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A GENETIC ANALYSIS OF THE _UNC-22_ REGION IN _CAENORHABDITIS ELEGANS_.

by

Donald Gordon Moerman

B.Sc. (Hons.), Simon Fraser University, 1973

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

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November, 1979

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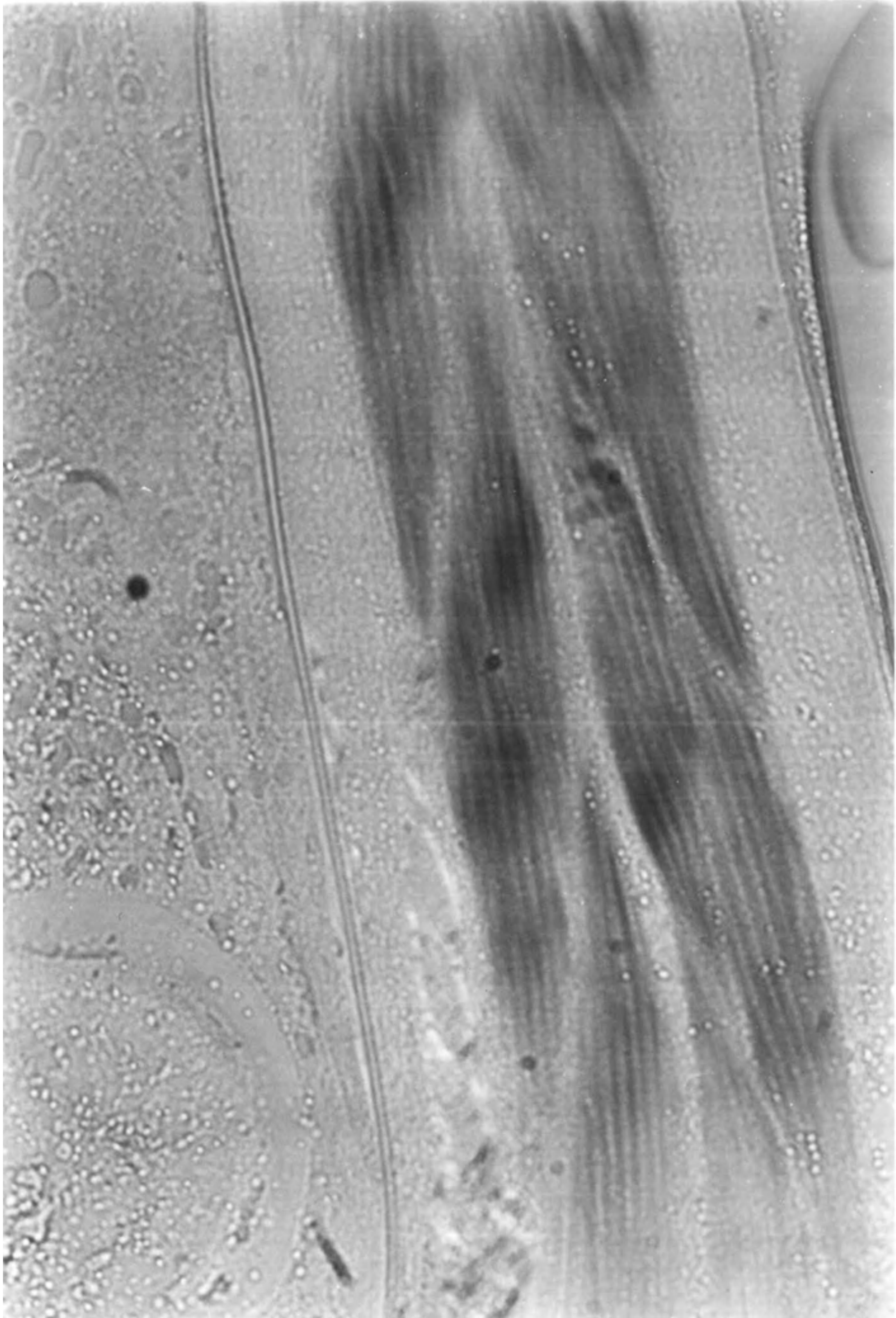

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Abstract

The purpose of this thesis was to investigate the genetic organization of the unc-22 region on chromosome IV in the free-living nematode *Caenorhabditis elegans*. Further, it was hoped that some understanding of the role of the unc-22 gene product in the ontogeny of the nematode might emerge from this study.

A genetic fine-structure analysis of the unc-22 gene showed several sites to be separable by recombination. Fifteen alleles were mapped within the locus, one of which was the dominant allele m52. Map distances between alleles were comparable to those associated with intragenic recombination in *Drosophila melanogaster*, indicating that genetic fine-structure analysis is feasible in *C. elegans*. Evidence of possible gene conversion was also found. A preliminary estimate of the unc-22 gene size is 1-2 x 10^{-2} map units.

A number of unc-22 alleles were tested for suppression by the informational suppressor sup-5(e1464). Of eleven mapped alleles and fifteen unmapped alleles, only one, s32 was suppressible.

To examine the region around the unc-22 locus, lethal mutations and deficiencies were isolated. Lethals were induced using the mutagen, ethyl methane-sulfonate (EMS), while deficiencies were induced with 0.1% formaldehyde. The effective lethal phase of the lethal mutations ranged from mid-embryonic to adult and three of these mutations were temperature sensitive. Two of the lethal mutations appeared to identify the adjacent genes to either side of the unc-22 site (let-56 to the left of unc-22 and let-52 to the right). One of the lethal mutations, s65, which mapped within 0.5 map units of unc-22, was epistatic to the unc-22 locus. While isolating the lethal mutations, four new EMS induced visible mutants linked to chromosome IV were isolated. Two of these 'displaced vulva' and 'spotty', represented new phenotypes.

Reversion studies of unc-22(s12) revealed six F1 'revertants' in 8 x 10^5 tested chromosomes. All six mapped to linkage group I and were allelic to unc-54, a gene coding for myosin. Morphological studies suggested these new alleles were unlike any previously identified alleles of this gene. One of these mutations, unc-54(s74), was tested for its ability to suppress

muscle twitching induced by various unc-22 alleles. Although suppressing all unc-22 mutations, s74 exhibited an allele specific pattern of suppression. That is, depending on the unc-22 allele, the pattern of suppression was dominant, semi-dominant, or recessive. These different patterns of suppression allowed the unc-22 mutations to be ranked according to their degree of severity. No correlation was found between the map position on a fine-structure map of an unc-22 allele and its pattern of suppression.

These genetic studies, as well as evidence from morphological observations and pharmacological tests, suggest that the unc-22 gene product is localized to muscle cells, and that it is intimately involved in the process of contraction.

To Jan

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It is a pleasure to acknowledge the help given to me by my senior supervisor, Dr. D. L. Baillie, and by my supervisory committee; Dr. M. J. Smith, Dr. D. G. Holm (U.B.C.), Dr. J. M. Webster and J. S. Barlow. I would also like to thank the following people: Dr. D. Popham for doing the electron microscopy on the twitcher mutations and for generously providing figure 13 of the text; Dr. C. Kreis for helpful discussions concerning muscle biochemistry and the possible nature of the unc-22 disorder; Dr. J. E. Rahe for an introduction to Nomarski optics; Ms. A. Rose, Mr. K. Reikki and Ms. T. Rogalski for help in isolating many of the lethal mutations; Ms. R. Rosenbluth for technical assistance and helpful discussions; Ms. S. Liner and Ms. C. Salamanca for technical assistance; and Mr. R. Long for reproducing the pictures contained in the text. Finally, I would like to thank all the worms who took part in this study.

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Introduction.

The process of cellular differentiation is one of the most intriguing problems in contemporary biology. The importance of understanding the process of differentiation has been recognized for some time, as is evident from the following statement by Auguste Weisman, "If we could see the determinants, and recognize directly their arrangement in the germ-plasm and their importance in ontogeny, we could doubtless understand many of the phenomena of ontogeny and their relationship to phylogeny which must otherwise remain a riddle." (Weisman, 1904; cited in Gould, 1977).

Weisman recognized that the problems of development and heredity are intimately connected. Several of the early geneticists recognized this relationship and tried to explain developmental processes based on what was then known about the gene (Weisman's determinants). L. C. Dunn (1917, cited in Dunn, 1965) was, perhaps, the first to suggest that chromosomal genes control steps in development, and later Richard Goldschmidt hypothesized the 'rate gene' to explain how a gene could control development (1918; 1938; cited in Hadorn, 1961). His idea was that, "The

mutant gene produces its effect, the difference from the wild-type, by changing the rate of partial processes of development. These might be rates of growth or differentiation, rates of production of stuffs necessary for differentiation, rates of reactions leading to definite physical or chemical situations at definite times of development, rates of those processes which are responsible for segregating the embryonic potencies at definite times." (Goldschmidt, 1938; cited in Gould, 1977.) The most penetrating suggestion concerning genes and developmental processes, was offered by T. H. Morgan (1934) who wrote: "The visible differentiation of the embryonic cells takes place in the protoplasm. The most common genetic assumption is that the genes remain the same throughout this time. It is, however, conceivable that the genes also are building up more and more, or are changing in some way, as development proceeds in response to that part of the protoplasm in which they come to lie, and that these changes have a reciprocal influence on the protoplasm."

Morgan realized that genes must somehow change during development. Today this concept is known as the variable gene activity theory of differentiation

(Markert and Ursprung, 1971; Davidson, 1976). This theory states that cell differentiation is based on the regulation of gene activity i.e. for each state of differentiation a certain set of genes is active in transcription and other genes are inactive. Over the years support for this view has come from many lines of research (reviewed in Davidson, 1976). Crucial support comes from the work of Mirsky and Ris (1949) who demonstrate that the DNA content among differentiated diploid cells of an organism is constant. This effectively rules out chromosome diminution as a common means of differentiation. The nuclear transplantation experiments of Gurdon and his co-workers (1974, for a review) makes this same point by demonstrating that differentiation is not an irreversible event, but that nuclei from differentiated cells when injected into a mature egg can direct the entire developmental program.

Variability in transcription has been demonstrated by RNA-DNA hybridization studies and by <u>in vitro</u> translation of specific mRNA's from differentiated cells. Evidence from RNA-DNA hybridization studies indicates much of the DNA in differentiated cells is not transcribed. For example, mouse liver (nuclear) RNA hybridizes with only 2-5% of mouse single copy DNA

(Brown and Church, 1972; Grouse et al., 1972). Comparisons of RNA populations among differentiated cell types shows that the RNA is qualitatively different, although there are some overlaps. Both repetitive and nonrepetitive sequence DNA transcripts demonstrate this difference (McCarthy and Hoyer, 1964; Davidson et al., 1968; Brown and Church, 1972; Grouse et al., 1972). Specific mRNA's, for example hemoglobin and ovalbumin mRNA, are tissue specific, (Axel et al., 1973; Palmiter, 1973; Rhoades et al., 1973; Hunt, 1974; Groudine and Weintraub, 1975) and also, the production of these mRNA's is an inducible phenomena (Palmiter, 1973; Gilmour et al., 1974).

All these observations point to differential gene transcription as the method of cell differentiation. These observations do not elucidate any of the molecular mechanisms by which gene expression is controlled. Several models for transcriptional control in eucaryotes have been proposed (Britten and Davidson, 1969; Georgiev, 1969; Crick, 1971). These models are, for the most part, modifications of the operon theory originally proposed by Jacob and Monod (1961) to explain control of gene transcription in procaryotes. Evidence in support of any particular model of gene

regulation in eucaryotes is non-existent. At present we do not understand the method of transcriptional regulation of a single gene in eucaryotes. The objective of this thesis has been to develop a system which would allow for questions pertaining to genomic organization, transcriptional regulation and cell differentiation to be explored. The assumption inherent in this approach has been that it should be possible to dissect out the genetic specifications for development in the same way that the biosynthetic pathways in bacteria and the assembly pattern in bacteriophage have been analyzed.

The organism used in this study was the free-living nematode Caenorhabditis elegans (Maupas). The nematode has a long history as a useful organism for the study of development. As early as 1871 Otto Butschli was using free-living nematodes to study the mechanism of fertilization (cited in Goldschmidt, 1956). He chose the nematode because it was transparent. Theodor Boveri also chose to work with a nematode for his studies on cell-division and nuclear morphology (1887-1907). For his work he used an ascarid parasite of horses (for details, see Jacob, 1973). Karl Belar, between 1910 and 1930, apparently was the first

to recognize the potential usefulness of the nematode for genetic studies (recorded in Goldschmidt, 1956). Belar used free-living nematodes in his studies on mitosis and the genetics of nutrition, and according to Goldschmidt he cultured them on agar plates. Not until 1950 (Nigon and Dougherty, 1950) was the first nematode mutant isolated, and this arose spontaneously. At this time Nigon and Dougherty pointed out that the free-living nematode had several traits that made it useful for genetic studies. However, it was not until the publication by Brenner (1974) of his work on the genetics of C. elegans, in which he identified over 100 genes affecting morphology and behaviour, that the worm became established as an organism for the study of genetic organization and development.

There are several reasons for using the nematode in a developmental genetic study. The adult worm has only about 800 somatic cells (Sulston and Horvitz, 1977). This can be compared to Drosophila which has about 3,200 to 3,500 cells at the blastoderm stage alone (Sonnenbick, 1950). Although having few cells, the anatomy of the worm is sufficiently complex to have the major differentiated tissue types of nerve, muscle, hypodermis, intestine and gonad. Perhaps the most

important point is that the cell lineage is almost entirely described (Deppe et al., 1978; Sulston and Horvitz, 1977). This has been possible because of the worms anatomical simplicity, its transparency, and because of the use of Nomarski interference microscopy, all of which make it relatively easy to monitor cell division and follow the movement of individual cells. The nematode is easily cultured in large numbers. Its short generation time (3 1/2 days at 20 degrees centigrade), large brood size (approximately 250 progeny) and the ease with which it can be mutated make it ideal for genetic studies (Brenner, 1974). Since it is a self-fertilizing hermaphrodite, mutant stocks are easily maintained and males are readily inducible, or can be maintained as stocks. Chromosome rearrangements, including translocations, inversions and deficiencies, are available in the worm (Herman et al., 1976; Herman, 1978; Meneely and Herman, 1979; Moerman and Baillie, in press). The DNA content of the worm is known (haploid DNA content equals 8 x 10^7 base pairs) and some data are available on its molecular organization (Sulston and Brenner, 1974; Schachat et al., 1978; Emmons et al., 1979). All together this makes the nematode an important organism for studying the fundamental laws governing development.

My approach to the problem of genomic organization and regulation owes much to the previous work done on _Drosophila melanogaster_ by Hochman (1971), Judd et al. (1972) and Chovnick et al. (1977). Indeed, their techniques for analyzing lethal mutations and for genetic fine-structure mapping have been essential in this research. The central locus investigated in this study was the _unc-22_ gene on linkage group IV (Brenner, 1974; Moerman and Baillie, 1979). This gene is believed to have a role in muscle structure and function. Worms homozygous for a mutation in this gene display a characteristic twitching pattern along the body-wall musculature, and morphological studies of these worms reveal that their body-wall muscle filament structure is disorganized.

The eventual goal is to understand how the _unc-22_ gene is regulated during development; but first it is necessary to understand the genetic organization of this locus and its relationship to other elements within the genome. This study has accomplished a significant portion of this latter task through the constructionn of an extensive fine-structure map of the _unc-22_ gene, the identification of several essential

loci linked to it, and the demonstration of gene interaction involving unc-22 and various genes affecting muscle structure and function.

Materials and Methods.

(i) Nematode strains, media and culture conditions: NG agar as described by Brenner (1974) was used throughout this study. *E. coli* (OP-50; a uracil auxotroph) in nutrient broth (Difco) at 2-3 x 10^8 cells/ml were used for streaking plates. 100 mm Petri plates were used for the maintenance of the strains (at 16 degrees centigrade) and for the isolation of mutations. *C. elegans* has two sexes: self-fertilizing hermaphrodites and males. The wild-type hermaphrodite stock, N-2-S, was derived from a single N-2 from the Cambridge stock collection in 1973. N-2-S male stocks were maintained by crossing males (5AA,XO) to N-2-S hermaphrodites (5AA,XX) each generation. The mutant strains employed in this study are listed in Table 1. Basic methods of nematode genetics are described by Brenner (1974).

(ii) Induction and isolation of unc-22 mutations: The mutagen ethyl-methane sulfonate (EMS) was used. N-2-S worms were washed from stock plates with M-9 buffer (Brenner, 1974) and collected after centrifugation (200 x g). These worms were then suspended in either 0.05M EMS (20 lambda EMS into 4 ml

Table 1.

Mutations used. *

Linkage group	gene	allele
X	unc-3	** e151
I	dpy-5	e61
	lev-11	88B
	unc-15	e73, e1214
	unc-35	e259
	unc-54	e190, s74, s75, s76, **** s77, s78, s95
	unc-59	e261
II	dpy-10	e128
III	dpy-18	e364
	sup-5	e1464
IV	dpy-4	e1166
	dpy-9	e424
	unc-5	e152
	unc-22	e66, s7, s8, s11, s12, s13, s14, s15, s16, s17, s18, s19, s20, s21, s22, s23, s32, s34, s35, s36, s55, m52 ***
	unc-26	e345, e205
	unc-30	e318
	unc-31	e169
	unc-43	e266
	tra-3	e1107
V	dpy-11	e224
	sup-3	e1405, e1407

*This table does not include the lethal mutations. These are listed in Tables, 11 and 12.

** e; isolated at Cambridge, England.

*** m; isolated at Columbus, Missouri, U.S.A.

**** s; isolated at Simon Fraser, Canada.

of M-9 buffer), 0.025 M EMS, or 0.0125 M EMS. After 4 hrs. at 20 degrees C. they were removed from the EMS solution with a Pasteur pipette and spotted onto a Petri plate. After approximately one hour, fourth stage larvae and young adult worms were placed (20/plate) on Petri plates with lawns of OP-50. These worms were left to lay eggs for 24 hrs. and were then removed. Three days later the F1 progeny were screened for putative unc-22 mutants.

To screen the F1 progeny of the mutagenized worms for putative mutations in the unc-22 gene I took advantage of the different behaviours exhibited by wild-type and twitcher worms in a 1% solution of nicotine alkaloid (Sigma Co.). Worms, when either homozygous or heterozygous for an allele of the unc-22 gene, twitch in a 1% solution of nicotine, whereas wild-type worms in this solution become rigid. Using nicotine, I could screen for twitchers in the F1 generation. To insure that all new mutations were independently induced alleles, only one mutant was taken per plate. Prior to any further examination these new mutants were outcrossed to wild-type males and allowed to resegregate from the F1 progeny. A stock of the mutant self-fertilizing hermaphrodite was then

established.

(iii) Construction of triple mutants: Triple mutants of the following genotype, unc-5(e152),unc-22(sx),dpy-4(e1166), were needed to position the twitcher alleles relative to one another. These triple mutants were constructed using the following protocol. Homozygous twitcher hermaphrodites were outcrossed to N-2-S males. The F1 male progeny were then mated to hermphrodites that were homozygous for dpy-4(e1166). From this cross young hermaphrodite progeny that twitched in a solution of 1% nicotine were selected and allowed to lay eggs. When the latter had grown they were screened for dumpy worms that twitched in the 1% nicotine solution. Once found, the dumpy mutants were placed on a plate and allowed to lay eggs, 1/4 of which were expected to be homozygous dumpy and twitcher. The double dumpy twitchers were then outcrossed to N-2-S males. The F1 male progeny this time were crossed to hermaphrodites homozygous for unc-5(e152). A 1% nicotine solution was added to the progeny and again the young hermaphrodites that twitched were selected and allowed to lay eggs. Progeny from these eggs were allowed to mature and unc-5 hermaphrodites that twitched in nicotine were

selected and placed on fresh plates. Approximately 1/4 of these unc-5 hermaphrodites progeny were triple mutants. Individuals of the genotype, unc-5(e152), unc-22(sx),dpy-4(e1166), were selected and established as a stock.

