy progeny. If *q* complements *m324*, all of the Dpy progeny (both males and hermaphrodites) will show wild-type coordination and growth. In the case of noncomplementation, virtually all of the Dpy animals will be uncoordinated, small, and slow growing (the phenotypes of *cha-1* and *unc-17* animals). In this case, there will be a few (less than 2%) Dpy animals with normal coordination, which arise by recombination in the male germline.

Four-factor crosses to determine the left-right order of a pair of noncomplementing alleles (*q* and *r*) were conducted according to the following scheme. Wild-type males (or, in most cases, *osm-3* males—see below) were mated to hermaphrodites homozygous for one of the alleles to be tested (the *q* allele) and the linked marker *lin-1*. Male cross-progeny (which were *lin-1 q +/+ + osm-3*—see map in Figure 1) were then mated to hermaphrodites homozygous for the other allele to be tested (the *r* allele) as well as the linked marker *dpy-13*. Approximately 200–600 Unc nonDpy hermaphrodite progeny from this cross (almost all of which were *lin-1 q +/+ r dpy-13*) were picked as virgins and allowed to lay eggs in three successive 24-hr periods; all of their subsequent progeny (15,000–150,000 animals) were then examined. In the absence of recombination, all such progeny will be uncoordinated, small, and slow growing (the phenotypes of *cha-1* and *unc-17* animals; see RAND and RUSSELL 1984). A rare intragenic recombination event between the *q* and *r* alleles, producing a chromosome completely wildtype in the *cha-1–unc-17* region, will lead to a nonUnc animal, which may readily be observed even when surrounded by several thousand Unc animals.

If the *q* allele is to the left of the *r* allele, then the wild-type recombinant chromosome will also carry the wild-type alleles of *lin-1* and *dpy-13*; animals carrying such a chromosome will be phenotypically (nonUnc), nonDpy, nonLin. If the *q* allele is to the right of the *r* allele, the recombinant chromosome will carry both the *lin-1* and *dpy-13* mutant alleles; animals carrying such a chromosome will be either Lin or Dpy, depending on which of the two parental chromosomes is also present. All nonUnc animals were cloned, and their progeny examined to confirm their genotype. This procedure is basically similar to that used by MOERMAN and BAILLIE (1979) to determine allele order within the *unc-22* gene.

Identification of artifacts: Since the scheme described above involves very large numbers of animals and extremely rare events, it is useful to have some way of making sure that putative "recombinants" are not artifactual. Such rare artifacts might arise, for example, if an injured wild-type animal were mistakenly scored as uncoordinated, or if a wild-type egg were inadvertently transferred along with an uncoordinated adult. In all such cases, the wild-type (*i.e.*, *cha-1*$^+$) allele is derived from the males used in the original mating (the other animals in the mating scheme contain either *cha-1* or *unc-17* mutant alleles). To help identify such artifacts, males homozygous for *osm-3* were used in most of the mapping experiments described above. *osm-3* is approximately one map unit from *cha-1* (RAND and RUSSELL 1984; and see Figure 1), and thus allows us to mark the *cha-1*$^+$ chromosome. [*osm-3* mutants have defects in certain sensory neurons, and these neurons fail to accumulate exogenously applied fluorescein isothiocyanate (HEDGECOCK *et al.* 1985); the mutation does not affect male mating behavior or reproduction (CULOTTI and RUSSELL, 1978).] All nonLin nonUnc nonDpy putative recombinants were therefore tested for uptake of fluorescein isothiocyanate (using the method of HEDGECOCK *et al.* 1985) to confirm that the animals were not carrying the *osm-3* mutation.

RESULTS

Properties of new mutations: A summary of *cha-1* and *unc-17* mutations, along with their phenotypes, is given in Table 1. Severe *cha-1* mutations, when homozygous, cause profound reduction in ChAT activity (≤2% residual activity for some alleles), as well as a characteristic jerky, coiling uncoordinated locomotion, resistance to cholinesterase inhibitors, slow growth, and small adult size (RAND and RUSSELL 1984). All of these phenotypes, except for the decrease in ChAT activity, are shared by most *unc-17*

TABLE 1

Properties of *cha-1* and *unc-17* mutations[a]

Class[b]	Alleles	ChAT[c]	Unc[d]	Complementation group[e]
"Pure" *cha-1*	*b401*	Low	A	*cha-1*
	cn101			
	m324			
	p503			
	p1152			
	p1154			
	p1182			
	p1186			
α	*p1156*	Low	A	Both
β	*e113*	Intermediate	B	Both
	e876			
"Pure" *unc-17*	*e245*	Normal	A	*unc-17*
	e283			
	e284			
	e327			
	e334			
	e335			
	e359			
	e464			
	e795			
	p300			
	p1160			

[a] Includes data of RAND and RUSSELL (1984).

[b] The α and β alleles were collectively referred to as "overlap" alleles in RAND and RUSSELL (1984).

[c] ChAT activity in extracts or in assays of individual animals; "low" means less than 15% of wild type, "intermediate" means 30–60% of wild type, and "normal" means 80–150% of wild type.

[d] Type of uncoordinated behavior displayed by mutant homozygotes (except for *p503*—see below): the A phenotype is characterized by small, slow growing animals with a coiling, jerky behavior; the B phenotype is characterized by normal growth and size, and a more "kinked" sort of uncoordination. There is a considerable range of severity within each phenotype class.

[e] In general, assignment is on the basis of uncoordinated behavior; however, since *p503* homozygotes have wild-type coordination, they were assigned to the *cha-1* complementation group because they failed to complement the other *cha-1* mutations for the ChAT deficiency.

homozygotes; *unc-17* animals contain normal levels of ChAT (RAND and RUSSELL 1984, 1985b). The presence (or absence) of ChAT activity and the complementation properties of each mutation allow assignment into the discrete *cha-1* or *unc-17* complementation groups, or one of the two classes of anomalous mutations, α or β (see Table 1). In addition to the previously described mutations, five new alleles of interest have recently been isolated and characterized. These mutations were tested for complementation with each other and with all the previously characterized *cha-1* and *unc-17* alleles (RAND and RUSSELL 1984), and assignment of each mutation to the *cha-1* and *unc-17* categories was made on the basis of these complementation tests as well as determinations of ChAT activity (see below).

cha-1(m324): The *m324* allele when homozygous leads to arrested development at all temperatures (ROGALSKI and RIDDLE 1988) and is therefore maintained over the *nT1* balancer chromosome (FERGUSON and HORVITZ 1985). Animals homozygous for *m324* progress through embryogenesis and hatch. The newly hatched animals are extremely small and coiled, and resemble an extreme version of young larvae homozygous for other severe *cha-1* and *unc-17* mutations. However, although *m324* homozygotes are capable of some movement, and can survive for as long as 3 weeks, they do not appear to grow or develop at all during this period.

ROGALSKI and RIDDLE (1988) showed that *m324* failed to complement the β allele *e113*, and was therefore in the *cha-1–unc-17* complex. In the present studies, *m324* failed to complement the behavioral and developmental phenotypes of *cha-1* mutations, but did complement all previously identified *unc-17* alleles. In fact, heteroallelic animals containing *m324* in *trans* to any other severe *cha-1* allele (*e.g.*, *m324/p1152*) were smaller, more uncoordinated, and slower-growing than the control homozygotes (*e.g.*, *p1152/p1152*).