(iv) Mapping and complementation of unc-22 mutations: To determine if a mutation was on linkage group IV, males of the putative genotype unc-22(sx) IV,+/ +,+ were crossed to hermaphrodites that were +,dpy-4(e1166)IV/ +,dpy-4(e1166)IV. Young F1 hermaphrodites that twitched in nicotine were picked. This insured that these hermaphrodites would be heterozygous for both the twitcher and the dumpy mutations. In a self-fertilizing hermaphrodite if the twitcher and the dpy-4 gene were not linked one would expect 1/16 of the F2 progeny to be both dumpy and twitching. The unc-22 gene, however, is about 5 map units from dpy-4 (see Figure 1). This means mutations defective in unc-22 should produce double mutant segregants with a frequency of only 1/1600 in the F2 generation.

Genetic complementation tests were done using the method described by Brenner(1974). Heterozygous males

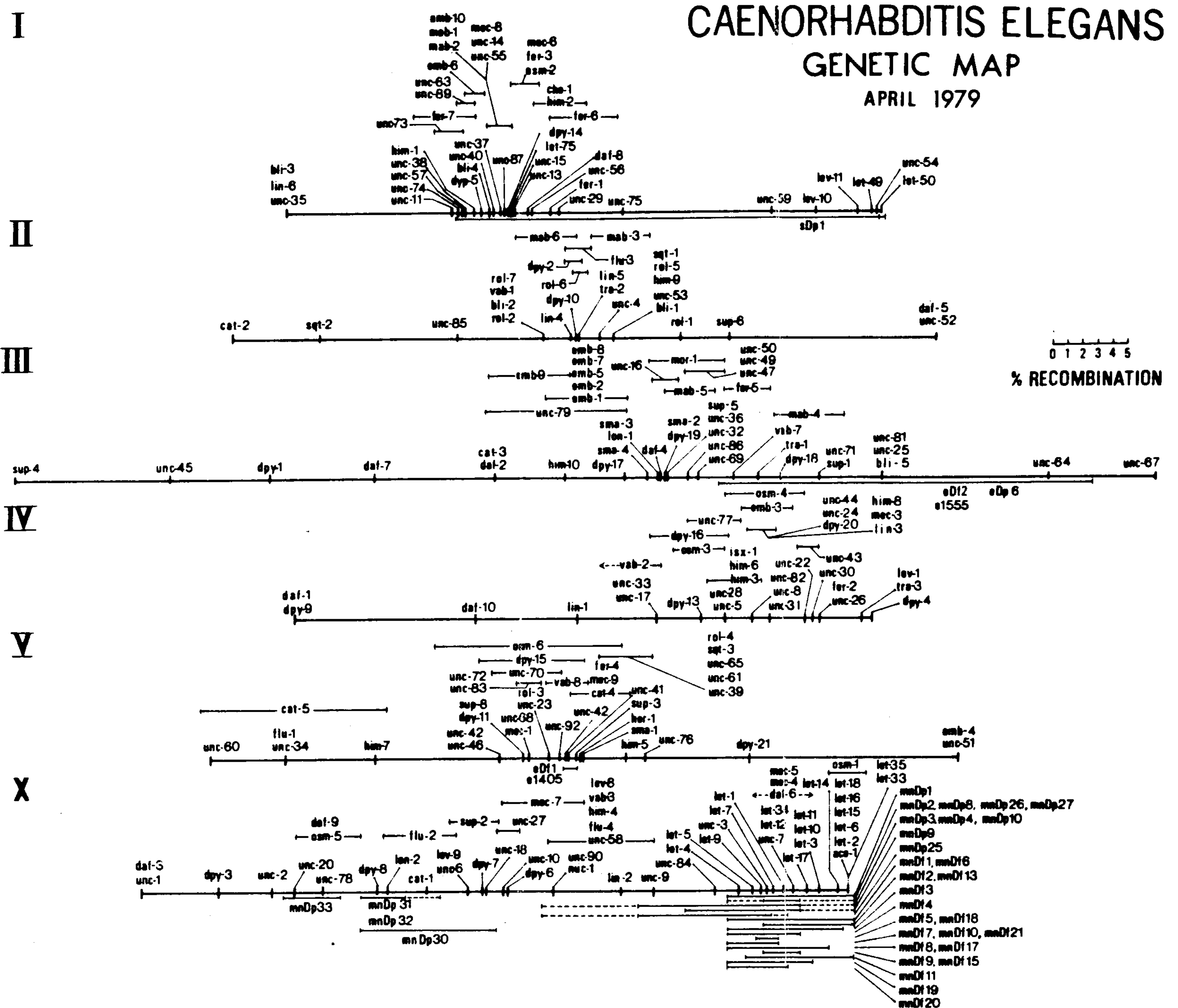


Figure 1. Genetic map of Caenorhabditis elegans.

of the genotype unc-22(sx)/+ were crossed to hermaphrodites of the genotype unc-22(sy). The F1 males were screened to determine if half of them were twitchers.

(v) Mapping within the unc-22 gene: Intragenic mapping was done in a manner similar to a method described to us by Dr. R. H. Waterston (personal communication). Young adult hermaphrodites of the genotype

+,unc-22(sy),+/unc-5(e152),unc-22(sx),dpy-4(e1166) were placed, one per 100 mm plate, at 20 degrees C. and allowed to lay eggs for 12 hrs. They were then transferred to a new plate, again for 12 hrs. These 12 hr transfers were done until there were six broods established for every heterozygous parent. The plates were left until the F2 generation matured and then scored. An estimate of the total number of progeny screened was obtained by the following method. For each brood progenies were counted from a random sample of plates (approximately 10%). The mean number of progeny per plate was determined and this number was multiplied by the number of plates in the brood. The broods were then summed. Only worms in the third larval or a later stage were counted, since screening of the plates was

done primarily at a low magnification and recombinants in earlier larval stages would possibly have been missed. To score for recombinants we looked for worms that were either wild-type, dumpy, _unc-5_, or dumpy _unc-5_ in appearance, that is, all non-twitcher phenotypes.

(vi) _Isolation, mapping and characterization of the unc-22 linked lethal mutations_: EMS treated (0.05M or 0.025M) _unc-22(s7)_ worms were mated to wild-type males. In the F1, young adult outcross hermaphrodites were selected (Figure 2) and placed one per plate at 26 degrees C. After 24 hrs. of laying eggs these worms were removed. The F2 worms were examined to determine the ratio of wild-type to mutant (_unc-22_) progeny. The rationale behind this procedure was that if a lethal arose closely linked to _unc-22_ on linkage group IV, a distortion was expected in the ratio of uncoordinated to wild-type worms. That is, there should have been a distortion in the ratio towards wild-type. Individuals showing a wild-type to uncoordinated ratio of 100 to one were designated as linked lethals. To insure also that sterile mutations in the region were isolated, all plates giving a 3:1 ratio were examined for the presence of fertile twitchers.

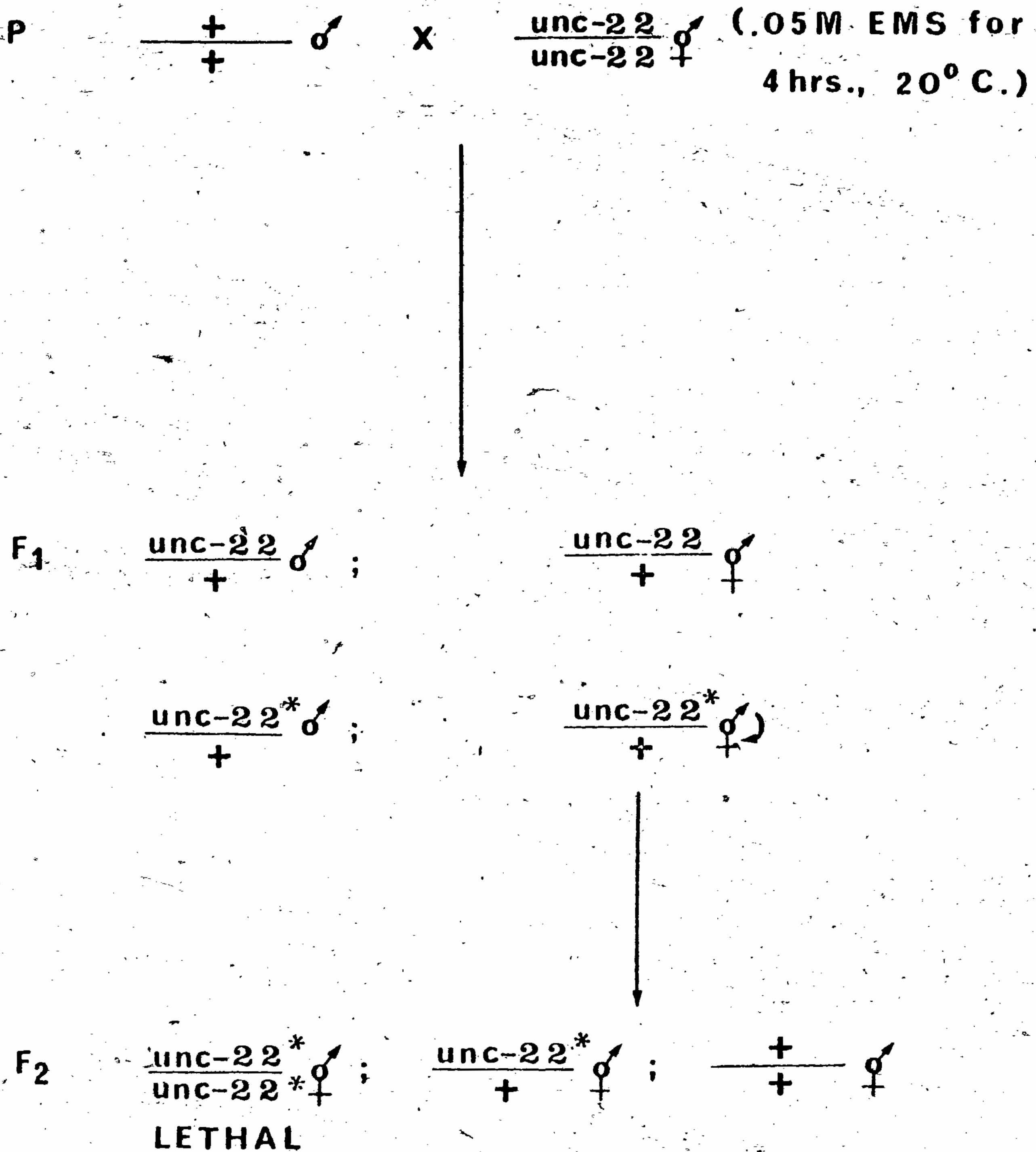


Figure 2. Selection protocol for unc-22 linked lethal and sterile mutations.

To reconfirm the presence of a linked lethal, F2 heterozygotes from plates showing a distortion in the phenotypic ratio were removed and again plated at 26 degrees to observe there segregation ratio. Ordinarily, the probability of picking a heterozygote would be 2/3 since the twitching phenotype characteristic of unc-22 is recessive in the heterozygote. However, as described earlier, worms heterozygous for a mutation in the unc-22 gene show a different behaviour in a solution of 1% nicotine than do wild-type worms. Wild-type worms go into a rigid paralysis while the heterozygotes tend to stiffen but continue to twitch or vibrate like tuning forks.

Any confirmed lethal mutation was maintained at 13 degrees C. Many, but not all, of the lethals were outcrossed to make certain they were not carrying any other mutations. Culturing the worms at 13 degrees made it possible to determine if any of the worms were temperature sensitive (ts). After a generation at 13 degrees if any of the lethals were ts, the ratio would return to a 3:1, wild-type to twitcher. Some ts lethal mutations were isolated in this way.

Lethal mutations were mapped relative to unc-22 by constructing the following strain; +,unc-22(s7),let-?,+/unc-5(e152),+,+,dpy-4(e1166). If the lethal mutation was on the unc-5 side of unc-22 then all the unc-22 recombinants would segregate only twitcher and unc-5,unc-22 progeny. If the lethal mutation was on the other side of the unc-22 gene, then the unc-22 recombinants would segregate twitcher and unc-22,dpy-4 progeny. To measure the distance from unc-22 to the lethal mutation, young adult unc-22,let-?/ + + hermaphrodites were placed, five or ten per plate (100 mm), and transferred every 24 hours to establish four broods. All progenies were counted and the map distance was determined by the following formula,

R = 2 x twi / 4/3 (wild + twitchers)   where,

R = recombination distance from unc-22 to let-?, twitchers = unc-22 mutations and wild = wild-type progeny.

Complementation tests between the lethal mutations were accomplished by crossing male unc-22,let-x/+,+ to unc-22,let-y/+,+ hermaphrodites and screening the progeny for adult males that twitched.

To determine the effective lethal phase of the lethal mutations it was first necessary to construct growth curves for N-2-S and unc-22(s7) worms. This was done by placing several N-2-S or unc-22(s7) worms on separate 100mm Petri plates and allowing them to lay eggs for 2 hrs. Adults were then removed. This insured that the progeny would be reasonably synchronous in their hatching and in their growth. The time of the removal of the parents was considered 0 hrs. The larvae were measured every 8 hrs until 96 hrs after 0 time. Larvae were measured after they were killed by placing the point of a hot soldering iron into the agar next to the worm. The dead worms laid straight and could be measured using an ocular micrometer at a magnification of 50x. All dead worms were then removed from the plate.

The effective lethal phase of the unc-22 linked lethal mutations was determined by taking young twitcher larvae from the plate containg the lethal mutation stock held at 13 degrees C. and placing them on another plate at 20 degrees C. for three days. At the end of this time they were measured and their lengths were recorded on the unc-22(s7) growth curve.

(vii) Mutagenesis with formaldehyde and isolation of deficiencies: Formaldehyde was prepared by warming 5 gm of paraformaldehyde (Fisher Co.) in 50 ml of 65 degrees C. distilled water and adding sodium hydroxide to clear the solution. After clearing, the solution was adjusted to pH 7.2, diluted to 150 ml with distilled water, further diluted to 500 ml by adding M9 buffer (Brenner, 1974) and this 1% solution was kept as a stock solution. All further dilutions were done by adding M9 buffer.

N-2-S worms were mutagenized by washing worms from stock plates with M9 buffer and collecting after centrifugation (200 x g). These worms were then suspended in either a 1%, 0.1% or 0.01% solution of formaldehyde. After 4 hrs. at 20 degrees C. they were removed from the formaldehyde with a Pasteur pipette and spotted onto a Petri plate. After approximately 2 hrs., fourth stage larvae and young adult worms were placed on Petri plates (either 10/plate or 25/plate) with lawns of OP-50 (at 26 degrees C.). These worms were left for 3 days and the F1 progeny were screened for putative deletions in the unc-22 region.

The screen for deletions takes advantage of the fact that mutations in the unc-22 gene, although mostly recessive under standard conditions, are also conditional dominants. That is, as previously described, worms when either homozygous or heterozygous for an allele of the unc-22 gene twitch in 1% nicotine, whereas, N-2-S worms in a solution of nicotine become rigid.

The protocol was to screen the F1 progeny for worms that twitched in nicotine and then to see if these worms segregated individuals with a twitcher phenotype. Individuals that did not were candidates for deficiencies in the region and were crossed to a series of lethal and visible mutations known to map in this area. Matings were done on 40 mm plates with the lethal or visible mutation being the male. An example cross is; male let-56(s46),unc-22(s7)/+,+ times putative deficiency. In the F1, the plates were examined for males to confirm that the putative deficiency bearing hermaphrodite outcrossed, and also for twitcher adults or twitcher larvae depending on whether the putative deficiency only exposed the twitcher gene or also the lethal gene (in this instance a lethal that blocks in the fourth larval stage).

(viii) Construction of sup-5;unc-22 double mutants, mutagenesis of sup-5 (e1464) and testing for suppression: Heterozygous males for a mutation in the unc-22 gene were crossed to homozygous sup-5(e1464) hermaphrodites at 20 degrees C. Because heterozygous worms for an unc-22 mutation twitch in nicotine, hermaphrodite worms were picked in the F1 of the cross, that twitched in the nicotine, and placed on separate 40 mm plates at 20 degrees C. The progeny of these worms were examined for twitchers. Since I was unaware of the dominant effect of sup-5 on some genes (Waterston and Brenner, 1978) the following scheme was devised to distinguish a 3:1 (wild-type: twitcher) ratio indicating no suppression, from a 13:3 (wild-type: twitcher) ratio indicating recessive suppression;

Twitcher progeny from the heterozygote sup-5;unc-22 were placed, one per plate, at 20 degrees for 24 hrs. After this time they were transferred to plates at 13 degrees C. and left. The purpose of this was two-fold. Firstly, although the suppressor is sterile at 13 degrees C. (Waterston and Brenner, 1978), it also suppresses better at this temperature (Dr. R. H. Waterston, personal communication). Secondly,

doing this gave a means independent from suppression to insure that I was studying the double mutant since only about 1/4 of the twitchers would have been homozygous for the sup-5 mutation as well. Sterility was not a problem because transferred worms lay a few eggs. Any 13 degree plates that were found with many twitcher progeny and eggs inside these were assumed not to be homozygous for the sup-5 mutation, because it induces sterility, and were discarded. The progeny from both the 13 and the 20 degree C., plates were examined for signs of suppression. If no suppression was seen under standard conditions, 1% nicotine was added to see if the frequency of twitching was altered. Worms on 13 degree C. plates that demonstrated suppression were rescued from the 20 degree C. plate.

The above scheme was used on a series of twitchers isolated prior to receiving the suppressor stock. An easier and more direct test was to isolate new twitcher mutations in a sup-5(e1464) background. Mutagenesis of sup-5 was done by washing off stock plates of sup-5(e1464) with M9 buffer (Brenner, 1974) and collecting the worms after centrifugation (200 x g). These worms were then suspended in 0.025M EMS. After 4 hrs. at 20 degrees they were removed from EMS with a

Pasteur pipette and spotted onto a Petri plate. After approximately one hour, fourth stage larvae and adult worms were placed, either as 10 per plate, or as drops from a pipette, on Petri plates with lawns of OP-50. After 24 hrs. of egg laying the worms were removed. The F1 progeny were later screened for worms that twitched in 1% nicotine. Any worms that twitched in nicotine were isolated and checked to see if they segregated twitchers in the F2 at 20 degrees C. These double mutant stocks, carrying both sup-5 and a twitcher mutation, were then tested for suppression following the protocol described earlier.

Twitchers that appeared to be suppressible by sup-5 were separated from it by, outcrossing to N-2-S males, selecting the young heterozygous hermaphrodites and placing them at 13 degrees C. Because of sterility the sup-5 mutation would eventually be lost from the strain and the true breeding twitcher could then be stocked. To obtain a double mutant sup-5;unc-22 with no background mutations the double mutant was outcrossed to a sup-5(e1464) male stock at 20 degrees C, Young hermaphrodite progeny were then picked that showed no twitching and allowed to lay eggs. Their progeny that twitched were then established as a true breeding sup-5;unc-22 stock.

(ix) Induction of revertants: Mutant stocks were washed from plates and mutagenized with 0.05M EMS and worms for the unc-22 alleles, s8 and s18, were placed 20/100 mm plate, while s12 worms were spotted onto the plates with a Pasteur pipette. Total counts were made on a small sample of the s8 and s18 plates to give an estimate of the total progeny screened. The s12 plates were too crowded to make a total count so a rough estimate was made by counting 1/4 of one plate, estimating the number per plate, and multiplying this by the total number of plates. This was compared to a second method where the worms were removed from the plates by washing with M9 buffer, concentrated in 1 ml of buffer, taken up in a Pasteur pipette and then put as single drops on four separate plates. The worms on each of the four plates were then counted and the approximate number of worms per plate estimated. From this the total number of worms screened could be determined. The second method gave a value 20% lower than the first and was used for the determination of the total number of progeny screened.

The screen for revertants involved examining the F1 progeny of the three twitcher mutations for worms

that failed to twitch. Any individuals found were isolated and established as a separate strain. The initial screen was done in the F1, therefore these mutations were dominant and needed to be made homozygous to stock. They were transferred over a number of generations until they gave progeny, all of which failed to twitch. Only one of the 'revertants' (s74) has been segregated away from a twitcher background. This was done by outcrossing the double mutant stock to N-2-S males and selecting young hermaphrodites which then produced progeny. Sluggish worms among these were outcrossed again, and the young hermaphrodites again were removed and allowed to lay eggs. Their progeny had nicotine added to them, and from plates that did not have worms that twitched in nicotine, a few hermaphrodites were selected and put on individual plates to lay eggs. Any of these that gave slow uncoordinated worms were used to establish the 'revertant' stock.

(x) Mapping of 'revertants' and construction of strains for gene interaction tests: The mapping of twitcher 'revertants' was done according to the methods for mapping and complementation described by Brenner (1974). The double mutations unc-15;unc-22 and

unc-54(e190);unc-22 were constructed by crossing male unc-22/ + to unc-15 or unc-54 hermaphrodites, and picking young F1 hermaphrodites that twitched in nicotine. Their progeny were examined for twitchers which were subsequently set up on individual plates. Some of the progeny of these individuals failed to twitch if unc-15 or unc-54 inhibited twitching. These were the double mutants.

Once it was determined that the 'revertants' were alleles of unc-54 it was desirable to test the degree of suppression of a number of different alleles of unc-22. To construct the heterozygous double mutants, male unc-54(s74)/ + ; +/+ were crossed to various twitcher hermaphrodites, and the F1 progeny were screened in 1% nicotine for worms that did not twitch. These young hermaphrodites were brooded, one per 40 mm plate, for three successive broods of 24 hrs each. Their mature progeny were then scored for the various phenotypes and from the resulting ratios the form of suppression was determined.

(xi) Construction of triploid and tetraploid stocks for dosage studies: Tetraploid stocks were constructed by using a scheme similar to one described

by Dr. R. Herman and Mr. Jim Madl (personal communication). Males heterozygous for unc-22(s7) were crossed to +/+/+/+ hermaphrodites. Triploid hermaphrodite progeny that exhibited an unusual twitch in nicotine (see Results) were placed on a plate and left until the next generation. Some of their progeny were twitchers and these were left, one per plate, until the following generation. The progeny of these worms were examined for twitchers that were larger than normal and these, once found, were kept for a few generations until they only gave large twitchers. They were then stocked as s7 tetraploids.

The triploid s7/s7/+ was constructed by crossing N-2-S males to the tetraploid s7 stock. The triploid m52/+/+ was constructed by crossing m52/+ males to +/+/+/+ hermaphrodites.

(xii) Construction of sup-3(e1405)/+;unc-15(e73);unc-22(s7) stocks and sup-3(e1407);unc-54(e190);unc-22(s7) stocks: The first stock was constructed by crossing a male unc-54(e190)/+;unc-22(s7)/+ to a hermaphrodite that was unc-15(e73)/unc-15(e73);sup-3(e1405),+/ +,dpy-11, and in the F1 worms that twitched were placed on individual

plates. In the next generation the plates were examined to find plates that had twitchers present but not dumpys. If the plates had unc-15 mutants, but no unc-54 mutants the slow moving worms with a wild-type looking body (unc-15 and sup-3) were placed in 1% nicotine. If they showed even the slightest twitch, they were placed on individual plates and allowed to lay eggs. Their progeny were then examined for twitchers and if found these twitchers were placed on individual plates and checked to see if they laid eggs (e73 could only lay a few eggs while the putative triple mutant was laying approximately 125 eggs). If a worm laid many eggs and had a less severe twitch than unc-15;unc-22 worms, the worm was considered to have the genotype e73;s7;sup-3(e1405)/+. This was confirmed by examining the percentage of the eggs that developed to adulthood.

To establish an unc-54;unc-22;sup-3 stock I used a different protocol. Male unc-54(e190)/+;unc-22(s7)/+ worms were crossed to hermaphrodites homozygous for sup-3(e1407)/sup-3(e1407) and in the F1 young hermaphrodites were placed one per 40 mm plate. In the F2, plates were examined to find one that gave slow wild-type looking worms and twitchers. Once found, the slow, wild-type worms were placed on separate plates.

Their progeny were then examined for twitchers since these slow worms were the double mutant unc-54;sup-3 and may have been heterozygous for unc-22 as well. When the triple mutant unc-54;unc-22;sup-3 was identified it was maintained as a stock for further study.

(xiii) Cut worm assays: These were done by cutting the worms while they were on the agar plate and placing them in the appropriate test solution. Worms were cut with a pair of Martin #1501 micro-dissecting scissors.

(xiv) Microscopy: Most of the routine processing and observational work was done using a Wild dissecting microscope. For polarized light microscopy a Leitz Orthomat with polarizing filters was used. Nematodes were immobilized in a 1% solution of nicotine and a cover slip was placed over the suspension. Nomarski pictures of living organisms at different stages of development were obtained by following the methodology of Sulston and Horvitz (1977) and by using a Zeiss Universal microscope equipped with Nomarski optics.

Results.

I. Fine structure analysis of the unc-22 gene.

(i) Mapping of the twitcher mutations to linkage group IV and the unc-22 gene: Several new recessive twitcher mutations have been isolated (Table 1) and all of these twitcher mutations map to linkage group IV. Complementation tests with all pair-wise combinations of e66, (the canonical allele of the unc-22 locus), s7, s8, s12, s13, s14, s17 and s18 have been done. All combinations give twitcher males in the F1 and therefore, these mutations fail to complement one another. The alleles, s16, s32, s34, s35, s36, s55 and m52 have been tested only with the alleles to which they were mapped (see Table 2) and, as expected, fail to complement. These two pieces of evidence, map location and complementation, confirm that these twitcher mutations are all alleles of the unc-22 locus.

(ii) Mapping within the unc-22 gene: Fifteen alleles were mapped within the unc-22 locus (Table 2). Eleven of thirteen tested alleles were separated from s8. Also, s14, s18, s34, s35, s36, s55 and m52 were separated from s12. Since in all crosses the parent

Table 2.

Recombination data between the various alleles of the unc-22 gene.

Allele pairs tested*	Frequency of unc-22+	Map distance (in map units)	L	R**
s8/s7	0/84,000	-		-
s8/s12	5/72,000	1.4×10^{-2}	s8	s12
s8/s13	0/64,000	-		-
s8/s14	5/74,000	1.4×10^{-2}	s8	s14
s8/s17	1/58,000	3.4×10^{-3}	s8	s17
s8/s18	2/52,000	7.7×10^{-3}	s8	s18
s8/e66	8/70,000	2.3×10^{-2}	s8	e66
s18/s12	4/235,000	3.4×10^{-3}	s18	s12
e66/s12	0/224,000	-		-
s12/s14	5/252,000	4.0×10^{-3}	s14	s12
s8/s16	1/262,000	7.6×10^{-4}	s16	s8
s12/m52	1/48,000 ***	8.3×10^{-3}	m52	s12
s8/s32	8/221,000	7.2×10^{-3}	s8	s32
s12/s36	8/224,000	7.1×10^{-3}	s36	s12
s8/s36	2/190,000	2.1×10^{-3}	s8	s36
e66/s16	2/20,000****	1.0×10^{-2}	s16	e66
s12/s35	7/184,000	7.6×10^{-3}	s35	s12
s8/s35	6/168,000	7.1×10^{-3}	s8	s35
s18/s32	2/212,000	1.9×10^{-3}	s18	s32
s18/s14	1/158,000	1.3×10^{-3}	s18	s14
s18/m52	0/162,000	-		-
s12/s34	7/178,000	7.9×10^{-3}	s34	s12
s8/s34	4/126,000	6.4×10^{-3}	s8	s34
s12/s55	6/160,000	7.5×10^{-3}	s55	s12
s8/s55	2/132,000	3.0×10^{-3}	s8	s55

$$\text{map distance (d)} = \frac{2(\text{unc-22+ recombinants}) \times 100}{\text{total offspring}}$$

*Mutation at left was in triple; unc-5,unc-22,dpy-4
**L=left, R=right
***Because m52 is a dominant mutation the number of recombinants is quadrupled, not doubled.
****In this experiment all 20,000 worms were counted individually.

hermaphrodites were heterozygous for the flanking outside markers, the left/right position for any two twitcher alleles that exhibited recombination could be determined. That is, in the genotype +,unc-22(sy),+ / unc-5(e152),unc-22(sx), dpy-4(e1166), if sy were to the left of sx, then the recombinant chromosomes scored were detected as either wild-type or unc-5 worms. If sy were to the right of sx, then the recombinant chromosomes appeared as either wild-type or dpy-4 worms. To determine the genotype of the recombinant chromosomes, and to insure that we were observing recombinants, all exceptional individuals were progeny tested.

The results of the progeny tests showed that of the 104 putative recombinants recovered, two were sterile, 87 segregated the markers in a manner that was compatible with chromatid exchange and 15 were recovered either with no flanking marker, both flanking markers or with an unusual flanking marker (Table 3). Only the 87 confirmed recombinants were used to determine the map distances in Table 2. The 14 exceptional individuals which had lost or carried unusual flanking markers could have resulted from outside marker exchange or gene conversion. The data

Table 3.

Progeny testing of putative recombinants.

Allele pair tested *	Total putative recombinants	Recombinant phenotype	Sterile**	Outside marker segregated
s8/s12	5	2 dpy		dpy
		3 wild		"
s8/s14	5	4 dpy		"
		1 wild		"
s8/s17	1	1 wild		"
s8/s18	2	2 wild		"
s8/e66	8	3 dpy		"
		5 wild		"
s18/s12	6	6 wild		4 dpy
			+	-
				1 none
s12/s14	7	3 unc		4 unc
		4 wild		3 none
s8/s16	2	1 unc	+	-
		1 wild		unc
s12/m52	1	1 unc		unc
s8/e32	9	6 dpy		8 dpy
		3 wild		1 unc
s12/s36	11	5 unc		8 unc
		6 wild		3 none
s8/s36	2	2 dpy		2 dpy
e66/s16	3	2 unc		2 unc
		1 wild		1 none
s12/s35	10	6 wild		7 unc
		3 unc		2 none
		1 dpy-unc		1 dpy-unc
s8/s35	8	4 dpy		6 dpy
		4 wild		2 none
s18/s32	2	1 dpy		dpy
		1 wild		
s12/s34	9	3 unc		7 unc
		4 wild		2 dpy-unc
		2 dpy-unc		
s8/s34	4	2 dpy		dpy
		2 wild		
s12/s55	6	4 unc		unc
		2 wild		
s8/s55	2	1 dpy		dpy
		1 wild		

*Mutation on left was in the triple mutant unc-5, unc-22, dpy-4.

**Sterile recombinants and putative convertants were not included in the calculation of map distances.

cannot unequivocally distinguish between these alternatives. Since gene conversion is well documented in fungal species (Mitchell, 1955; Fogel and Mortimer, 1969), as well as in D. melanogaster (Smith et al., 1970), we would expect it to occur in C. elegans as well.

The number of exceptional progeny with lost or parental flanking markers was higher than expected from double crossover events. The flanking markers unc-5 and dpy-4 are both about five map units from unc-22. I expected, therefore, one in 40 of the recombinants to have unusual flanking markers (2.5%) if there were no interference whereas, about three in 20 with unusual markers were observed (15%). This strongly suggests that these exceptional individuals were conversion products (see Table 4).

The 87 confirmed recombinants give the left/right position of the various alleles. From this information an intragenic map of the unc-22 gene can be constucted (Figure 3). On this map the alleles that have been positioned unambiguously are shown above the line. These include s16, s8, s18, s14 and s12, the allele e66 is included above the line because it is closely linked

Table 4.

Putative conversion rates per unc-22 allele.

Allele	Total progeny	Putative recombinants	c/o*	conversions allele	conversions other	conversion rate
s12	1,600,000	54	43	4	7	5×10^{-6}
s14	500,000	13	11	2	0	1×10^{-5}
s36	400,000	13	10	3	0	1×10^{-5}
s35	350,000	18	13	3	1	2×10^{-5}
s16	300,000	4	3	1	0	5×10^{-6}
s32	400,000	11	10	1	1	5×10^{-6}
s8	1,600,000	38	35	0	3	-
s7	85,000	0	0	0	0	-
s13	65,000	0	0	0	0	-
s17	60,000	1	1	0	0	-
s18	800,000	11	10	0	1	-
s66	300,000	11	10	0	1	-
m52	200,000	1	1	0	0	-
s34	300,000	13	11	0	2	-
s55	300,000	8	8	0	0	-

* c/o = crossover

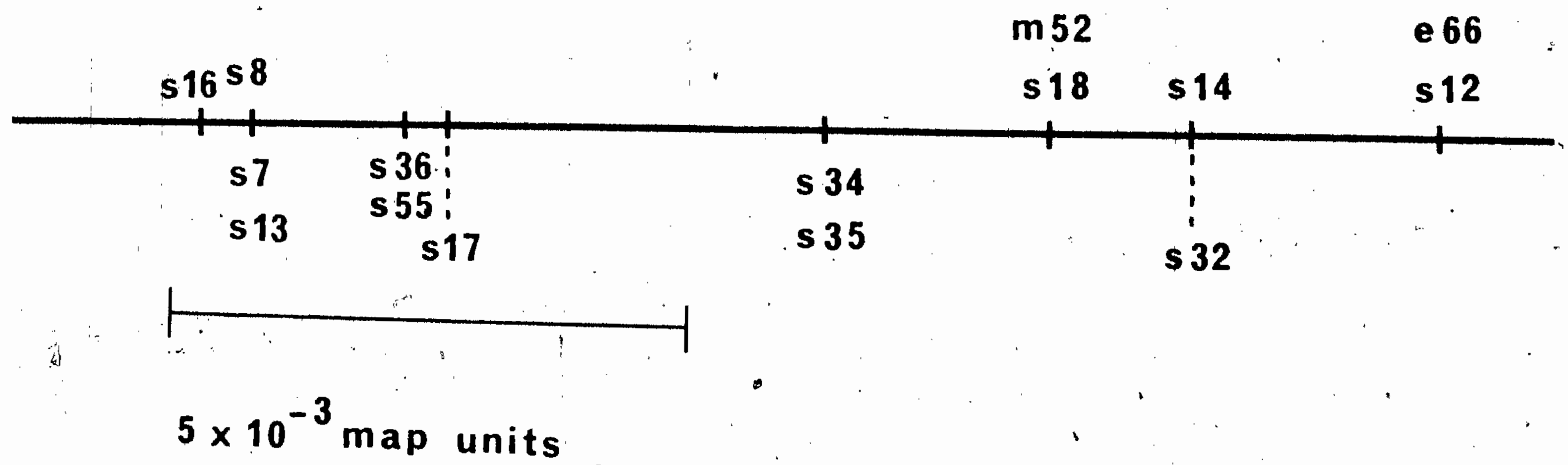


Figure 3. Fine structure map of the *unc-22* gene.

to s12. Although the alleles s13 and s7 have not been separated from s8, they are listed below the line since they have not been positioned relative to s16 or s17. The allele s17 has been positioned relative to s8 only. The position of s17 is, therefore, shown with a dotted line. The same is true for the formaldehyde induced alleles, s34, s35, s36 and s55 which are known to be between s8 and s12 but have not been positioned relative to s18 or each other. The position of s32 is fairly firm since it is based on very large numbers, but it still needs to be positioned relative to s12. Its position is also shown with a dotted line. The same holds for m52, a dominant allele of unc-22 (isolated by Dr. D. Riddle), which appears to be near s18. The distances shown between the alleles should be considered as tentative since they are based on few recombinants.

On the plates containing putative recombinants there were periodically clones of non-twitcher worms. This occurred 16 times among the 104 putative recombinants. In 13 cases dpy's or unc's as well as wild-type worms were seen and these were classified as recombination events that had taken place in the F1 generation thus giving rise to many non-twitchers in

the F2 generation. Three of the events involved either double crossovers or conversions because there were, in one case, many wild-type worms on the plate, and in the other two cases, many dpy-unc's on the plates. The explanation of an F1 event having occurred can account for some of these observations but it seems highly improbable that it can account for all of them (see Table 5).

In the case of recombination between s18 and s14, one F1 event was observed. On the plate there were 35 wild-type worms, 15 dumpy worms and one large wild-type worm. This presumably was the F1 recombinant. Although unlikely, it is not impossible that such an event could occur. In other cases this was not a satisfactory explanation. For example, in recombination between s8 and s17, there was one recombinant found in 58,000 progeny screened. It was an F1 crossover. In recombination between s8 and s18 there were two crossovers in 52,000 progeny screened. Again, one of these appeared to have happened in the F1 generation. In a recombination experiment between s8 and s36 two recombinants were found in 200,000 worms examined, and both of these were presumably the products of F1 events. Finally, in a recombination run between s18 and

Table 5.

Occurrence of putative F1 recombinants.

Allele pair tested***	Progeny in F2	Putative recombinants	c/o	convertants	F1 events no.	F1 events type
s8/e66	7 x 10^4	8	8	0	2	c/o*
s8/s17	6 x 10^4	1	1	0	1	c/o
s8/s18	5 x 10^4	2	2	0	1	c/o
s12/s14	2.5 x 10^5	7	5	2	1	c/o
s12/s36	2.2 x 10^5	11	8	3	1	**conv
s8/s36	2 x 10^5	2	2	0	2	c/o
s18/s32	2 x 10^5	2	2	0	2	c/o
s12/s34	1.8 x 10^5	9	7	2	3	1 c/o 2 conv
s12/s55	1.6 x 10^5	6	6	0	2	c/o
s18/s14	1.6 x 10^5	1	1	0	1	c/o

* c/o = crossover

** conv = convertant

*** Allele listed at left was in the triple mutant

unc-5,unc-22,dpy-4.

s32 where 212,000 progeny were screened, two recombinants were found, both of which appeared to arise as F1 events. These last two observations make it impossible to consider these clones of non-twitchers as the result of crossover events that occurred in the previous generation.

On some of the plates showing a number of non-twitchers the exceptionals were counted. As stated earlier, on the s18/s14 plate there were 51 non-twitcher progeny. One of the s18/s32 plates had 36 non-twitchers, while an s12/s34 plate had 19 non-twitchers and an s12/s55 plate had only 4 non-twitchers. An F1 recombinant would give far more than 4 non-twitchers. Three or four possible explanations for this phenomenon exist and will be examined in the General Discussion.

(iii) The size of the unc-22 gene: An earlier estimate of the unc-22 gene size was 2.4×10^{-2} map units (Moerman and Baillie, 1979). This was based on the distance between the outermost alleles, s16 and e66, and was determined by adding the distance from s16 to s8 to the distance from s8 to e66. However, an examination of Table 6 suggests that this is an

Table 6.

Recombination size of the *unc-22* locus.

Alleles summed	Additive total (in map units)	No. of c/o **	c.l. *
s16..............e66***	1×10^{-2}	2	(0-9) .005 (0.5-5) .1
s16...s8...s18...s12	1.2×10^{-2}	7	(2-17) (3-12)
s16...s8...s34...s12	1.5×10^{-2}	12	(5-24) (8-18)
s16...s8...s36...s12	1.0×10^{-2}	11	(4-23) (7-17)
s16...s8...s35...s12	1.5×10^{-2}	14	(6-27) (9-20)
s16...s8...s55...s12	1.1×10^{-2}	9	(6-27) (6-20)
s16...s8..........e66	2.4×10^{-2}	9	(3-20) (5-14)
s16...s8..........s12	1.5×10^{-2}	6	(2-16) (3-10)
s16...s8...s14...s12	1.9×10^{-2}	11	(4-23) (7-17)
Average distance	1.5×10^{-2}		
Average distance excluding s16...s8..........e66	1.4×10^{-2}		

* c.l. = confidence limits. ** c/o = crossovers.

*** In this experiment all progeny were individually tested.

overestimate of the size of the locus which is probably due to the anomalously high recombination rate observed betwen s8 and e66. This can be seen when one compares the s8-s12 distance to the s8-e66 distance. There is almost a two-fold difference, yet s12 and e66 cannot be separated.

A closer estimate of the actual gene size is 1 - 2 x 10^{-2} map units. This is a compromise between the distance obtained, when one examines the distance from s16 to e66 and counts all the progeny individually, and when one uses the estimation procedure for total progeny. The fact that 1.0 x 10^{-2} map units is in closer agreement with the other distances listed in Table 6 than is 2.4 x 10^{-2} map units may be misleading because the confidence limits on all these values allows for overlap (see the final column of Table 6). No convincing evidence of map expansion which is so characteristic of fungal fine structure mapping studies (Hastings, 1975 for a review) has been found.

II: Deficiencies, recessive lethal, sterile and visible mutations in the unc-22 region.