cha-1(cn101): Isolated by HOSONO, KUNO and MIDSUKAMI (1985), *cn101* is temperature-sensitive; homozygotes grow and move normally at temperatures up to 20° (and also have wild-type sensitivity to cholinesterase inhibitors at 16°), but at 25° have the behavioral and developmental characteristics of severe *cha-1* mutants, including drug resistance. In addition, *cn101* displayed a complementation pattern which was temperature-dependent: at 25°, *cn101* failed to complement all *cha-1* mutations but did complement *unc-17* alleles, while at 20° or below, *cn101* complemented all the mutations tested (*i.e.*, *cha-1*, *unc-17*, α, and β). Using standard assay conditions (see MATERIALS AND METHODS), *cn101* homozygotes grown at 20° contained approximately 3–4% of the wild-type ChAT activity (Table 2). However, the measured amount of ChAT activity was dependent on the exact assay conditions; it was possible, by altering the pH and salt concentration of the assay, to demonstrate as little as 1% or as much as 12% of the wild-type activity in *cn101* animals (RAND and RUSSELL 1985b). In preliminary experiments, *cn101* homozygotes grown at 16° or 25° had comparable ChAT activity to animals grown at 20°, but since the residual enzyme was extremely labile and all assays were conducted at 5°C, it is unclear to what extent such data reflected *in vivo* enzyme levels.

cha-1(p1182) and cha-1(p1186): These are both temperature-sensitive lethal mutations—animals homozygous for either of these mutations behave like mild *cha-1* mutants at 16° or 20°, but within 15 min after transfer to 25°, such animals become virtually paralyzed. After 2–3 days at 25°, they are still unable

TABLE 2

ChAT activity in mutants

Allele	ChAT in homozygotes (% of wild type)	ChAT in heterozygotes (% of wild type)
+	100 ± 5.7	100 ± 14
cn101	3.6 ± 0.8	52.9 ± 8.3
p1182	11.1 ± 1.5	47.2 ± 15.3
p1186	7.8 ± 1.8	48.9 ± 8.8
m324	ND	47.2 ± 14.9
p300	109 ± 10.7	ND

All animals were grown at 20°. Homozygote data: animals were 84 ± 4 hr from hatching (except *p300* homozygotes, which were 104 ± 4 hr from hatch—a comparable developmental stage); assays were conducted at 5° for 22.5 hr; results are given as the mean ± SD of six independent measurements (11 measurements for control) with 4 nematodes for each activity measurement. Heterozygote data: all of the *cha-1* heterozygotes, as well as the wild-type control, were also heterozygous for the morphological marker *dpy-13(e184)*; animals were 90 ± 2 hr from hatch; assays were conducted at 5° for 24 hr; results are given as the mean ± SD for 10 independent measurements of one animal each. ND, not determined.

to move, they have not grown at all, and they begin to die. The temperature sensitivity of these strains is present at all developmental stages. The alleles *p1182* and *p1186* failed to complement each other as well as all other *cha-1* mutations for uncoordinated behavior, small size, and slow growth, but they did complement all *unc-17* mutations for these phenotypes. In addition, the temperature-sensitive lethality associated with *p1182* and *p1186* failed to complement the lethality of *m324*: animals which were *p1182/m324* or *p1186/m324* were uncoordinated at 20° and did not grow at 25°. Both alleles, when homozygous, led to significant reductions in ChAT activity (Table 2).

unc-17(p300): This mutation is phenotypically similar to other severe alleles, and leads to extreme resistance to cholinesterase inhibitors. In complementation tests, *p300* displayed the properties of an *unc-17* mutation. Animals homozygous for *p300* had approximately wild-type ChAT activity (Table 2), a result similar to those obtained with previously characterized *unc-17* alleles.

In agreement with previous results (RAND and RUSSELL 1984), the anomalous alleles *p1156, e113,* and *e876* failed to complement all of the new alleles.