(i) Lethal and sterile mutations: Recessive lethal and sterile mutations were isolated as described in Materials and Methods and illustrated in Figure 2. Of 3094 individuals screened, 49 were found to have lethal or sterile mutations in the unc-22 region. Three of these mutations were found to be temperature sensitive (this has been confirmed by Mr. K. Reikki). All mutations were induced either with 0.05M EMS or with 0.025M EMS. Although 0.05M EMS appears to give a higher mutation rate per chromosome, the difference is not statistically significant (Table 7). The forward mutation rate for the induction of lethals in this region using 0.025M EMS was approximately 1.5%. This region represents about 1% of the genetic map of the nematode.

Of the original eight mutations isolated with 0.05M EMS, six have had all pair-wise complementation tests done. These are let-51(s41), let-52(s42), let-53(s43), let-54(s44), let-55(s45) and let-56(s46). As well, let-51(s41) and a 0.025M EMS induced lethal, let-59(s49), complement. All seven of these mutations

Table 7.

Screen for unc-22 linked lethal and sterile mutations.

EMS concentration	Total progeny screened	Lethals	95% confidence	%
0.05M	320	8	(4-16)	2.5
0.025M	2774	41*		1.5

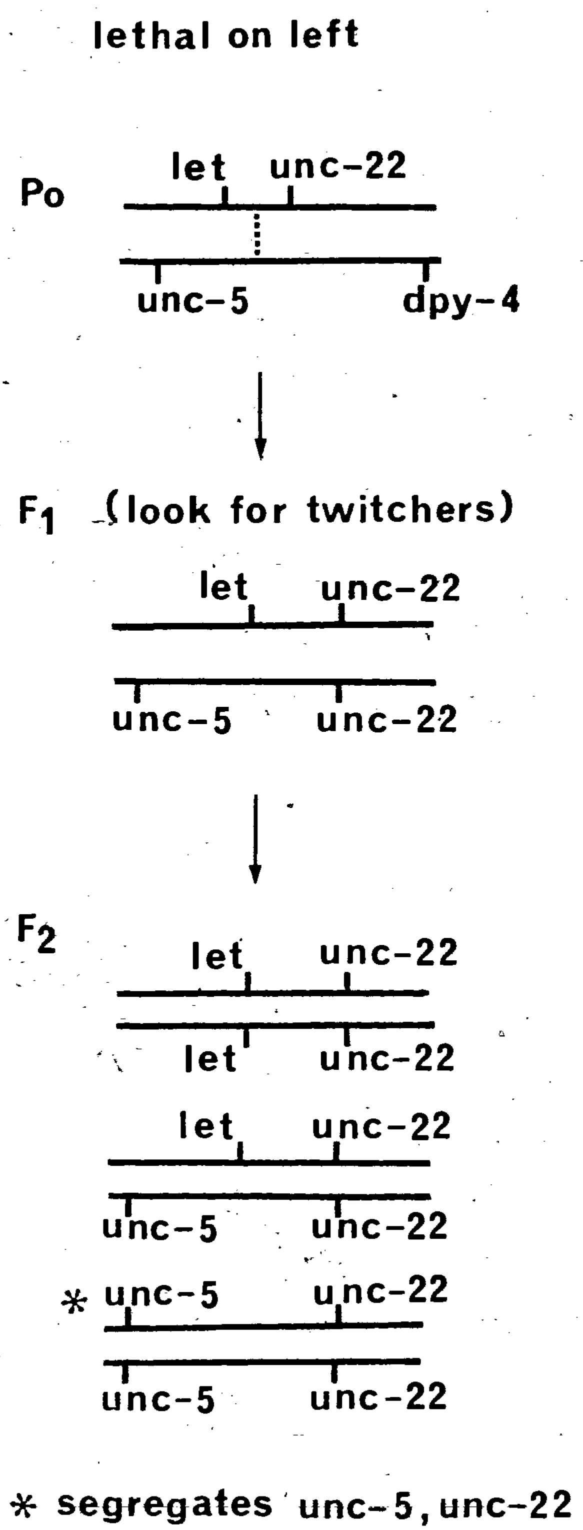
*3 of these are temperature sensitive.

This gives a rate of ts lethals/total lethals of 6%

have been mapped relative to unc-22 by the method shown in Figure 4. The mutations, let-58(s48) and s51 have also been positioned by this method. The mutation let-60(s59) has been positioned relative to unc-22 by using two deficiencies in this region, sDf1 and sDf2. Map distances from unc-22 to these nine mutations are shown in Table 8. With map distances, complementations tests and positioning information relative to unc-22, a cursory map of this region has been constructed (Figure 5). On this map six of the lethal sites lie to the left of the unc-22 site and three lie to the right.

Of the 40 other lethal and sterile mutations, one, s53, is an allele of let-54. The three lethals, s54, s57 and s72 are all temperature sensitive(ts) and no mapping or complementation tests have been done with these. Of the other mutations, eleven have had only their effective lethal phase determined. The others will be described in context with the deficiencies.

(ii) Deficiencies: To position the lethal mutations relative to one another and to separate tightly linked genes it was necessary to obtain deficiencies in this region. The lethal and visible mutations already identified helped characterize these



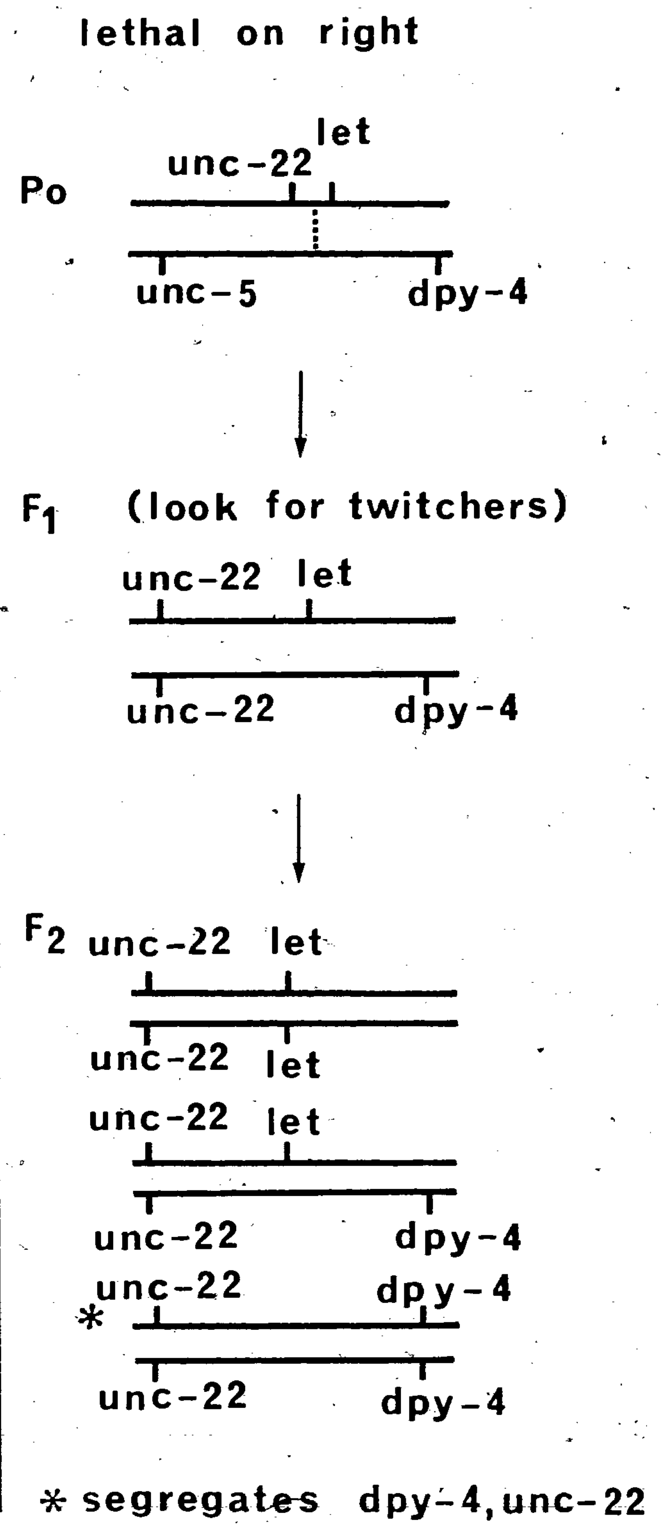


Figure 4. Method of positioning the lethal mutation sites relative to the unc-22 site.

Table 8.

Map distances from unc-22(s7) to various lethal mutations.

Genotype tested	Phenotype of progeny wild-type	twitcher	Distance (in map units)
let-53(s43),unc-22(s7)/+ +	4582	22	0.7
let-54(s44),unc-22(s7)/+ +	3400	32	1.4
let-55(s45),unc-22(s7)/+ +	3861	19	0.6
let-52(s42),unc-22(s7)/+ +	4512	0	0.1 *
let-51(s41),unc-22(s7)/+ +	4456	11	0.4
let-59(s49),unc-22(s7)/+ +	2225	6	0.4
let-60(s59),unc-22(s7)/+ +	975	3	0.5
let-58(s48),unc-22(s7)/+ +	519	5	1.5
let-56(s46),unc-22(s7)/+ +	26,000	1	0.01 **

* With this pair I also set up 10 plates with 1 worm per plate and left them till the F2 generation. Two crossovers were found. If one uses the formula, recombination = 2 x c/o / 2/3 total progeny and assumes 2,500 worms per plate, then the map distance between let-52 and unc-22 is approximately 0.03 map units.

** number of total progeny determined as in above.

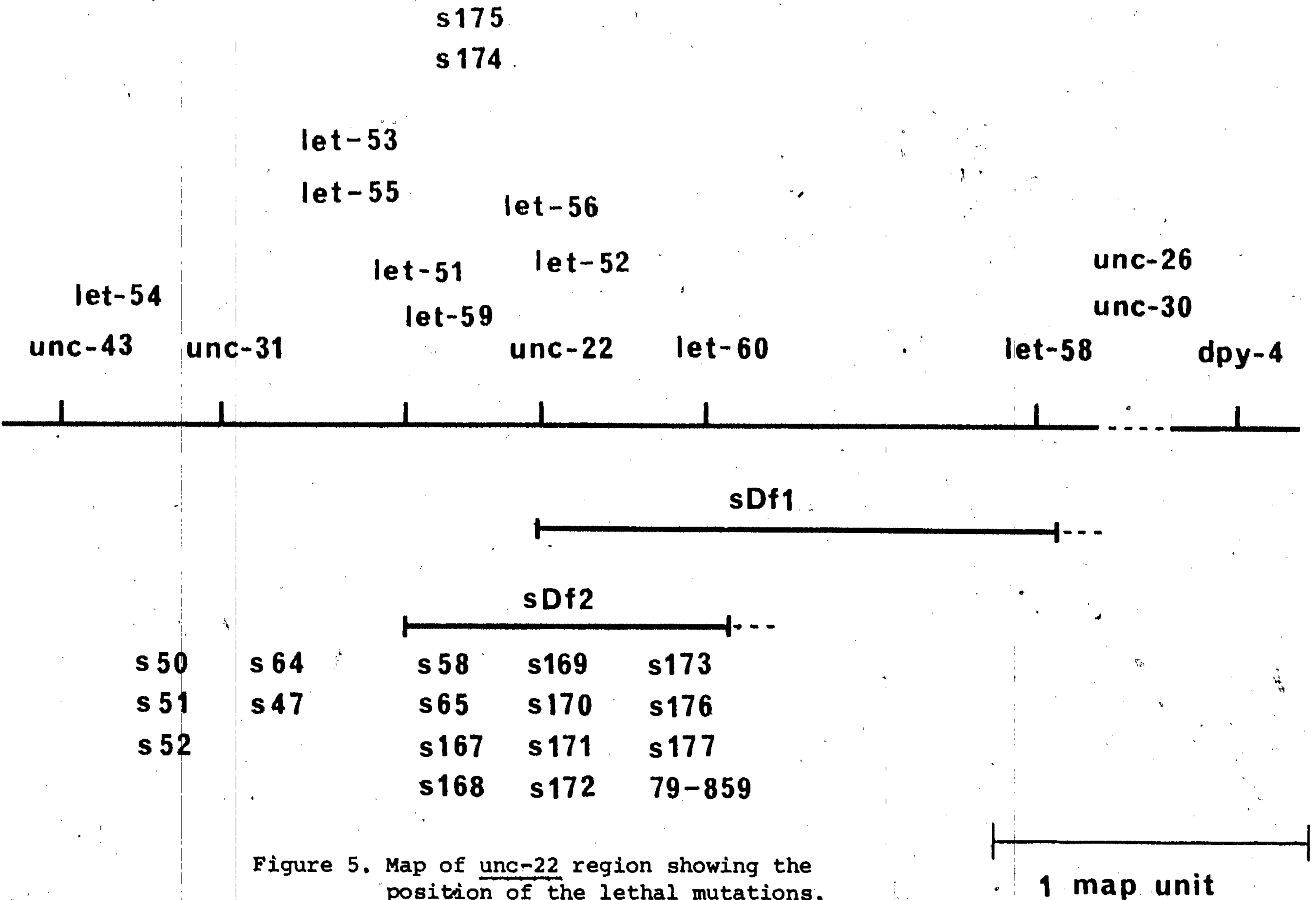


Figure 5. Map of *unc-22* region showing the position of the lethal mutations.

deficiencies, which were then used to limit new lethals to specific regions, and confirm lethal map positions that were based on distances from the unc-22 site. To generate the deficiencies formaldehyde was used following the protocol described in Materials and Methods. Although X-rays can cause deficiencies in the nematode (Meneely and Herman, 1979), note was taken of the early results of Slizynska (1957) and the more recent findings of O'Donnell et al. (1977) demonstrating formaldehyde induced deficiencies in D. melanogaster. These observations led me to examine its mutagenic effects in C. elegans and I found it capable of inducing both point mutations and deficiencies in the nematode.

Three concentrations of formaldehyde were tested: namely 1%, 0.1% and 0.01%, but mutations were found with only 0.1%. The high dose of 1% killed the worms. On examining worms plated from this concentration only a few larvae were alive after 2 days. Possibly these were protected as either dauer larvae or eggs. With 0.01% no mutations were identified in approximately 6 x 10^4 tested F1 chromosomes, nor were any visible mutations observed in the F2 generation after setting up 625 F1 worms.

Using 0.1% formaldehyde approximately 270,000 F1 chromosomes were examined (Table 9) and seven mutations were obtained. These were separated into two groups; one group segregating homozygous twitchers (_unc-22_), the other group giving progeny that appeared wild-type and progeny that twitched in 1% nicotine. The group of formaldehyde induced _unc-22_ mutations, _s34_, _s35_, _s36_ and _s55_, were all phenotypically indistinguishable from EMS induced _unc-22_ mutations (see Results I and III for further characterization of these mutations). It was the second group that contained possible deficiencies in the _unc-22_ region. With 0.01% formaldehyde 225 of the F1 worms were placed on plates and their progeny were examined for any type of visible mutation. Three uncoordinated worms were found on separate plates. These mutations were not mapped.

The characterization of the deficiencies is still in progress. Of the three, _sDf1_ and _sDf2_, were confirmed as deficiencies in the _unc-22_ region. The other putative deficiency, _sDf3_, gave puzzling results in the complementation tests with the lethal mutations. I also found a spontaneous twitcher mutation in this

Table 9.

Formaldehyde mutagenesis in the unc-22 region.

Type of disorder	Formaldehyde concentration	chromosomes tested	number of isolates	frequency
unc-22 mutations	0.1%	135,000	4	3×10^{-5}
deficiencies	0.1%	135,000	3 *	2×10^{-5}

* one of these may be some other type of rearrangement.

stock which suggests that it contains more than a simple deficiency.

Deficiencies sDf1 and sDf2 have been examined in some detail. Both appear to be 1-2 map units in length, and both uncover unc-22 but do not expose dpy-4(e1166), the outside gene on the linkage group. The two deficiencies overlap by at least 0.5 map units and reciprocal crosses produce eggs that fail to hatch. Deficiency sDf1 appears to be larger than deficiency sDf2. Worms with this deficiency also grow more slowly and give fewer progeny (Table 10). Results of the initial complementation tests on these deficiencies are shown in Table 11. All uncovered genes are lethal or sterile except unc-22. The deficiencies have been initially complemented with unc-22, dpy-4 and eight of the nine (excluding let-60) lethal sites to characterize their extent. As can be seen from Figure 5 the right boundary has not been determined for either deficiency. The left boundary is better defined and illustrates one of the important uses of deficiencies. sDf1 ends between unc-22 and let-56 which are tightly linked, and sDf2 breaks between let-51 and let-59 which map the same distance from unc-22, 0.4 map units. The deficiencies, therefore, allow for the positioning of

Table 10.

Fecundity of formaldehyde induced deficiencies.

Deficiency	eggs	hatchability number	hatchability %	Number with deficiency	No deficiency
sDf1	69	10	15%	4	6
sDf2	65	46	75%	30	16
sDf3	116	28	23%	19	9

Table 11.

Complementation tests of deficiencies, sDf1 and sDf2, with various genes in the unc-22 region. *

Gene in unc-22 region tested **	sDf1	sDf2
unc-22(s7)	-	-
let-51(s41) ***	+	+
let-52(s42)	-	-
let-53(s43)	+	+
let-54(s44)	+	+
let-56(s46)	+	-
let-?(s47)	n.d.	+
let-58(s48)	-	+
let-59(s49)	+	-
dpy-4(e1166)	+	n.d.****

* + = complement; - = fail to complement.

** All genes tested were the male stock.

*** All lethal mutations were kept in a stock linked to unc-22(s7).

**** n.d. = not done.

tightly linked genes relative to one another.

Once the extent of the deficiencies was determined they were used to position the new lethal sites (Table 12). Of 14 lethal and sterile mutations tested with sDf1, only three failed to complement. Thirty lethal and sterile mutations were tested with sDf2, and 18 of these failed to complement. The two deficiencies allowed for the region around the unc-22 gene to be divided into five zones: Zone 1, an area to the left of unc-22 but beyond the extent of sDf2; Zone 2, an area to the left of unc-22 but uncovered by sDf2, Zone 3, an area to the right of unc-22 uncovered by both sDf2 and sDf1; Zone 4, an area to the right of unc-22 exposed by sDf1 only, and finally; Zone 5, an area to the right of unc-22 but beyond the extent of sDf1.

Zone 1 includes the let sites, let-54, let-53, let-51 and probably let-55. It also contains s47, s50, s51 and s52. Zone 2 encompases the let sites let-59 and let-56. The mutations 27-360 and s174 are also in this zone. Zone 3 contains let-52 and let-60. Zone 4 contain let-58 and zone 5 does not contain any lethal or sterile mutations. This is understandable since the screen is limited to isolating mutations that are

Table 12.

Characterization of lethal and sterile mutations isolated in the unc-22 region.

Mutation	length (in mm)	Larval blockage stage	Comments
s41	-	egg	
s42	0.28	L-1	
s43	0.80	L-4	
s44	0.30	L-1 to L-2	blocks in the moult
s45	0.32	L-1 or L-2	
s46	0.78	L-4	
s47	0.98	adult	sterile
s48	-	?	no unc-22 larvae
s49	0.28	L-1	
s50	0.72	L-4	
s51	0.56	L-3	
s52	0.76	L-4	
s53	0.28	L-1 to L-2	blocks in the moult
s54	-	Late larval	temperature sensitive
s57	-	Late larval	temperature sensitive
s58	0.60	L-3	fails to complement sDf2
s59	0.44	L-2	fails to complement sDf2 and sDf1
s60	0.16	L-1	
s61	0.76	L-4	
s62	-	adult	sterile and complements sDf2
s63	0.55	L-3	complements sDf1
s64	0.50	L-3	complements sDf1 and sDf2
s65	0.76	L-4	modifies twitch and fails to complement sDf2
s72	-	egg	temperature sensitive
s166	0.33	L-1 or L-2	complements sDf2
*s167 (60-829)	-	egg?	fails to complement sDf2
s168	0.65	L-3	fails to complement sDf2
s169	0.25	L-1	fails to complement sDf2

Table 12 continued.