ChAT levels and gene dosage: ChAT activity for each of these new mutants is given in Table 2. It was impossible to measure ChAT activity directly in adult *m324* homozygotes, but measurements from heterozygous and heteroallelic animals (see below) indicate that *m324* leads to near-total ChAT deficiency. The four new mutations with *cha-1*-like complementation properties all caused large decreases in ChAT activity. In addition, ChAT assays demonstrated that the alleles *cn101, m324, p1182,* and *p1186* all failed to complement each other as well as all previously identified *cha-1* alleles for the ChAT deficiency (data not shown). Gene dosage experiments for all four of these new *cha-1* alleles showed that heterozygotes had half the ChAT activity of wild-type controls (Table 2). These data are comparable to results obtained with the previously described *cha-1* alleles (RAND and RUSSELL 1984).

Mapping of alleles: Because of the unusual complementation pattern shown by *cha-1* and *unc-17* alleles, mapping experiments were conducted to locate representative alleles from each complementation category. We had previously determined that the *cha-1* allele *p1152* was located approximately 0.01–0.02 map unit to the left of the *unc-17* allele *e245* (RAND and RUSSELL 1984).

The specific protocol used to map the mutations is described in MATERIALS AND METHODS. The basic strategy was to construct 200–600 heteroallelic animals, carrying two noncomplementing *cha-1* and/or *unc-17* alleles, with the left-flanking marker *lin-1* on one chromosome and the right-flanking marker *dpy-13* on the other chromosome. Because noncomplementing alleles were used, these animals were uncoordinated. Approximately 15,000–150,000 progeny from such animals were screened for wild-type (*i.e.*, coordinated) recombinant progeny. Depending on the left-right order of the two alleles being tested, the recombinant chromosome would carry both or neither of the flanking markers. The genotypes of all putative recombinants were then determined by evaluation of their progeny.

The results of 23 mapping crosses are presented in Table 3. Most crosses yielded unambiguous results, *i.e.*, recombinants were identified, and a left-right order could be assigned to the two mutations being tested. The number of recombinants given in Table 3 is based on confirmed recombination events involving exchange of *both* flanking markers, and excludes questionable events which might be due to conversion or double events (see below). The calculated recombination frequency is meant to be approximate only, especially in those cases where four or fewer recombinants were identified. Since several of the experiments yielded only one or two recombinants, it is not particularly surprising that no recombinants were observed in 4 of the 23 experiments; this was probably due to close proximity of the mutations being mapped and/or sampling effects, and should not be construed to indicate anything unusual about the alleles involved.

The genetic map derived from these crosses is shown in Figure 1. It is clear that mutations with common complementation properties (*i.e., cha-1* or *unc-17*) were clustered on the fine-structure map (see DISCUSSION). As soon as this pattern became apparent, it permitted a more efficient strategy for subsequent mapping experiments. In particular, new alleles to be

TABLE

Summary of mapping crosses

Allele pair	Total progeny (estimated)	No. of recombinants	Double events or convertants	Approximate recombinant frequency (%)	Allele on left
p1152/cn101	35,400	1	0	0.003	*cn101*
p1156/cn101	15,200	2	0	0.013	*cn101*
p1152/b401	48,000	2	1	0.004	*p1152*
p1152/m324	23,500	2	0	0.009	*p1152*
p1156/p1152	63,000	5	1	0.008	*p1152*
b401/m324	36,600	1	0	0.003	*b401*
p1156/b401	77,700	9	1	0.012	*b401*
p1156/m324	17,200	3	0	0.017	*m324*
p1156/p1182	55,400	3	3	0.007	*p1182*
p1156/p1186	44,100	5	0	0.011	*p1186*
p1156/p1154	156,000	≥2	2	≥0.001	*p1154*
e359/p1156	40,000	0	0	?	?
e245/p1156	110,000	1	0	0.001	*p1156*
e876/p1156	62,300	5	0	0.008	*p1156*
e113/p1156	82,000	18	0	0.022	*p1156*
e359/e245	115,200	0	0	?	?
p300/e245	36,600	0	0	?	?
p1160/e245	60,200	7	1	0.012	*e245*
e795/e245	73,400	8	1	0.011	*e245*
e113/e245	56,000	19	1	0.034	*e245*
p1160/e795	46,600	1	0	0.002	*p1160*
e795/e876	86,900	16	1	0.018	*e795*
e876/e113	73,000	0	0	?	?

The allele listed on the left of each allele pair was in *cis* to *lin-1*; the allele listed on the right of each pair was in *cis* to *dpy-13*. The total number of progeny in each experiment was estimated by counting representative subsets of progeny. For technical reasons, it was difficult to identify the recombinants in the cross involving *p1154* and *p1156*. Thus, the two observed recombinants in that experiment represent a lower limit. All of the "double event or convertant" chromosomes carried the *dpy-13* marker, except for the one arising from *e795/e876*, which carried *lin-1*.

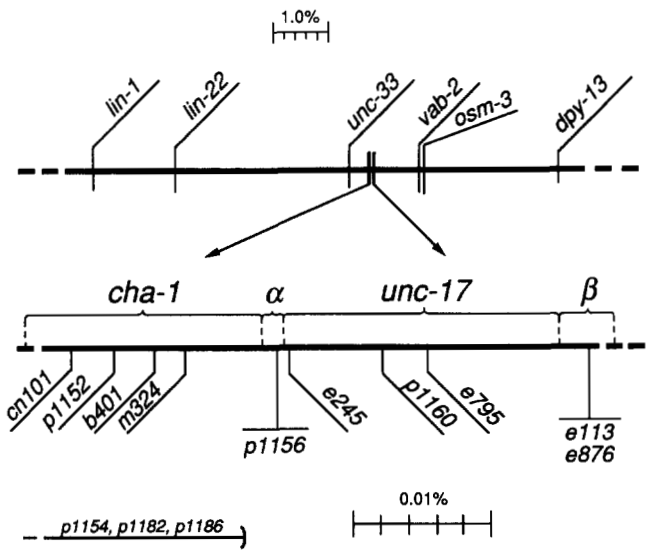

FIGURE 1.—Genetic map of the *cha-1–unc-17* region and surrounding loci. Top, Map of the genes near *cha-1* (modified slightly from RAND and RUSSELL 1984). Bottom, Fine-structure map of the *cha-1–unc-17* complex. Those alleles which are shown could be ordered unambiguously, with the distance between them corresponding to the approximate recombination frequency derived from the data in Table 3. The three alleles (*p1154, p1182,* and p1186) which are to left of *p1156*, but which were not otherwise ordered, are indicated below the map. *e113* and *e876*, which could not be ordered with respect to each other, but which were ordered with respect to other mutations, are shown at the same map position. Two additional mutations, for which sufficient data do not yet exist to assign any map position, are not shown: *p300* could not be ordered with respect to *e245*, and *e359* could not be ordered with respect to either *p1156* or *e245* (data in Table 3).

mapped were first positioned with respect to *p1156*. This not only helped to confirm the emerging pattern (*cha-1* alleles to the left of *p1156*, and *unc-17* alleles to the right of *p1156*), but gave a rough estimate of the distance of the new allele from *p1156*, which helped in the choice of alleles for the next experiment. The left-right ordering of the different mutations was completely self-consistent; however, the additivity of the derived intervals was (predictably) not perfect. In several cases, the spacing between mutations in the lower part of Figure 1 represents a compromise among data from several experiments. It therefore appears that the leftmost and rightmost alleles are 0.03–0.04 map unit apart.

Double events and/or genetic conversion: In several of the mapping experiments, nonUnc animals were recovered carrying only one of the flanking markers (*dpy-13* or *lin-1*), rather than both or none (the expected results for reciprocal exchanges). These data are presented in Table 3. The genotypes of these animals suggested that they could have resulted from gene conversion. Apparent convertants have been observed in *C. elegans* at the *unc-13, unc-15, unc-22, unc-54* and *unc-60* loci (MOERMAN and BAILLIE 1979; ROSE and BAILLIE 1980; WATERSTON, SMITH and MOERMAN 1982; MCKIM *et al.* 1988). However, in the present study, similar results would have been obtained by a pair of reciprocal exchanges, one between the two *cha-1* or *unc-17* alleles, and another between the *cha-1–unc-17* region and one of the flanking markers. The methods used in these experiments cannot distinguish between conversion events and double crossovers, and in the absence of information on interference in *C. elegans*, it is not possible to decide which of these explanations is the more likely.