Mutation	length	Larval blockage stage	Comments
*s170 (15-1605)	0.57	L-3	fails to complement sDf2
s171 (89-228)	-	adult	sterile but 'leaky' fails to complement sDf2
s172 (94-267)	small	L-1	fails to complement sDf2
s173 (10-1594)	0.58	L-3	fails to complement sDf2
s174 (57-797)	0.60	L-3	complements sDf1 and fails to complement sDf2
s175 (27-360)	-	larval	lethal larvae are dark multiple blocks, fails to complement sdf2 but complements sDf1
s176	small	L-1	fails to complement sDf2
s177	small	L-1	fails to complement sDf2
s212	-	egg?	no twitcher larvae seen
s213	-	egg or L-1	no twitcher larvae seen complements sDf2
s214	1.00	adult	sterile
s215	0.26	L-1	complements sDf2
s216	0.98	adult	sterile
93-1725	0.48	L-2 or L-3	
24-77	0.40	L-2	complements sDf1
41-1805	0.80	L-4 to adult	complements sDf1 and sDf2
27-359	0.70	L-4	
79-859	1.10	adult	sterile, fails to complement sDf2
58-412	1.10	adult	sterile
93-1450	-	egg?	no unc-22 larvae seen
28-339	-	-	

* The numbers without an 's' in front of them are isolation numbers. In the text some of the mutations are referred to by their isolation number as well as their 's' number.

within about one map unit of the *unc-22* locus. Eleven mutations, *s65*, *s58*, *s177*, *s173*, *s168*, *s171*, *s172*, *s169*, *s176*, *60-829* and *s170*, fail to complement with *sDf2* but these have not been tested with *sDf1*. Therefore, I do not know if they are in zone 2 or zone 3. Five other mutations, *s60*, *s61*, *s62*, *s63* and *s64*, are presumably in zone 1 or 5 because they are particularly difficult to maintain as stocks; the lethal keeps separating from the *unc-22* marker.

Zones 2 and 3 contain 17 lethal and sterile mutations plus the *unc-22* site. Four of the mutations define the let sites, *let-59*, *let-56*, *let-52* and *let-60*. Initial complementation tests indicate that; *s170* and *s173* fail to complement (this should be repeated), *s168* and *s58* fail to complement, *s174* and *s168* complement, *s46* and *s65* complement and, *s168* and *s170* complement. If it is assumed that the individual lethal sites have the same forward mutation rate as the *unc-22* site, 6×10^{-4} using 0.025M EMS (Table 13), then the sample of 3094 tested chromosomes should have included hits at most of the lethal and sterile sites in this region.

Table 13.

Forward mutation rate at the unc-22 locus.

EMS concentratiion	Type of screen	Total tested chromosomes	Twitcher	Estimated mutation rate
0.025 M	sDf2	3,274	2	6×10^{-4}
0.025 M	Sup-5(e1464)	6,000	5	8×10^{-4}
- -	N-2-S	64,000	0	1.6×10^{-5}

(iii) Characterization of the lethal and sterile mutations: The effective lethal phase for each mutation is listed in Table 12 and shown in Figure 6. This list includes one confirmed embryonic lethal, eight first larval stage lethals (L-1), two lethals that block in the moult from the L-1 to the next larval stage, two lethals that block in second stage larvae (L-2), eight lethals that block in third stage larvae (L-3), seven lethals that block in fourth stage larvae (L-4), seven sterile adults and one lethal that does not have a fixed stage of blocking. As well, there are four other possible embryonic lethals, two lethals that have not been confirmed as either L-1 or L-2 at their time of blockage, one lethal that could be either an L-2 or an L-3 blocker and one mutation that has not been confirmed as either an L-4 blocker or a sterile adult. One mutation, 28-339 has not had its time of developmental arrest determined. The effective lethal phase of the three ts lethals has been determined, with one blocking in the egg and the other two blocking in a late larval stage (Mr. K. Reikki, personal communication).

Only two of the mutations were examined with Nomarski optics. The confirmed embryonic lethal, s41,

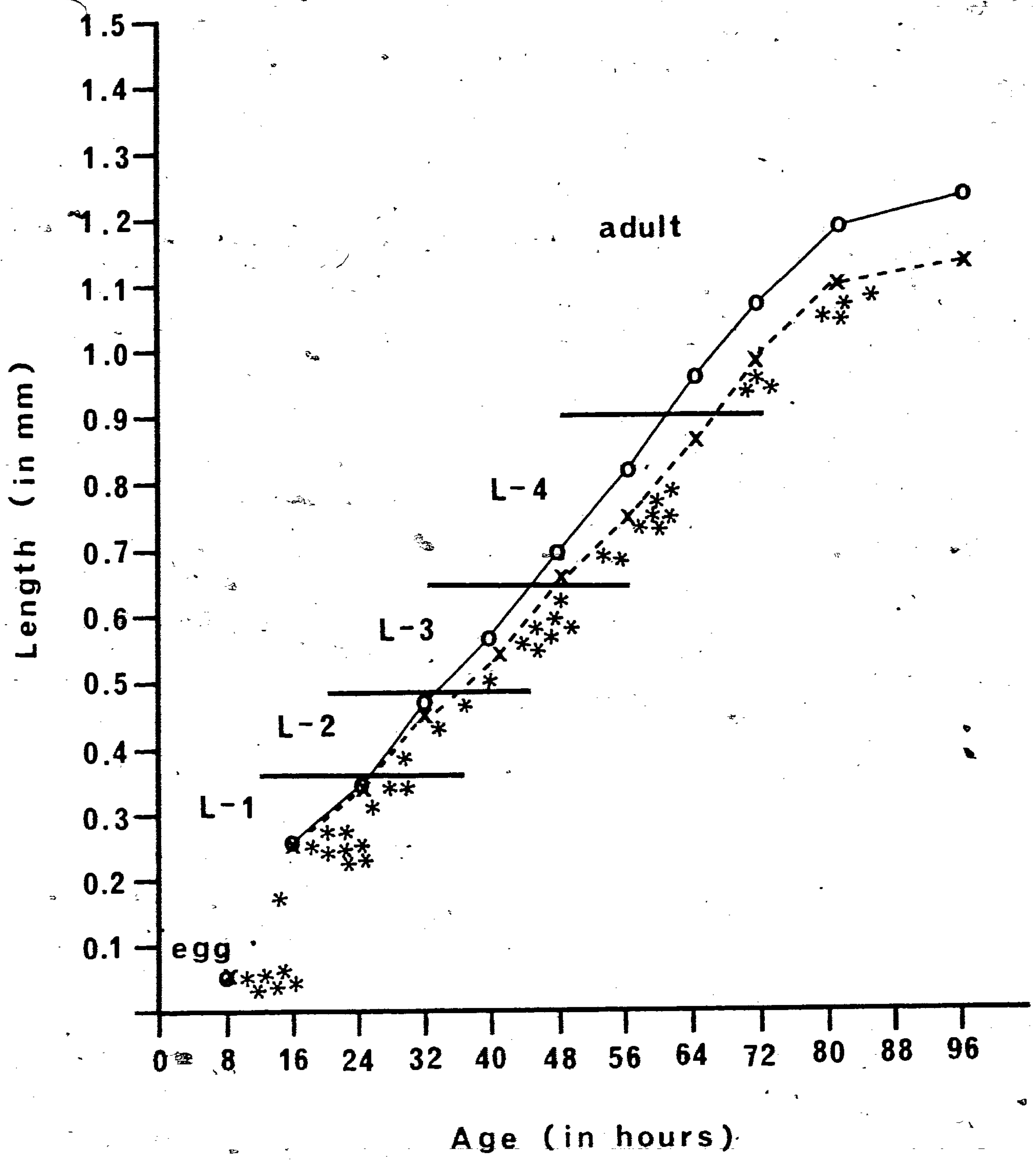


Figure 6. Growth curve of N-2-S vs unc-22(s7) and distribution of developmental lethal and sterile mutations.

N-2-S growth curve is solid line. unc-22(s7) growth curve is dotted line. Blocking time of lethal mutations is indicated by stars. The figure does not include s48, s54, s57, s175 or 28-339.

was found to block in a late embryonic stage. One of the adult sterile mutations, s47, was examined. Its oocytes appeared morphologically normal, but they were not fertilized and no zygotes were seen. To examine the possibility that this was a fertilization defective mutant (see Ward and Miwa, 1979), a male rescue experiment was done but no outcross progeny were found. It is presumed, therefore, that the problem is in oogenesis.

Among the L-1 lethals, many of the lethals block in very early L-1 just after hatching. Let-59(s49) does not even straighten out after hatching but lies in a curl. Two of the L-1 blockers that can move are s42 and s169. The let-52 gene is important because its map position indicates that it is the adjacent gene to the right of unc-22.

The gene, let-54, is important for two reasons. First, because it has a unique time of blockage, the L-1 to L-2 moult. This was determined by observing that all the let-54,unc-22 worms after reaching a length of 0.3 mm become shiny and straight. The shiny suface indicated that the worms were moulting. However, none of the worms proceeded any further in development than

this stage. I have recovered two alleles of this gene, s44 and s53. Other mutations similar to these have been isolated but because of their loose linkage to the unc-22 gene they have been impossible to keep. The identification of two alleles of the let-54 gene in such a small sample suggests that this gene may be more easily mutated than some of the other genes in this area. A second reason for the interest in this gene is that it gives an indication of the time of moulting in unc-22 mutants. The horizontal lines in Figure 6 show when the moults occur in an N-2-S worm (Byerley et al., 1976). The twitcher and twitcher-lethal combinations would be expected to be somewhat different from this. The fact that let-54(s44),unc-22(s7) worms block at approximately 0.3 mm suggests that the bar should be lowered slightly when estimating moult times for unc-22 linked lethal mutations. Because there will be differences of an individual nature in the different mutant combinations, each double mutant should be followed individually through the moulting cycle. This is tedious and probably need only be done with mutations that fall in the borderline area between two larval stages. If the length of 0.3 mm does indicate the time of the L-1 to L-2 moult, then s45 and s166 block in the L-2, not in the L-1.

There are two mutations that definitely block in the L-2, let-60(s59) and 24-77. Three other mutations, s45, s166 and 93-1725 block in either late L-2 or in early L-3. Among these five mutations there are at least two genes, let-60 to the right of unc-22 and let-55 to the left of unc-22. These genes are at least one map unit apart. From the complementation tests done with the deficiencies it can be assumed that s166 and 93-1725 are not alleles of let-60.

Lethals that arrest development in the L-3 stage have a heterogenous blocking time from 0.50 mm to 0.65 mm covering the whole time of growth during this period. The eight or nine lethal mutations that block in this stage define a minimum of three essential sites. This is based on the complementation tests of the lethal mutations with the deficiencies and some selected complementation tests between various lethals. Based on the deficiency tests, s64 must define a site outside of zones 2 and 3, while s168 and s170 define a group within these zones. These latter two mutations complement each other and, therefore, define separate sites.

The lethal mutations that block in the L-4 arrest at a variety of lengths throughout the whole larval stage. The lethal let-56(s46) is important because it is most likely the gene adjacent to the left of unc-22. It gave few recombinants in a large scale mapping experiment but was separated from unc-22 by sDf1. The plot of points in Figure 6 is misleading because it indicates that the lethals follow an unc-22 growth curve. This has not been the case for s43 or s46 which grow slower than does unc-22(s7). The lethal s65 shows signs of gene interaction with unc-22. The stock is difficult to keep because s65,s7/+,+ worms do not twitch well in 1% nicotine. Even the homozygous twitchers have a subdued twitch in nicotine. Both let-56 and s65 are uncovered by sDf2, but they complement and are, therefore, treated as separate genes. The essential gene, let-53, is another L-4 blocking gene, which means there are at least three defined L-4 complementation groups. The fact that s50 and s52 complement suggests that there are possibly five L-4 complementation groups.

The sterile adult mutations (st), based on the complementation tests of s47 and s171 with sDf2, must form at least two complementing groups. These

mutations are more difficult to map than let mutations because the twitchers must be progeny tested. Consequently, little has been with this group of mutations. The mutation, s171, which is uncovered by sDf2, is a 'leaky' mutation. That is, although most of the homozygous s171 worms are sterile, some lay a few eggs. These hatch but as adults they are sterile.

The results of the complementation tests of the lethal and sterile mutations with sDf2 made it clear that a wide range of lethal phenotypes can be exhibited by different alleles of any single locus. Crosses of the mutations with the deficiencies which gave hemizygous progeny revealed something of the nature of the mutations at these sites. If the mutation at a locus was such that the allele was a nullo (amorphic) i.e. no gene product was made, then a hemizygous worm for that mutation should not block at a different stage of development from a worm homozygous for that mutation. If the mutation only partially inactivated the gene product, then decreasing the amount of the gene product by 1/2 might shift the stage of developmental arrest. Examples of both types of mutation were found. Table 14 shows that s42, s49, s65, s170 and s174 are possibly nullo mutations. As either

Table 14.

Lethal and sterile mutations in the homozygous and hemizygous state . Characterization of developmental blockage stages.

Mutation	Homozygous (let/let)	Hemizygous (let/sDf2)
s46	L-4	L-3
s42	L-1	L-1
94-267	L-1 (early)	egg
89-228	adult (sterile,leaky)	adult (sterile)
10-1594	L-3	L-1
15-1605	L-3	L-3
57-797	L-3	L-3
s59	L-2 (late)	L-2 - L-3 (hermaphrodite)
		L-4 (male)
s49	L-1 (early)	L-1 (early)
s65	L-4	L-4

homozygotes or hemizygotes, worms with these mutations blocked at the same stage of development. Worms carrying the mutations, s46, s172, s173 and s171 did show a difference when either in the homozygous or the hemizygous state. Each of the larval mutations (s46, s172, s173) blocked one larval stage earlier, while the adult sterile mutation (s171), behaved as though it was no longer leaky. An examination of these data shows that there is no bias for nullo mutations to be in a particular larval stage.

An unexpected sidelight from testing the lethal and sterile mutations with sDf2 was that the effective lethal phase for s59 appeared to be later in males than in hermaphrodites. This needs to be retested since even the hermaphrodites blocked at a later stage than I had originally characterized the lethal.

The indication that s42 was a putative nullo led me to examine its interaction with sup-5(e1464), a possible informational suppressor (Waterston and Brenner, 1978). One characteristic of sup-5 is its ability to suppress a sub-class of nullo mutations of the unc-54 locus as well as individual alleles of many other genes. To see if it could suppress a lethal

mutation, _s42_ was initially selected. The stock was structured such that the parent was _e1464/+;s7,s42/+,+_. The parents were allowed to lay eggs at 20 degrees C. for 24 hrs. and then at 15 degrees C. for the rest of their egg-laying time. The progeny were examined for adult twitchers. None were found with _s42_. Moreover, two other lethals tested, _s41_ and _s43_, did not yield twitchers either. These three mutations, therefore, do not belong to the sub-class of nullo mutations that are suppressible by _sup-5_.

(iv) _Other screening methods_: Since the objective in isolating these mutations was to obtain mutations in each of the genes in the _unc-22_ region, it was necessary to vary the types of screening procedures employed. Hence, screens were done for maternal lethals, grandchildless lethals and for linked visible mutations. Only one maternal lethal was found in 184 chromosomes examined and no grandchildless mutations were found in 182 tested chromosomes. The maternal lethal has since been lost.

Four visible mutations have been found in the region. Two of these, _s93_ and _s94_, are uncoordinated. They impede the movement of the twitcher but do not

have any obvious effect on the twitch itself. How close they map to the unc-22 gene is unknown. Two other mutations appear to be new phenotypes. One of these is the mutation I call 'spotty'. It is called this because the intestinal cells have black 'pigment-like' granules, either in them, or just on the cell surface. The mutation is recessive and is most easily seen in a twitcher background. The double mutation bearing strain, spotty-twitcher, grows very slowly at 20 degrees C. and appears to be lethal at 13 degrees C. It is unable to lay eggs and, therefore, the progeny hatch internally. Consequently the double mutant strain produces few progeny. As yet the map position of spotty relative to the twitcher locus is unknown. The other new mutation is called 'displaced-vulva'. In the wild-type worm the vulva is placed approximately midway on the body. In worms homozygous for this mutation the vulva is closer to the posterior of the animal. The expression of the phenotype is variable, from a slightly displaced vulva to a worm resembling a pseudomale. It seems that the more posterior the vulva is placed the more the worm resembles a male in the posterior region. The mutation is ts, penetrance is best at 25 degrees C., but it also can be seen in some individuals at either 20 degrees or 13 degrees C. The

mutation maps to a position half-way between unc-22 and unc-5. Because of the dual problem of penetrance and expressivity this positioning should be considered tentative.

A screen using 0.025M EMS in which 3274 chromosomes were examined for visible mutations was done with the deficiency sDf2. With this stock I was able to look for visible mutations in the F1 generation. Two twitchers but no other visible mutations were found. It is too early to tell if this means that there are no visible mutations close to the unc-22 locus. It could mean only that other sites in the area are less sensitive to EMS. Evidence in favour of this view is presented in the General Discussion.

Prior to the commencement of this work four visible mutations were known to map in this region in addition to the unc-22 site. These were mapped relative to the unc-22 gene. The genes unc-43 and unc-31 map to the left of the unc-22 site while unc-30 and unc-26 map to the right. There relative positions are shown on the genetic map in Figure 5.

III: Suppression, dosage effects and gene interaction involving the unc-22 locus.

(i) Suppression by sup-5(e1464)III: Sup-5(e1464) is an informational suppressor that maps on linkage group III in *C. elegans* (Waterston and Brenner, 1978). Its characteristics are that it suppresses specific alleles in a wide variety of genes. It appears to act as a dominant suppressor of some genes and a recessive suppressor of others. No doubt this is related to the function of the gene product of the individual loci. Of 26 unc-22 alleles tested for suppression by sup-5, only s32, an allele isolated in an e1464 background, showed signs of diminished twitching. The sup-5 mutation suppresses homozygous unc-22(s32) only when it is homozygous and at 13 degrees C. The difference in phenotypic expression of the double mutant sup-5; unc-22 is that the worm has a reduced frequency of twitching in 1% nicotine even though, without nicotine, it is a visible twitcher. The suppression is much more visible when one compares the double mutant e1464/e1464; s32/+ to a mutant that is s32/+. Worms of the first genotype have a greatly reduced twitch when compared to worms of the second genotype, even at 20 degrees centigrade.

(ii) Dosage effects at the unc-22 locus: Table 15 shows the results of using various tetraploid, triploid, deficiency and dominant mutation combinations to analyze dosage effects at the unc-22 locus. The dosage effect at this locus is very marked and can be most clearly seen in the diploid s7/+, the deficiency sDf2, and the two triploids, s7/+/+ and s7/s7/+. These data suggest the amount of unc-22 gene product that must be 'inactivated' to give, either a twitch in 1% nicotine or, a visible phenotypic twitch. The breakpoint for a 1% nicotine induced twitch lies between 33% and 50% gene product inactivation, whereas, to obtain a visible twitch requires about 66% gene product inactivation in males and a somewhat higher percentage of inactivation in hermaphrodites. This differential effect according to sex in the s7/s7/+ triploids was unexpected. All the males twitched whereas, only the early larval stages of the hermaphrodites twitched. The adult hermaphrodites did not twitch. This may be a size related phenomenon rather than a sexual one i.e. possibly there is more inertia in the larger adult hermaphrodites for muscle contraction simply because of their bulk, since when they are the same size as the males, they do twitch.

Table 15.

Gene dosage at the unc-22 locus.

Genotype	Normal environment	1% nicotine	Presumed % of protein inactive
+/+	-	-	0
s7/+	-	+	50
s7/s7	+	+	100
s7/+/+	-	+ *	33
sDf2	-	+	50
s7/s7/+	+ (males)	+	66
	+ (young hermaphrodite)		
	- (adult hermaphrodite)		
m52/+	+	+	-
m52/+/+	-	+	-
s32/+	-	+	50
s32/s32	+	+	100
e1464;s32	+	+	- **
e1464;s32/+	-	+ (slow)	- ***

* These worms stop twitching after a few minutes.

** This pair and the following pair were done at 20 degrees C.

***From the efficiency of suppression of unc-54 and unc-15 alleles by e1464 (Waterston and Brenner, 1978), a rough calculation of the amount of normal unc-22 protein present in e1464;s32 and in the e1464;s32/+ worms can be made. This would be about 10% in e1464;s32 and 60% in e1464;s32/+.

On the plates containing the <u>s7/+/+</u> triploid worms most of the worms twitched in nicotine for a few minutes and then stopped as shown in Table 15. These were the worms used to make tetraploid twitchers. Usually diploid worms homozygous or heterozygous for an <u>unc-22</u> mutation twitch in nicotine for hours. A puzzling result was that, on these plates with worms that twitched for a few minutes and stopped, there were always one or two worms that would continue to twitch. These latter worms behaved as if they were <u>s7/+</u>. Since there were so few of them I can only assume that they were the products of an unusual segregation in the tetraploid parent such that, in the cross <u>+/s7</u> males times tetraploid wild-type hermaphrodites, not all the progeny were truly triploid, but some were diploid or at least aneuploid for chromosome IV.

(iii) <u>Gene interaction involving the unc-22 alleles</u>: An important goal of this thesis was to look for mutations that might control the disorder caused by the mutations at the <u>unc-22</u> locus. The approach used was to try and obtain 'revertants' of some of the alleles of the locus and analyze their properties.

Three unc-22 alleles, s8, s18 and s12 were examined for F1 revertants after being treated with 0.05 M EMS. No revertants of s8 were found in 1.8 x 10^5 tested chromosomes, and none were found for s18 in 1.1 x 10^5 tested chromosomes. Allele s12, however, gave six 'revertants' in 7.8 x 10^5 tested chromosomes (Table 16). These revertants had a dual phenotype. They were dominant suppressors of s12 and looked wild-type when heterozygous but, when homozygous, they were slow and stiff except for s75 which had only a weak homozygous visible phenotype. Their suppression of s12 was quite strong since the double mutant strains did not twitch even in 1% nicotine. Again, s75, was the exception because such individuals did twitch in 1% nicotine when a double mutant with s12.

All six 'revertants' segregated independently of unc-22 and were, therefore, not true revertants i.e., they were not second site mutations within the unc-22 gene. These new mutations all mapped to linkage group I and were loosely linked to dpy-5, being separated by about 23 map units. Allelism tests with some of the uncoordinated genes on chromosome I gave an unexpected result. All the unc genes tested complemented except one, unc-54, a gene that codes for a heavy chain of

Table 16.

Screen for revertants at the unc-22 locus.

allele tested	EMS concentration	Chromosomes tested	Number of isolates	Frequency
s8	0.05 M	177,000	0	-
s18	0.05 M	110,000	0	-
s12	0.05 M	780,000	6	1×10^{-5}

myosin (MacLeod et al., 1977), and which maps approximately 25 map units from dpy-5 (Brenner, 1974). One of the new mutations, s74, when heterozygous over unc-54(e190) had a phenotype that resembled the homozygous s74 phenotype. In an initial experiment, about 25,000 chromosomes were examined for a recombinant between s74 and e190, and none were found. Since then a large scale screen of this heteroallele was done. Two crossovers were found in 77 plates. I estimate that between 2×10^3 and 4×10^3 worms were on each plate in this screen. This new mutation, s74, appeared to be an allele of unc-54 and to map relatively close to the allele, e190. Table 17 shows that the other mutations are all allelic to s74.

Animals homozygous for all unc-54 alleles so far described in the literature (Epstein et al., 1974; MacLeod et al., 1977; Waterston and Brenner, 1978) are characterized as being thin, transparent, almost totally paralyzed, unable to lay eggs and incapable of promoting muscle birefringence under polarizing light microscopy. The six new unc-54 alleles, s74, s75, s76, s77, s78 and s95 appear to be quite different. Most animals of such strains are fairly normal in size, none is clear and all are capable of movement to some

Table 17.

Allelism tests of various suppressor mutants with s74.

Heterozygote	fast male	slow male	Phenotype of slow male *
e61,s74/+ +	+	-	all males normal
e61,s74/+,s76	+	+	(++) slow, slightly unc
e61,s74/+,s75	+	+	(+) only slightly slow
e61,s74/+,s78	+	+	(++++) almost stopped
e61,s74/+,s77	+	+	(+++) slow and unc
e61,s74/+,s95	+	+	(+++) slow and unc

* There are two interrelated phenotypes to look for; slowness of movement, and an uncoordinated (unc) movement. The scale + to ++++ refers to slowness, the ++++ being the slowest.

degree, although s78 and s95 animals are almost totally paralyzed. The ability to lay eggs is concomitant with the degree of paralysis, s75 laying many eggs, s78 laying few eggs and s95 laying none. On examining s74 under polarizing optics the muscle appears to be normal, or if not normal, at least laid down within the cell appropriately. Similar to wild-type muscle, repeating anisotropic and isotropic zones arranged longitudinally are observed (see Frontispiece). These are the A bands and dense bodies and the H zones and I bands. Dr. R. H. Waterston (personal communication) has since examined s74 worms under higher magnification (1,000x as compared to my observations at 400x) and states that the A zone is not discrete although one can make out the dense bodies quite clearly. The edge of the A zone appears to be fuzzy which, he says, indicates that the myosin molecules may be splayed out. Even with this degree of disorganization these mutations still have good birefringence. The unc-54 alleles like e190 do not show this organization and have a markedly diminished birefringence (Epstein et al., 1974). It seems these new mutations represent a novel class of myosin mutation where the myosin is sequestered and organized correctly into actin and myosin bundles but has lost a portion of its ability to contract.

An examination of the interaction of these new myosin alleles with various alleles of the unc-22 gene has proved revealing. Most of the work has been done using unc-54(s74) and so the comments will reflect this. Double mutant heterozygotes of s74 and various twitcher alleles show that even one dose of s74 is sufficient to suppress twitching in nicotine of a large number of twitcher alleles as heterozygotes (Table 18). The exception is m52, a dominant allele of unc-22, but note that the suppressor was capable of suppressing the visible twitch in this instance (Table 18).

The manner of suppression of unc-22 alleles by unc-54(s74) is allele specific (see Table 19). Heterozygous doubles of s74 and various unc-22 alleles give progeny with a wide range of phenotypic ratio's between wild-type, what I call rigid-slow and twitcher. These ratio's extend from an 11:4:1 ratio of wild-type : unc : twitcher, an example of dominant suppression; to a 9:4:3 ratio of wild-type : unc : twitcher, which indicates that the suppression is recessive. A ratio intermediate between these two would indicate that the suppression is semi-dominant.

Table 18.

Effect of 1% nicotine on heterozygous mutant individuals.

Genotype tested	Phenotype *	
	wild-type hermaphrodite **	twitch in 1% nicotine ***
+ ; +/s8	+	+
+/s74;+/s8	+	-
+/s74;+/s34	+	-
+/s74;+/s36	+	-
+/s74;+/m52	+	+ ****
+/s74;+/s18	+	-
+/s74;+/s14¹	+	-
+/s74;+/s32	+	-
+/s74;+/s7	+	-

* The test involves looking for wild-type worms on the plate and, when these are found putting them in a 1% nicotine solution to see if they twitch.

** A + sign means wild-type hermaphrodites were present

*** A + sign means the worm twitched in nicotine.

**** A jerking twitch that seemed to be somewhat slower.

Table 19.

Suppression of unc-22 alleles by s74.

Parent genotype		Phenotypic ratio in F1			Type of suppression
		wild	s74 (unc)	twitcher	
s74/+;s12/+		11	4	1	dominant
s74/+;s8/+	*	11	4	1	dominant to
		9	4	3	semi-dominant
s74/+;s7/+	*	11	4	1	dominant to
		9	4	3	semi-dominant
s74/+;s18/+	*	11	4	1	dominant to
		9	4	3	semi-dominant
s74/+;s36/+	*	11	4	1	semi-dominant
		9	4	3	
s74/+;s34/+	*	11	4	1	semi-dominant
		9	4	3	
s74/+;s14/+		9	4	3	recessive
s74/+;s32/+		9	4	3	recessive
s74/+;m52/+	**	9	4	3	dominant to
		8	4	4	semi-dominant

* All these pairs fit a 10 : 4 : 2 ratio which is an impossible configuration.

** The allele m52 is a dominant allele of unc-22. The 8 : 4 : 4 ratio shown here is not possible.

The way this suppression works is illustrated in the series of Punnett squares (Figures 7,8,9 and 10). Dominant suppression allowed only 1/16 of the progeny to be twitchers since only that proportion of the progeny were homozygous for the twitcher mutation and also homozygous for the wild-type allele of unc-54. The suppression of s12 was of this type (Figure 7). To confirm that this was the case, several s12 twitchers from the double mutant were progeny tested. None of the twitchers gave progeny that were suppressed. Recessive suppression means that s74 stopped the twitching induced by the unc-22 locus only when it was homozygous, and, therefore, s74 inhibited only 1/4 of the twitchers, still leaving 3/16 of the total progeny as twitchers. The interaction of s74 and s32 or s14 was an example of this type of suppression (see Figure 8). Several s32 twitchers were progeny tested. Some of these twitchers had progeny that were suppressed. The unc-22 alleles, s7, s8, s18, s34 and s36 did not fit either an 11:4:1 ratio nor a 9:4:3 ratio, but instead exhibited intermediate ratio's between these. This was most probably due to the fact that in these cases s74 behaved as a semi-dominant suppressor. This suggested that it was a 'leaky' dominant suppressor i.e. some of the twitchers that were 'supposed' to be suppressed

	s74 ; s12	s74 ; +	+ ; s12	+ ; +
s74 ; s12	$\frac{s74}{s74};\frac{s12}{s12}$ sup	$\frac{s74}{s74};\frac{+}{s12}$ sup	$\frac{+}{s74};\frac{s12}{s12}$ wild	$\frac{+}{s74};\frac{+}{s12}$ wild
s74 ; +	$\frac{s74}{s74};\frac{s12}{+}$ sup	$\frac{s74}{s74};\frac{+}{+}$ sup	$\frac{+}{s74};\frac{s12}{+}$ wild	$\frac{+}{s74};\frac{+}{+}$ wild
+ ; s12	$\frac{s74}{+};\frac{s12}{s12}$ wild	$\frac{s74}{+};\frac{+}{s12}$ wild	$\frac{+}{+};\frac{s12}{s12}$ twi	$\frac{+}{+};\frac{+}{s12}$ wild
+ ; +	$\frac{s74}{+};\frac{s12}{+}$ wild	$\frac{s74}{+};\frac{+}{+}$ wild	$\frac{+}{+};\frac{s12}{+}$ wild	$\frac{+}{+};\frac{+}{+}$ wild

Figure 7. Punnett square of *s12* and *s74* interaction.

	s74 ; s32	s74 ; +	+ ; s32	+ ; +
s74 ; s32	s74/s74 ; s32/s32 sup	s74/s74 ; +/s32 sup	+/s74 ; s32/s32 twi	+/s74 ; +/s32 wild
s74 ; +	s74/s74 ; s32/+ sup	s74/s74 ; +/+ sup	+/s74 ; s32/+ wild	+/s74 ; +/+ wild
+ ; s32	s74/+ ; s32/s32 twi	s74/+ ; +/s32 wild	+/+ ; s32/s32 twi	+/+ ; +/s32 wild
+ ; +	s74/+ ; s32/+ wild	s74/+ ; +/+ wild	+/+ ; s32/+ wild	+/+ ; +/+ wild

Figure 8. Punnett square of s32 or s14 and s74 interaction.

actually 'leaked' through and appeared as twitchers (see Table 19 and Figure 9). The number of twitchers was a measure of the ability of the s74 mutation to suppress a particular unc-22 allele. The raw data indicated that s74 suppresses s8 fairly well but that it suppressed s34 poorly as a dominant.

The interaction of s74 and m52, the dominant twitcher, gives almost the same ratio of progeny in the F1 from a double heterozygote as recessive suppression does, but for a totally different reason (Table 19). The Punnett square in Figure 10 illustrates what is occurring. In the F1 progeny from the double heterozygote 3/16 are obviously twitchers. An extra 1/16 is necessary to fit an 8:4:4 ratio. Again, the easiest assumption is that the s74/+;m52/m52 group is leaky such that a few are scored as twitchers. This seems to be what has happened, since of five twitchers that I progeny tested, one gave slow-rigid progeny as well as twitchers. These slow and rigid worms produced progeny that were phenotypically similar to themselves in the next generation. It would seem that, even when heterozygous, s74 is a fairly effective suppressor of a homozygous dominant twitcher.

	s74 ; s7	s74 ; +	+ ; s7	+ ; +
s74 ; s7	s74/s74 ; s7/s7 sup	s74/s74 ; +/s7 sup	+/s74 ; s7/s7 wild-twi	+/s74 ; +/s7 wild
s74 ; +	s74/s74 ; s7/+ sup	s74/s74 ; +/+ sup	+/s74 ; s7/+ wild	+/s74 ; +/+ wild
+ ; s7	s74/+ ; s7/s7 wild-twi	s74/+ ; +/s7 wild	+/+ ; s7/s7 twi	+/+ ; +/s7 wild
+ ; +	s74/+ ; s7/+ wild	s74/+ ; +/+ wild	+/+ ; s7/+ wild	+/+ ; +/+ wild

Figure 9. Punnett square of s7, s8, s18, s34 or s36 and s74 interaction.

	s74 ; m52	s74 ; +	+ ; m52	+ ; +
s74 ; m52	$\frac{s74}{s74}$; $\frac{m52}{m52}$ sup	$\frac{s74}{s74}$; $\frac{+}{m52}$ sup	$\frac{+}{s74}$; $\frac{m52}{m52}$ wild-twi	$\frac{+}{s74}$; $\frac{+}{m52}$ wild
s74 ; +	$\frac{s74}{s74}$; $\frac{m52}{+}$ sup	$\frac{s74}{s74}$; $\frac{+}{+}$ sup	$\frac{+}{s74}$; $\frac{m52}{+}$ wild	$\frac{+}{s74}$; $\frac{+}{+}$ wild
+ ; m52	$\frac{s74}{+}$; $\frac{m52}{m52}$ wild-twi	$\frac{s74}{+}$; $\frac{+}{m52}$ wild	$\frac{+}{+}$; $\frac{m52}{m52}$ twi	$\frac{+}{+}$; $\frac{+}{m52}$ twi
+ ; +	$\frac{s74}{+}$; $\frac{m52}{+}$ wild	$\frac{s74}{+}$; $\frac{+}{+}$ wild	$\frac{+}{+}$; $\frac{m52}{+}$ twi	$\frac{+}{+}$; $\frac{+}{+}$ wild

Figure 10. Punnett square of m52 and s74 interaction.

By using the *unc-54(s74)* mutation, it is possible to separate various *unc-22* alleles according to their response to suppression. The suppression by *s74* of *s12* and *s14* corresponds well with the birefringence patterns exhibited by individuals bearing these two mutations (see Figure 11). The allele, *s12*, for example, causes very little disorder in muscle structure while *s14* animals (Figure 11) appear quite disorganized. The myosin mutant, *s74*, is able to suppress the twitching and to reestablish normal birefringence. Individuals carrying the double mutants *s74;s12*, *s78;s12*, *s95;s12* and *s74;s32* all have near normal birefringence. The degree of suppression (*s12*>*s7*≥*s8*≥*s18*>*s36*≥*s34*>*s14*≥*s32*), does not seem to bear any relationship to the map position of the *unc-22* alleles, i.e., suppression does not seem to be polar. This can be illustrated by noting that *s12* and *s32* are at opposite ends of the the suppression spectrum but map next to each other (see Figure 3).

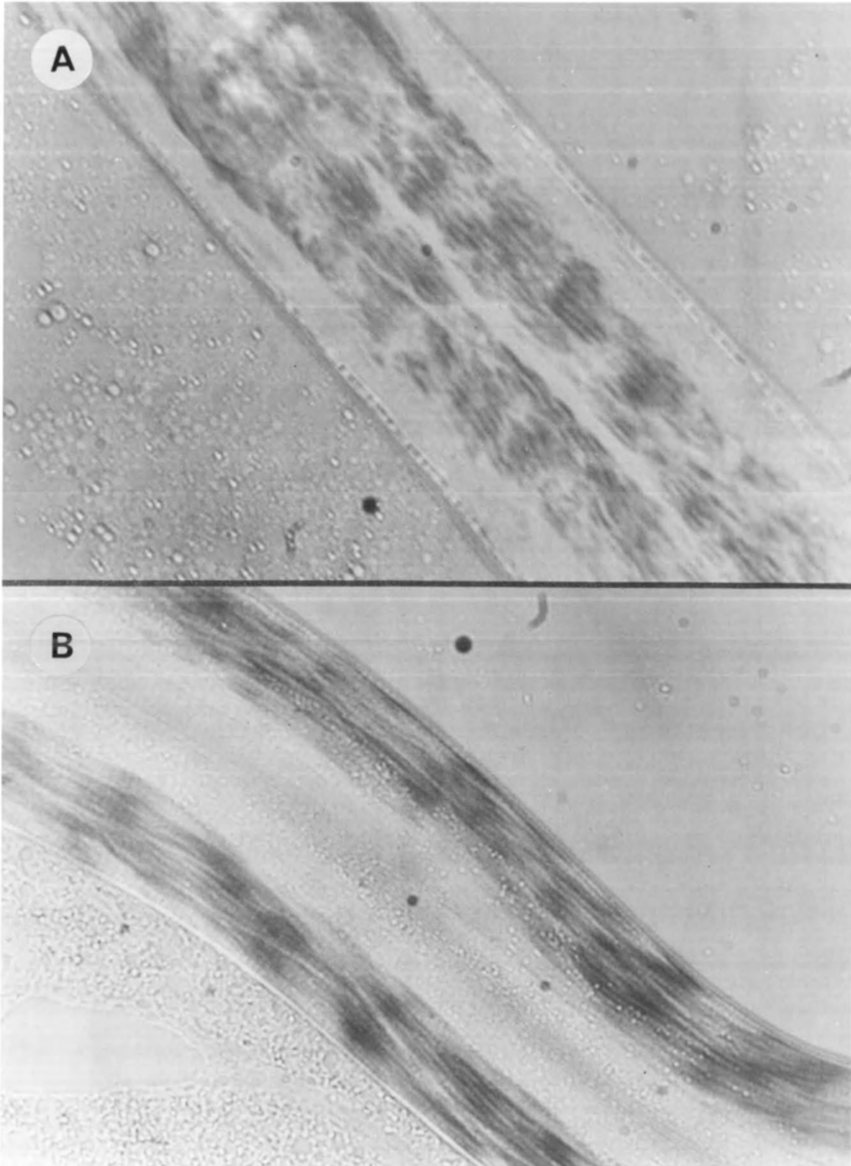
Since these new alleles affecting myosin have a range of homozygous phenotypes, and the *s74* strain appears to be in about the mid-range in severity of paralysis, I suspect that the other novel alleles of *unc-54* will have their own specific pattern of twitcher

Figure 11. Polarized light microscopy pictures of *unc-22(s12)* and *unc-22(s14)*.

a) *s14*

b) *s12*

Magnification 400x


A
B

suppression. In particular, the two extreme alleles for paralysis, _s75_ and _s95_ are probably worth examining. Preliminary experiments on _s77_ and _s78_ tend to support this idea (Table 20). The data are confounded by the fact that one is examining twitchers that are heterozygous for two different _unc-22_ mutations, but I believe the point to be valid.

Worms homozygous for the allele _s77_ phenotypically resemble _s74_ worms while _s78_ individuals are the second most severely paralyzed worms of the group. The crosses shown in Table 20 gave outcross F1 progeny with four genotypes as shown in Figure 12. Since self-crossed twitcher hermaphrodites could not be separated from outcrossed twitcher hermaphrodites only male progeny were scored. A 1:1 ratio of wild-type to twitcher progeny indicated no dominant suppression, a 2:1 ratio indicated semi-dominant suppression and a 3:1 ratio indicated that suppression was dominant. None of the combinations showed recessive suppression. The patterns of suppression illustrated by the two _unc-54_ alleles was different, with _s78_ being a more effective suppressor of alleles _s14_, _s8_ and _s35_. The _unc-54_ allele, _s77_, appeared in most cases to act as a semi-dominant suppressor while _s78_ behaved as a

Table 20.

Suppression by unc-54(s77) and unc-54(s78) of various unc-22 alleles.

Genotype tested			Number of males in F1 *	
			wild-type	twitcher
s77/+;s12/+	x	s14	36 (2-3)	14 (1) **
s78/+;s12/+	x	s14	58 (3)	17 (1)
s77/+;s12/+	x	s8	25 (2)	14 (1)
s78/+;s12/+	x	s8	60 (3)	17 (1)
s77/+;s12/+	x	s18	54 (3)	18 (1)
s78/+;s12/+	x	s18	38 (3)	12 (1)
s77/+;s12/+	x	s32	68 (2)	31 (1)
s78/+;s12/+	x	s32	42 (2)	20 (1)
s77/+;s12/+	x	s35	32 (2)	15 (1)
s78/+;s12/+	x	s35	39 (3)	13 (1)
s77/+;s12/+	x	m52	49	61

* A 1:1 ratio indicates no dominant supppression. A 3:1 ratio indicates that the suppresion is dominant. This is not the case for m52 a dominant allele of unc-22.