DISCUSSION

Lethal and null alleles: The results presented above include the first description of lethal *cha-1* alleles in *C. elegans*: the unconditional lethal mutation *m324* and also two temperature-sensitive lethal alleles, *p1182* and *p1186*. The fine-structure mapping experiments demonstrated that the lethality associated with *m324* homozygotes maps within the *cha-1* region. Recombination events to the left and to the right of *m324*, yet within *cha-1* (which allowed the ordering of the alleles), produced chromosomes which were wild-

type at *cha-1* and which contained no recessive lethality; there is thus no other recessive lethal mutation linked to *m324*. Similarly, the temperature-sensitive lethality associated with animals homozygous for either *p1182* or *p1186* appears to map within the *cha-1* region.

Phenotypically, *m324* appears to be either an extreme hypomorph or a null allele: heteroallelic animals containing *m324* in *trans* to another *cha-1* allele have a more severe phenotype than homozygotes of that allele. ChAT assays *in vitro* of *m324* heterozygotes and heteroallelic animals containing *m324* suggest that this allele contributes little or no ChAT activity to such animals, although it is possible that, *in vivo*, the *m324*-encoded protein possesses some enzyme activity. However, since *m324* homozygotes are capable of some limited movement (which probably reflects some minimal level of cholinergic function), it seems likely that *m324* is probably not a null allele, but rather an extreme hypomorph.

The genetic map: The most striking feature about Figure 1 is the correspondence between the complementation properties of the different mutations and their relative map positions. Thus, all seven of the *cha-1* alleles tested lie in one region, to the left of the α allele *p1156*; the three *unc-17* alleles for which unambiguous mapping data exist lie in a contiguous group to the right of *p1156*; and the two β alleles *e113* and *e876* lie to the right of the *unc-17* region. Of particular interest was the result that the three anomalous alleles *p1156*, *e113*, and *e876*, which were subdivided into two classes on the bases of behavioral phenotype and ChAT activity (Table 1), were also divided into the same classes by the mapping data.

These studies were undertaken, in large part, to resolve uncertainties resulting from the anomalous complementation behavior of *cha-1* and *unc-17* mutations (RAND and RUSSELL 1984). In particular, it was hoped to distinguish between one complex gene (predicted by our model) and two closely linked, genetically interacting, and perhaps functionally related genes. If the mapping had shown that *cha-1* and *unc-17* mutations were interspersed, it would have provided convincing evidence for a one-gene interpretation, but the clustering which was actually observed (all the *cha-1* mutations in one cluster and all the *unc-17* mutations in another) was consistent both with one-gene and two-gene models.

However, the mapping of the anomalous alleles (which fail to complement both *cha-1* and *unc-17* mutations) helps us to choose between the two alternatives. If *cha-1* and *unc-17* were two distinct genes, then the three anomalous alleles most probably would represent small deletions or other events *between* the two loci, extending into and/or disrupting both of them. However, the results presented above, which show that *e113* and *e876* do not map between *cha-1* and *unc-17* but rather to the right of both, argue strongly against such a two-gene interpretation. More generally, the result that the eight leftmost and the two rightmost mutations on the map all fail to complement each other, suggests that all of these mutations, as well as those in between, are part of a single complex gene.