** Numbers in ( ) indicate ratio.

Po ♂ $\frac{\text{unc-54(s78)}}{+}$; $\frac{\text{unc-22(s12)}}{+}$ X $\frac{+}{+}$; $\frac{\text{unc-22(sY)}}{\text{unc-22(sY)}}$ ⚥

F1 ♂ $\frac{\text{unc-54(s78)}}{+}$; $\frac{\text{unc-22(s12)}}{\text{unc-22(sY)}}$

♂ $\frac{\text{unc-54(s78)}}{+}$; $\frac{+}{\text{unc-22(sY)}}$

♂ $\frac{+}{+}$; $\frac{\text{unc-22(s12)}}{\text{unc-22(sY)}}$

♂ $\frac{+}{+}$; $\frac{+}{\text{unc-22(sY)}}$

recessive suppression = 1 : 1 wild : twi

dominant suppression = 3 : 1 wild : twi

Figure 12. Protocol for testing suppression by <u>unc-54</u> alleles of a heteroallelic mutant <u>unc-22</u>.

dominant suppressor in the majority of cases. Also of importance was the observation that s32 was the only allele not suppressed dominantly by s78; when heterozygous with s12, s32 was suppressed in a semi-dominant fashion. The allele, s32, was the one that s74 was the least successful in suppressing. It should be recalled that s32 is the suspected nullo allele.

The technique of testing different unc-54 alleles for their ability to suppress unc-22 alleles allows for an even finer discrimination between the twitcher alleles. For example, s74 grouped s14 and s32 together but the data from s78 suppression experiments suggest that s32 is a more severe mutation than s14. It should be obvious that this technique not only allows for the ranking of twitcher mutations according to their severity but also allows for the quantifying of the severity of unc-54 mutations.

IV: A phenotypic characterization of mutations at the unc-22 locus and further experiments on gene interaction.

The role of the unc-22 gene in the ontogeny of *C. elegans* is unknown. To gain some understanding of the function of this gene on linkage group IV, several observations have been made. The problem has been approached from a number of different directions; genetic, morphological, physiological and biochemical. The majority of the mutations at this locus are recessive, but there is one allele, m52, which is dominant (isolated by Dr. D. Riddle). Worms homozygous for an unc-22 mutation are characterized by a twitching pattern that extends along the body-wall musculature; hence these worms are referred to as 'twitchers'. Compared to N-2-S worms, these worms are thinner, move slower, have fewer progeny (N-2-S=280; s7=200; s32=180) and have an extended developmental time (Figure 6). A particularly useful characteristic of the unc-22 mutations is that they twitch in a 1% solution of nicotine as heterozygotes. This has allowed for a number of manipulations involving this gene that otherwise would not have been possible.

The twitching pattern of worms with an unc-22 mutation make it distinct and was one reason why it was chosen for this study. Since commencing this study a second 'twitcher' has been found. This is the gene designated lev-11 which maps on chromosome I near unc-54 (Dr. J. Lewis, personal communication). The single mutation known for this gene produces a weak visible twitch when homozygous, but as a heterozygote does not twitch at all in 1% nicotine. This may explain why I failed to recover any alleles of this gene since most of the unc-22 alleles were isolated using 1% nicotine in the F1 generation after EMS mutagenesis. Heterozygous double mutants of lev-11 and various unc-22 alleles failed to show any obvious signs of gene interaction.

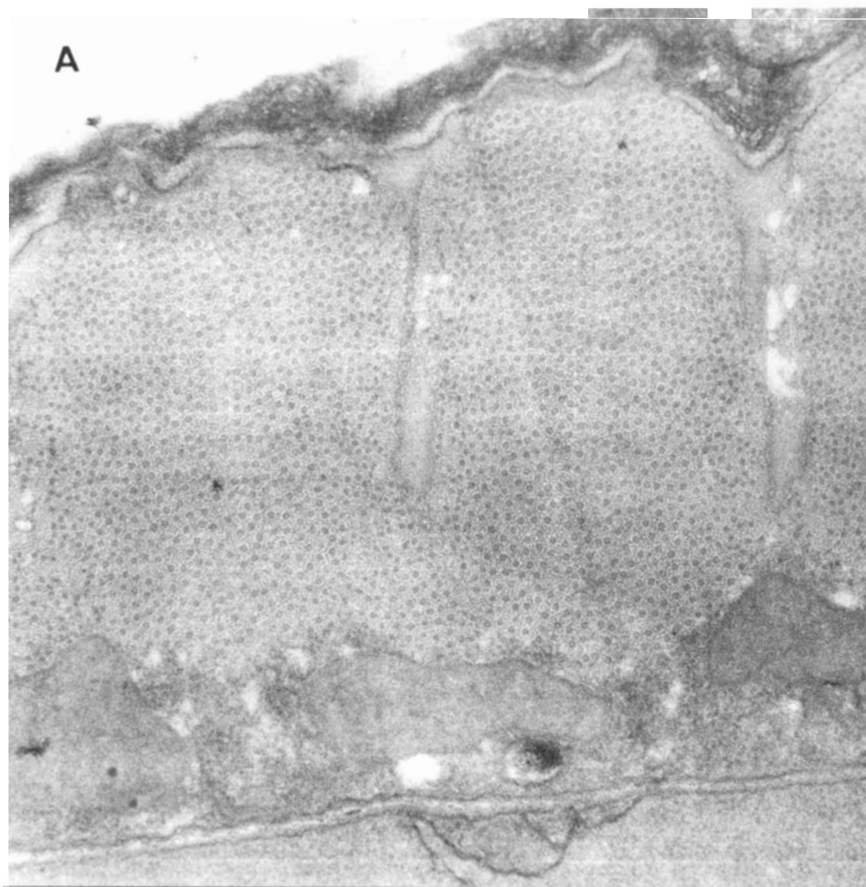
An electron microscopy (EM) study of the unc-22 alleles s7 and s32 was done (Dr. D. Popham, personal communication). The EM showed that the muscle in the adult twitcher was very disorganized, with clumps of actin and myosin in different parts of the cell. The muscle cell boundary was irregular, mitochondria were blown out and filaments were running at irregular angles (see Figure 13). Birefringence studies also indicated that the muscle was abnormal (Figure 11).

Figure 13. Electron microscopy pictures of N-2-S and unc-22(s7) in cross-section.

a) N-2-S

b) unc-22(s7)

Magnification 21,000x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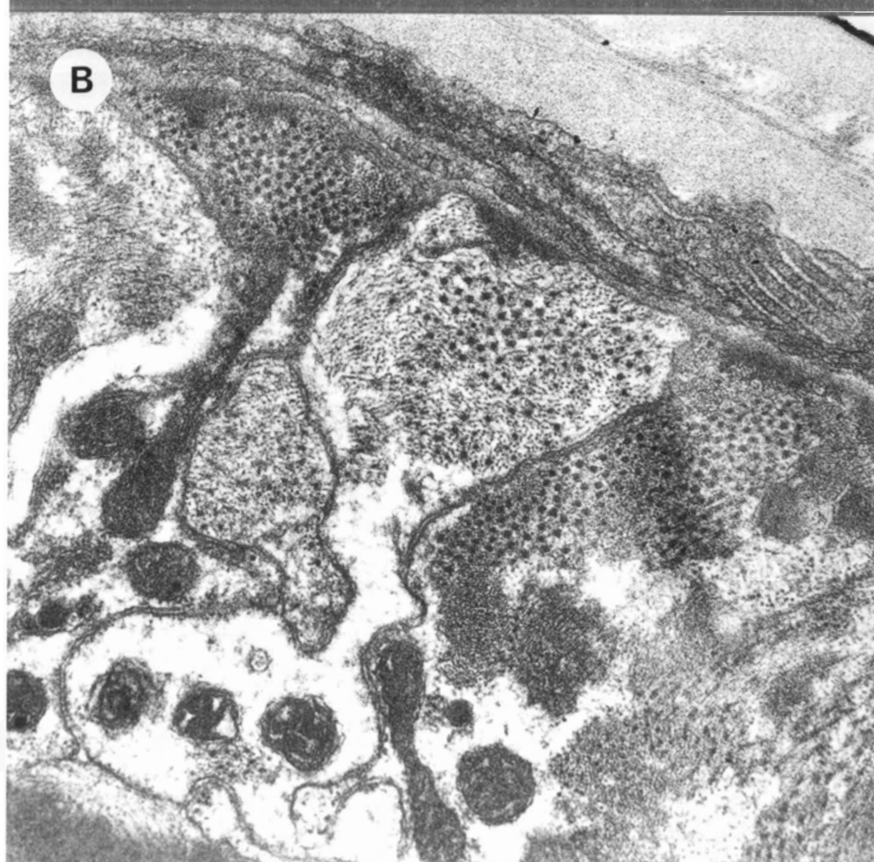
B

Under polarizing optics the muscle was thinner than wild-type muscle, and disorganized patches were seen along the entire length of the body. A qualitative separation of the various groups was made based on their degree of disorganization (s12 and s14 being near the extremes, see Figure 11).

The unc-22 mutations cause muscle twitching. This could be done via the nervous system or it could be due to a disorder in the muscle itself. The following two observations I believe make it impossible to consider the unc-22 mutations as a neural problem. The first observation is related to the effect of nicotine. Nicotine is a mimic of acetylcholine (Ach) in vertebrate nervous systems and causes contraction of skeletal muscle (see Albers, 1972, for an overview). The nematode seems to have Ach, or at least the enzyme, choline acetyltransferase, which is necessary to make it (Mr. Jim Rand and Dr. R. Russell, personal communication). As well, the addition of Ach causes the contraction of the body-wall musculature in cut worms (cited in Suston et al., 1976). Ach seems to act the same way in nematodes as in vertebrates. Nicotine causes a rigid paralysis in an N-2-S worm. It does the same in a twitcher, but the difference is that it still continues to twitch over and above the paralysis.

The second observation comes from a cut worm assay. If an N-2-S worm is cut into three pieces, with one incision being behind the pharynx and the other being posterior to the vulva, all the pieces will go flaccid and will not move. If a twitcher is cut in the same fashion each of the pieces will continue to twitch for at least several minutes. Each piece seems to be autonomous, not needing any central nervous system input. The twitching also does not seem to be the result of a contraction having been initiated in one muscle cell and then spreading to the others. This possibility might have been suspected since Debell et al. (1963) showed that *Ascaris lumbricoides* muscle cells are electrically coupled and White et al. (1976) have suggested that *C. elegans* muscle cells are the same.

These observations would argue that whatever causes the twitch, it is something other than the neural input, i.e., that it is 'downstream' from the neurotransmitter/muscle receptor protein junction. However, neural input does seem capable of eliciting an effect. 'Overstimulation' i.e. adding 1% nicotine can somehow stress the system such that a worm heterozygous

for an <u>unc-22</u> mutation will twitch. A clearer example of this can be seen when the nicotine is increased to the very high level of 5%. Even <u>N-2-S</u> worms vibrate. The vibration looks phenotypically similar to a twitch. A recent result obtained by Dr. J. Lewis (personal communication) is of interest here. He exposed worms to oubain, a cardiac glycoside which inhibits active sodium and potassium transport. He found that wild-type worms become paralyzed but that twitchers keep on twitching while in the chemical. This again suggests that the <u>unc-22</u> disorder is localized to the muscle cell.

The <u>unc-22</u> induced twitch is influenced by the contractile apparatus, most notably by the <u>unc-54</u> gene product, myosin (MacLeod et al., 1977). A double mutant of <u>unc-22(s7)</u> and <u>unc-15(e73)</u>, the gene for paramycsin (Waterston et al., 1977) appears paralyzed, but on closer examinatin one can see a slight surface twitch. This can be observed more easily if the worm is put in 1% nicotine. An <u>unc-22;unc-54(e190)</u> worm is totally paralyzed. There is no twitch, neither in 1% nicotine, nor in higher doses. The importance of the <u>unc-54</u> gene product for twitching has been demonstrated with the discovery of the novel alleles of myosin (see Results III).

There are at least three genes coding for a myosin heavy chain in the nematode (Epstein et al., 1974; and Dr. R. H. Waterston, personal communication), unc-54 and one other gene code for a 210,000 molecular weight polypeptide, while a third gene codes for a 206,000 molecular weight polypeptide. The unc-54 gene product is found along with the other 210,000 molecular weight myosin in the body-wall muscle, unc-54 producing about 75% of the heavy chain myosin. The 206,000 molecular weight myosin is found only in the pharynx while the other 210,000 molecular weight myosin is found in the body-wall muscle and in the pharyngeal muscle (Epstein et al., 1974; MacLeod et al., 1977; Schachat et al., 1977; Mackenzie et al., 1978). Since at least two myosin heavy chains are found in a body-wall muscle cell the question arises whether the twitch could be 'rerouted' through the other body-wall myosin. The aforementioned data suggest that it cannot be, but since this minor body-wall myosin forms only 25% of the total myosin in the cell, perhaps there is just not enough of it to generate a visible twitch. After all, homozygous unc-54(e190) worms are unable to move as adults even with 25% of their body-wall myosin intact.

An experiment to examine whether the twitch can be rerouted through the other body-wall myosin has been done using an indirect suppresor of unc-54 and unc-15 mutations, sup-3, which is located on chromosome V (Riddle and Brenner, 1978). The mode of suppression of sup-3 appears to be by causing an increase in the amount of the minor body-wall myosin. Riddle and Brenner (1978) suggested this and Dr. R. H. Waterston (personal communication) has shown that sup-3 worms have at least twice as much of the minor body-wall myosin. With this information an obvious question is, what does a worm of the genotype e190;s7;sup-3(e1407) look like? To be more specific, can it twitch? The answer is that the worm does have a very slight surface twitch which can be enhanced if the worm is put in 1% nicotine. Even then it is a rather weak twitch. The worms develop slowly and lay only a few eggs, the majority of which hatch internally.

The twitch can, therefore, be rerouted through the other body-wall myosin. The reason that the twitch is so weak is not clear but is most probably because the sup-3 mutation does not return the body-wall myosin levels to that of a wild-type worm. A worm with the genotype unc-15(e73);unc-22(s7);sup-5(e1405)/+ was also

examined for its ability to twitch and, as expected, it was a much better twitcher than an e73;s7 worm and was even capable of laying many eggs. Several lines of evidence converge to suggest that the discorder caused by the unc-22 mutations is related to muscle structure and function. To determine the role of this gene, its protein product must be isolated. Some preliminary one-dimentional sodium dodecyl-sulphate (SDS) polyacrylamide gels, which separate proteins according to their molecular weight, have been run on s32, a putative nullo allele of the unc-22 gene (Dr. C. Kreis, personal communication). At present no demonstrative difference can be seen in the proteins on gels of s32 and gels of N-2-S.

General Discussion.

(i) The size of the unc-22 gene: A preliminary estimate of the unc-22 gene size, based on the two outermost alleles, s16 and e66, is 1 to 2 x 10^{-2} map units. This is the first reported recombination map for any gene in C. elegans. Two other genes are presently being mapped, unc-54 and unc-15 (Dr. R. H. Waterston, personal communication; Ms. A. Rose and Dr. D. L. Baillie, personal communication), with estimates of their gene size ranging from 10^{-2} to 10^{-3} map units. It is too early to tell if the recombination size of these genes is comparable to that of unc-22. From the data of Sulston and Brenner (1974) on the amount of unique DNA in the worm, 6.7 x 10^{7} base pairs, and the total distance for the genome of 320 map units (Brenner, 1974; Riddle, 1978), I have calculated that the unc-22 gene contains approximately 2 to 4 kb of DNA. If this whole region codes for the structural element then that is enough DNA to code for a protein of a molecular weight between 70,000 and 140,000.

This calculation should be treated with caution since there is an inherent assumption here that DNA length and mapping distances have a direct correlation.

This is an oversimplification for there are a number of difficulties in equating mapping units with lengths of DNA or protein. This results from two aspects of the structure of the genome, one being the position of the gene along the length of the chromosome, and the other being, for lack of a better phraseology, the nuclectide composition of the gene. In the first case I am referring to the 'centromere effect' (Beadle 1932; Thompson, 1964) whereby, in D. melanogaster, genes more proximal to the centromere have been found to have a reduced rate of exchange when compared with that of more distal genes. This phenomenon also appears to take place at the tip of the chromosomes, in the telomeric region, where excess DNA per map distance has been found (Rudkin, 1965). As a consequence of this effect, genes that appear clustered on the genetic map around the centromère, or at the telcmere, are physically much further apart along the actual chromosome. This can be seen on a polytene map (see Lefevre 1976, page 58, Figure 21). What this means is that 0.01 map units does not contain the same amount of DNA in all parts of the chromoscme.

In C. elegans the chromosomal location of the centromeres is unknown, and, therefore, whether the

unc-22 locus is similar in recombination frequency to an area that is proximal to the centromere or to an area that is distal to it is inknown. If my statement about genetic maps is true, then there is a suggestion as to the location of the unc-22 gene relative to the centromere. A comparison of the linkage map of *C. elegans* (Figure 1) with that of *D. melanogaster* (see Herskowitz, 1965, page 185, Figure 13-4) illustrates an interesting similarity, this being a clustering of mutants in specific regions. In *Drosophila* the clusters are at the centromere and the telomeres with a string of sites joining these, and in the worm you see the same phenomena. This is particularly obvious on chromosome I of the worm, and suggests that the center of the clusters may be the site of the centromere (as originally proposed be Brenner, 1974). A caveat here is that, since Herman et al. (1976) have obtained free duplications in the worm, there is the possibility that the chromosomes have diffuse centromeres. If this is the case then it is impossible to know what to predict in regards to DNA content and map distances at this time.

If the chromosomes of the nematode do have localized centromeres, the position of unc-22 at the

right-hand edge of the cluster on linkage group IV puts it well away from the centromere and into the euchromatic portion of the chromosome. Rudkin (1965) has shown that in Drosophila this region of the chromosome shows a very good correlation between DNA content and mapping distance. Data from procaryotes also shows that the probability of crossing-over is about the same along the DNA molecule. Watson (1976) uses a very nice example to illustrate this point, a comparison of the genetic map of the phage lambda generated by Amati and Messelson (1965) with a physical map of the genome produced by Szybalski (cited in Watson, 1976). The correspondence between points on the two maps is amazingly accurate.

With these examples of close correlation between crossing-over and physical distance, is there really a need for caution in using the general calculation employed at the beginning of this discussion? I believe there is because, up to this point I have only been discussing the problems with correlating DNA content and crossover frequency at a medium level of resolution, but if one examines recombination at the level of individual nucleotides several phenomena occur which can have a drastic effect on the relationship

between DNA length and mapping distance. These observations are summarized below.

First, Ronen and Salts (1971) found that recombination rates for the separation of adjacent bases vary by at least three orders of magnitude in different parts of the rII cistron of bacteriophage T4. Second, they found that even their highest rate of crossing-over between adjacent bases was two orders of magnitude less than was predicted from experiments measuring average genetic distance. If there had not been other examples of this behaviour, these findings could be dismissed as being a special property of recombination between adjacent nucleotides. But there are other examples, such as the work of Norkin (1970) on marker specific effects in the lac operon of E. coli and the work of Moore and Sherman (1974, 1977) on recombination in the iso-1-cytochrome c gene of yeast. They too have found that physical distances are of secondary importance when examining recombination within a gene. What emerges from this work is the knowledge that the recombination distance within a gene is not strictly a function of the physical distance separating two markers, but that the ability of each marker to recombine is a function of its neighbouring

nucleotides, and that the type of base change within the codon itself can have a dramatic effect on the DNA's ability to recombine.