Alternatively, might the β mutations represent alterations in a 5′ regulatory element? Such a possibility could apply to either a one-gene or (less likely) a two-gene model. However, even though β mutants have approximately half of the wild-type ChAT activity and are uncoordinated, the behavioral phenotype cannot be due to the decrease in enzyme activity alone. This is because *cha-1* heterozygotes (*e.g.*, *p1152/+*) have half of the wild-type ChAT activity and are normally coordinated. Therefore, the behavioral defects associated with β mutants cannot be due solely to lowered levels of ChAT expression, and if these mutants are indeed regulatory, they must affect some type of regulation other than ChAT level.

The *cha-1* complex is large: It is noteworthy that the *cha-1* complex locus seems to be rather large as measured by recombination—approximately 0.035 map unit (or perhaps more) separate the leftmost and the rightmost mutations. This is more than the map distance across the other *C. elegans* genes for which data exist, even those encoding proteins considerably larger than the 71-kDa monomer of ChAT (RAND and RUSSELL 1985a). Thus, *unc-22*, which encodes a protein of grater than 500,000 kDa (MOERMAN *et al.* 1988) has a recombination-derived map size of approximately 0.024 map unit (MOERMAN and BAILLIE 1979); *unc-54*, which encodes a 228,000-kDa myosin heavy chain (KARN, BRENNER and BARNETT, 1983), has a map size of approximately 0.02 map unit, and published data for other genes include 0.012 map unit for *unc-60* (MCKIM *et al.* 1988), 0.0045 map unit for *unc-15* (ROSE and BAILLIE 1980), and 0.0024 map unit for *unc-13* (ROSE and BAILLIE 1980). Possible explanations for the large apparent size of *cha-1* are: (1) the gene contains many and/or large introns; (2) the gene includes one or several "hot spots" for recombination; and (3) the ChAT protein is synthesized as a very large precursor, which is cleaved into the 71-kDa active form. Cloning and molecular analysis of *cha-1* will undoubtedly help to resolve such questions.

Predictions of the multiple-domain model: As described in the introduction, our multiple domain model postulates that *cha-1* and *unc-17* represent parts of a single gene encoding different domains of a single polypeptide (RAND and RUSSELL 1984, 1985b; RUSSELL and RAND 1986), and the fine-structure data presented above support this model. In addition, this model makes three testable predictions.

Genetic analysis: Presumably, any true null mutation (*i.e.*, leading to total absence of gene product and concomitant loss of all function) would be lethal. This assumption is based on the lethality of *m324* homozygotes and the apparent hypomorphic nature of this allele (see above), and also by analogy with Drosophila, where null alleles of the *Cha* locus are recessive lethals (GREENSPAN 1980). Based on mutational analysis in many systems, it is likely that there are many distinct sites within a gene that can be altered by mutation to give a null phenotype. Since, according to our model, the complementation properties of the extant *cha-1* and *unc-17* mutations depend on their ability to dimerize, a true null mutation should fail to complement *all* the other alleles, *i.e., cha-1, unc-17,* α, and β. This predicts the existence of a fifth class of allele: lethal mutations with the complementation properties of "overlap" mutations but which lie in the *cha-1* and/or *unc-17* regions of the map. Presumably, members of this class would be candidates for null alleles.

It is possible that many lethal alleles will be identified with complementation and mapping properties which will place them into one of the four existing categories. In fact, *m324* is a case in point. The model does not claim that *all* lethal mutations will be in the new class, but merely that such a class does exist. The generation and mapping of a number of lethal alleles will test this prediction.

Molecular analysis: If the multiple-domain model is correct, the entire *cha-1–unc-17* region should comprise a single transcriptional unit. The test of this prediction will require the cloning and transcriptional analysis of the region, and such experiments are now underway.

Biochemical analysis: We have already shown that *cha-1* is the structural gene for ChAT (RAND and RUSSELL 1984). If (as the model states) *cha-1* and *unc-17* encode different domains of the same protein molecule, then presumably, some *unc-17* mutations should lead to qualitative alterations in the ChAT protein. This prediction is now being tested by biochemical analysis of ChAT purified from *unc-17* mutants.

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