From the aforementioned and adding to it statistical considerations I conclude that the estimate obtained on the amount of DNA within the *unc-22* locus is but an approximation. A further complication ensues when trying to estimate the molecular weight of the *unc-22* gene product. This arises because the structural and regulatory regions within the *unc-22* gene have not yet been delineated. At present we know only that the right portion of the gene is part of the coding element since *s32* maps into this region and it is suppressed by *sup-5*, the informational supressor. Of course the entire region may code for the structural element, and any cis-linked regulatory sites could be outside of this area, as was discovered at the *rosy* locus in *D. melanogaster* (Chovnick et al., 1976). I suspect this may be the case for the following reason: If one takes 0.01 map units as an average gene size and divides the total 320 map units by this number, one would estimate that this organism has 32,000 genes. This is a much higher number than Brenner's (1974) estimate of 2,000 genes or Baillie's (unpublished results) of 4,000

genes, which are based on forward mutation rates. If, however, this whole region were structural and the regulatory sites were outside of it, then the number of genes would be considerably less. Furthermore, the discovery that many genes of eucaryotes have inserts of stretches of DNA that are not translated within the structural element of the gene has demonstrated that there is no simple correlation between a DNA region, the primary transcript and the final protein product (see Crick, 1979, for a review).

An alternative method of obtaining an estimate of the unc-22 gene size is to use the approach of target theory. Simply stated the target theory assumes that the larger the mutational target, the more easily it will mutate. Forward mutation rates between various genes can be compared and the genes can be ranked in size; those with the higher mutation rates being the larger genes. By this criteria the unc-22 gene appears larger than the average gene in the nematode. I found that the forward mutation rate for this gene using 0.025M EMS was 1 per 1600 tested chromosomes. Since Brenner (1974) estimated the average forward mutation frequency for a gene in *C. elegans* using 0.05M EMS at 1 per 2,000 tested chromosomes, my data suggest the

unc-22 gene is more easily mutated than the average gene.

Evidence from another approach confirms this. J. Zengel and H. Epstein (personal communication from Dr. J. Zengel) found that, among 112 EMS induced muscle-defective mutants they isolated, there were 35 (31%) which were allelic to the unc-22 gene. All other genes, except one, were represented by less than 7 hits. In fact most genes had only one or no hits at all. A particularly interesting result was that the one exception to this hit frequency, the unc-54 gene, was as sensitive to EMS mutagenesis as the unc-22 gene (again, 35 unc-54 mutants in their sample which is 31% of the total). The unc-54 gene is a very large gene, coding for a 210,000 molecular weight myosin heavy chain (Epstein et al., 1974; MacLeod et al., 1977). A measure of the absolute forward mutation rate for the unc-22 locus, and a comparison of relative forward mutation rates among a number of loci suggest that the unc-22 gene is larger than the average gene. In fact, if the comparison with unc-54 is justified it is a very large gene, coding for a protein with a molecular weight of as much as 200,000 daltons.

But are the above statements justified? Target theory makes the assumption that all factors other than gene size are equal and the mutational hit frequency is strictly a response to the number of base pairs in the target, the more base pairs the higher the probability of it being hit. This is not the true situation. Several factors will vary between genes, the most important of these being the GC to AT ratio within the gene. This is important because EMS has a base specificity, causing primarily GC to AT transitions (Coulondre and Miller, 1977). A gene of low GC content will obviously have a reduced forward mutation rate with EMS. Genetic 'hotspots' within a gene will also bias the data (Benzer, 1961). Miller and his co-workers (see Miller, 1978) have demonstrated that small 4 nucleotide tandem repeats within a gene can act as mutational hotspots. Similarly, Coulondre et al. (1978) have shown that in the _lacI_ gene of _E. coli_, 5-methylcytosine is responsible for transition hotspots. This is an important observation when you consider that in many eucaryotes 5-methylcytosine comprises 5% of the cytosine in the DNA (data taken from Davidson's 'The Biochemistry of Nucleic Acids' 8 edition, 1976). On the other hand, from the limited data available, neither the _unc-22_ nor the _unc-54_

intragenic maps demonstrate evidence of mutational hotspots. Another criticism is that the protein itself may be insensitive to amino acid changes. A final criticism of the target theory is that since the higher order organization of DNA in the cell is unknown, we cannot be certain all genes are equally accessible to the mutagen.

(ii) The resolving power of genetic fine structure analysis in the nematode: Bearing in mind the criticisms I have stated in the previous section, one can use the value of 2 kb of DNA per 0.01 map unit to make an estimate of the resolving power of the mapping procedures used in this work. The two closest alleles so far resolved are s16 and s8. They are about 7.6×10^{-4} map units apart. A first order approximation of their physical separation is 160 nucleotides. It should be emphasized that 160 nucleotides is not the limit of resolution of this system and that it is quite possible to extend the resolution to at least 40 nucleotides simply by increasing the number of nematodes in the sample. This would involve the screening of 10^6 worms, a number well within the practical limits of this system. To increase the resolution further I believe it is necessary to vary

some of the present experimental conditions. Several possible manipulations are feasible. At present fine structure mapping is done at 20 degrees C. but it is known that increasing the temperature to 26 degrees C. will almost double the cross-over frequency in the nematode (Rose and Baillie, 1979). This same effect has been observed in *Drosophila* (Plough, 1917). Doing fine structure experiments at 26 degrees C. should increase the resolution a further two-fold.

It would be useful if a selective system whereby worms homozygous for either allele being examined die or are at least slowed down in their development sufficiently to minimize their contribution to the F2 generation. The mutation *unc-43*, which maps 1.6 map units to the left of *unc-22*, can be used in this way. As a double mutant with *unc-22* it has a particularly severe phenotype and grows very slowly.

Lethal and conditional lethal mutations can be used but their usefulness depends on the order of the alleles being tested. For example, by using the *unc-5(e152)* stock that carries a ts egg lethal that maps to the left of *unc-22*, one can make the following *unc-22* heterozygote,

unc-5(e152),ts?,unc-22(sx),dpy-4(e1166)/unc-43(e266),unc-22(sy). If the recombination experiment is done at 26 degrees C. both of the unc-22 homozygous alleles are effectively eliminated from contributing progeny to the F2 generation. Increasing the sample size in this manner increases the resolution a further two-fold, if sx is to the left of sy. If sx is to the right of sy then all wild-type cross-over chromatids for unc-22 will carry the ts lethal and if they segregate with the chromatid containing e152,ts?,sx, e1166 then that worm will die and the event will not be recorded. A ts maternal lethal would allow time to rescue the recombinant and shift it down to 13 degrees C. This way the recombinant could be seen and also progeny tested. Preliminary results indicate the unc-5 linked lethal mutation is a maternal lethal.

Another possibility for increasing the resolution is to use the recombination enhancer (Rose and Baillie, 1979). Stocks homozygous for the enhancer have an increased recombination frequency. Fine structure mapping could be done in the enhancer background. Unfortunately, it is not known if the enhancer increases intragenic recombination as well as intergenic recombination. If it does, in combination

with increased temperature and a 'balanced' lethal system the resolution of this system could be increased by an order of magnitude.

Another possibility would be to use the stock sDf1. Deletion mapping within a gene is a powerful tool and was very important in fine structure mapping in procaryotes and bacteriophage, most notably in the rII region of bacteriophage T4, and also in the tryptophan and lactose operons of E. coli (Benzer, 1959,1961; Nomura and Benzer, 1961; Yanofsky et al., 1964; Beckwith, 1978). This technique has been used little, however, in eucaryotes. This is primarily because intragenic deficiencies are not only difficult to obtain but also difficult to characterize. The exception to this is yeast where deletion mapping has been used extensively, most notably in positioning mutations in the HIS 4 complex (Fink and Styles, 1974). Deletions have been put to limited use in Drosophila to analyze the organization of the complex loci, white, notch and the achaete-scute system. Judd (1961) with the aid of a deficiency was able to split the white complex into two functional parts, and notch has been localized to salivary band, 3C7, using deficiencies (Welshons, 1974; Welshons and Keppy, 1975). An

intriguing study of the achaete-scute complex has been done with the aid of duplication-deficiency combinations and left-right inversion recombinants (Muller, 1935; Muller and Prokofyeka, 1935; Garcia-Bellido, 1979). This, however, is about the limit of the use of deficiencies in higher eucaryote fine structure analysis.

Whether sDf1 ends within the unc-22 gene is unknown, but it does not extend into what is thought to be the adjacent gene on the left-hand side, let-56. If the deficiency were shown to break within the gene it would be useful for improving the resolving power of the system and for analyzing the left-hand boundary of the gene. To examine this possibility the heterozygote unc-43,unc-22(s16)/sDf1 should be set up and the offspring examined for wild-type progeny, which if found, would confirm that the deficiency does not extend to the end of the gene.

With the simple modifications detailed here the resolution of the unc-22 gene could be pushed practically to the single nucleotide level. The work of Benzer (1959, 1961) on the rII locus in T4 first suggested that the basic unit of recombination is the

nucleotide itself and the work of Yanofsky (1963) demonstrated it at the molecular level by using mutants that alter the same amino acid in the _A_ polypeptide of tryptophan synthetase of _E. coli_. Few systems in eucaryotes have the resolving power to separate adjacent bases. As pointed out by Chovnick et al. (1962) this is largely due to a problem in experimental design, most workers simply cannot screen sufficient numbers of progeny. In this regard the nematode is a very promising organism.

(iii) _Conversion and a novel recombination event:_ The data accumulated by myself on the _unc-22_ gene and others on the genes, _unc-15_ and _unc-54_, (Ms. A. Rose, personal communication; Dr. R. H. Waterston, personal communication) indicate that conversion events do occur in this organism. This statement should be qualified. The data at present show high negative interference, that is, a much higher than expected frequency of apparent double crossover events. The rate of occurrence of these events, 10^{-5}, is comparable to rates of gene conversion obtained for _D. melanogaster_ (Chovnick et al., 1971).

The prevailing viewpoint at present is that all

crossing-over originates as a conversion event (Hurst et al., 1972; Hastings, 1975; Catcheside, 1977). Its detection in _C. elegans_ should, therefore, not come as a surprise. Of interest is that the ratio of non-reciprocal events to reciprocal events in the nematode is much lower than has been found in yeast or in _Drosophila_. Hurst et al. (1972) have found that half of all conversionary events are associated with a reciprocal recombination for neighbouring material in yeast. Chovnick et al. (1971) working on the _rosy_ locus in _Drosophila_ have concluded that the ratio of crossover events to conversion events is a reflection of the distance between two alleles, the closer the alleles, the greater the bias toward a conversion event. Only when alleles were at the extreme opposite ends of the gene did they find that reciprocal events occurred one-half of the time. In the majority of the cases reciprocal events were much rarer. In the nematode my observations were quite the reverse. In no instance did the convertants account for more than 30% of the exceptional individuals. Assuming that crossovers and convertants have the same relationship in the nematode as in _Drosophila_, the obvious implication of this data is that the _unc-22_ locus is quite large.

The other observation that should be mentioned, which again is based on limited data, concerns the conversion frequency and the mutant allele map position. The data that can be compared are the conversion frequency for the alleles s8 and s12. These alleles map to opposite ends of the locus (Figure 3). Although the data presented here are not sufficient to prove statistically significant differences in conversion frequencies, there is an indication that s12 is involved in and possibly promotes non-reciprocal exchanges. Of 15 exceptional individuals, 11 came from heterozygotes involving s12 (Table 4). Two interpretations of this observation are possible. The first suggests a polarity in conversion frequency within the locus going from s8 with a low frequency to s12 with a high frequency. The conversion rates of alleles between the two does not substantiate this interpretation. The other possibility is that s12 is a 'marker effect' allele (see Hastings, 1975, for a review). That is, there is something peculiar about s12 such that it has the ability to promote non-reciprocal exchanges. More data will be needed to clarify these speculations.

To detect and properly analyse gene conversion requires the recovery and examination of each of the four products of a single meiotic event. This is why the lower eucaryotes, particularly Neurospora and yeast, have been used to study non-reciprocal exchange. In lieu of tetrad analysis, Drosophila workers have developed a system through which they can examine two of the four products of a single meiotic event. This is the half-tetrad system which makes use of attached-X and attached-autosomes to analyze conversion events (Chovnick et al., 1970; Smith et al., 1970; Ballantyne and Chovnick, 1971). The development of a half-tetrad system for the nematode would be a useful contribution. At present we are limited to a random analysis of meiotic events since we can recover only one of the products of a single meiosis.

Periodically during the fine structure experiment clones of non-twitcher worms were observed on a single plate. However, less than one non-twicher per plate was expected, because of the rarity of recombination events. These 'bursts' of non-twitchers occurred 16 times among the 104 putative recombinants, and were the result of both reciprocal and non-reciprocal exchange (13 of 16 showed an exchange of outside markers). The

trivial explanation is that these clones were the result of crossing-over in the F1 generation. This explanation is incapable of accounting for the majority of the observations since an F1 recombinant was not found on most of the plates and the burst size in most cases was too small to be the progeny from an F1 recombinant. There is the possibility these clones were the result of an F2 event and that I was examining the F3 generation. I do not believe this was the case because the brood time was not long enough for a third generation to mature and non-twitcher recombinants do not grow much faster than a twitcher (see Figure 6).

A reasonable hypothesis is that these bursts of non-twitchers were the result of mitotic exchanges that occurred during gametogenesis. This exchange could have occurred in a stem cell involved in spermatogenesis or oogenesis. In the newly hatched L-1 larva there are 4 primordial cells which lay down the whole of the reproductive system (Nigon, 1965; Hirsh et al., 1976; Kimble and Hirsh, 1979). if at any time during the development of this system one of the two stem cells involved in gametogenesis underwent a mitotic exchange within the <u>unc-22</u> gene then there would be a 50% chance that this stem cell would be wild-type at the <u>unc-22</u>

locus. All future oocytes from this cell would also be wild-type.

Similar observations were reported for *Drosophila* but the size of the clones were much smaller, usually containing only two or three individuals. Differences in gonad morphology between *C. elegans* and *D. melanogaster* may account for this difference in clone size. Smith et al. (1970) found that one of their non-reciprocal events at the *maroon-like* locus in *Drosophila* was the result of a burst and concluded that this was due to an intragenic event having occurred in the previous generation. Ballantyne and Chovnick (1971) reported a similar occurrence at the *rosy* locus, and Chovnick et al. (1971) found several examples of clustering in a large experiment on recombination at the same locus. They examined 11 clusters of 2 exceptionals each and found that in 5 cases the two exceptionals were genetically identical but that in the other 6 the individuals were different. Chovnick et al. (1971) concluded that the clusters were not the result of a premeiotic gonial mitotic event. In contrast to this, Gelbart and Chovnick (1979) during a study on unequal crossing-over in the *rosy* region found three tandem duplications that appeared to be the result of a

single gonial event. The possibility of gonial mitotic events occuring in Drosophila is therefore still under debate.

Nevertheless, mitotic crossing over does occur in somatic tissue. It was observed in Drosophila (Stern, 1936) and was induced in the nematode (Babu and Siddiqui, unpublished results). As well, Roman (1956) observed that diploid yeast cells recombine while dividing mitotically and demonstrated that the recombinants were largely due to non-reciprocal exchange Roman, 1963). The clusters obtained from the unc-22 gene were of the reciprocal and non-reciprocal type. These observations, therefore, are not at variance with the above. In yeast a mitotic and a meiotic fine structure map of the iso-1-cytochrome c gene are available (Parker and Sherman, 1969; Moore and Sherman, 1975; 1977). Both methods separate the alleles within a gene in a similar manner, although X-ray mitotic mapping is more sensitive to marker effects. The positioning data from the clustered recombinants at the unc-22 site was consistent with the data obtained from the meiotic recombinants in every detail. I believe these observations make a strong argument for the usefulness of clustered recombinants

in determining the genetic organization of a locus.

Mitotic recombination in yeast is increased with ultraviolet light (Roman and Jacob, 1958) or X-rays (Manney and Mortimer, 1964) and in fact, this is part of the standard procedure for doing yeast mitotic fine structure mapping. Perhaps this technique could be adapted for use in the nematode if the occurrence of these clustered recombinants could be increased in the progeny of a heteroallelic worm by low levels of X-rays applied to the parent. This would be an additional method for increasing the resolution of fine structure mapping in C. elegans. However, this increase would not confirm that these clusters are the result of mitotic events because X-rays are also known to increase the meiotic recombinaton frequency (Muller, 1925).

How else might clustered recombination data be explained? A possible explanation comes from observations on recombination in Drosophila males. For a long time it was assumed that male recombination did not take place in D. melanogaster (Morgan, 1914). Recently, however, many natural populations of D. melanogaster were found which, when outcrossed, gave

males with a low level of recombination (Hiraizumi, 1971). Of relevance is that these males sometimes gave a cluster of recombinants. This observation was interpreted as indicating that male recombination was premeiotic in origin (Hiraizumi et al., 1973).

An alternative to this interpretation was proposed by Henderson et al. (1978). Their view is based on results they obtained from a series of experiments using a heterozygous paracentric inversion male recombining stock and a noninverted marker gene male recombining stock. The experimental rationale was that, if recombination in males was 'conventional' and premeiotic then no chromosome aberrations should appear during meiosis in either of these stocks. If recombination was the result of a 'conventional' meiotic event then the heteozygous inversion stock should give some cells that contained dicentric bridges and fragments (from crossing-over within the inversion), while the noninverted marker stock should appear normal.

The results were surprising, for they found that all the males that showed recombination, whether in inversion heterozygotes or not, also had anaphase

bridges associated with first and second meiotic division. This result suggested that the spontaneous chromosome breakage and male recombination were part of the same process. Furthermore, there was a lack of chromosomal disorders at the metaphase I stages which indicated that the breakage and reunion events did not occur during premeiotic gonial mitosis. From these data they concluded that the breakage and reunion events, which could result in bridge formation, fragmentation or recombination occurred during meiosis, and that the clustering of recombinants must be the result of a primary gonial cell 'disorder' which subsequently altered the meiotic behaviour of all the daughter cells. The possibility that recombination clusters in C.elegans are due to an analagous event cannot be discounted.

(ii) The essential genes in the unc-22 region: The lethal mutation analysis is the most difficult part of the thesis to comment upon because the analysis is incomplete. A number of complementation tests are still required and several lethals are yet to be positioned relative to the deficiencies or unc-22. Despite these reservations some general conclusions can be stated.

The purpose of this portion of the thesis was to try and identify the genes adjacent to the twitcher site, to determine the distance between these genes and unc-22 and further, to try and 'saturate' a limited region around unc-22 for mutational sites. Some success with the first two objectives was obtained but the completion of the third objective needs to await future research.

Genetic analysis identifies the genes adjacent to unc-22 to be let-56 on the left, and let-52 on the right. Judging from the recombinational size of the unc-22 gene (0.01 to 0.02 map units) these flanking genes are as close recombinationally, as unc-22 is in length. Multiple allelic series exist in Drosophila which contain lethal as well as visible alleles (see Hadorn, 1961; Welshons, 1965). Because these two lethal mutations map so close to the twitcher site there is the possibility that they are lethal alleles of the unc-22 gene. I believe this is rather unlikely for the following reasons. The most severe effect a mutation in the structural element of a gene could have would be to stop the formation of a functional polypeptide. I have previously argued that the allele s32 is just such a mutation, yet worms homozygous for s32 are not lethal,

although, they are slower in development and lay fewer eggs. As well, the dominant mutation, m52, is not lethal when homozygous and yet is obviously a very severe mutation. Further, since let-56 complements sDf1, we know it must also complement the unc-22 gene and therefore, by definition, it forms an adjacent, yet separate essential site. The let-52 site has not been defined as well as this. If a fine structure experiment were done to demonstrate that s42 were further to the right of s7 than s12, the argument for s42 being a separate gene would be strengthened. There is a possibility the let-52 site is a mutation in the control element of the unc-22 gene. This again seems unlikely since in other eukaryotes control elements for genes have been found intractable to mutagenesis (Dr. A. Chovnick and Dr. F. Sherman, personal communication).

With the preliminary identification of the adjacent genes to the twitcher site, the obvious query is whether their position near the unc-22 site means they have some special relationship to that gene? Observations of the homozygous double mutants let-56,unc-22 and let-52,unc-22 did not reveal any obvious interaction between these sites (unlike the

mutation s65 which, when homozygous with unc-22, is not only lethal but also suppresses twitching in the double mutant). This does not mean no interaction occurs between these three genes but only that it is not easily discernable. Perhaps one approach to investigating the relationship of these three sites would be to examine the homozygous let-?,unc-22 mutations in an unc-54(s74) background. My work indicates that the gene products of unc-22 and unc-54 interact. Possibly unc-54 and the essential sites in this region also interact.

In a region 1.5 map units to either side of the twitcher I identified at least nine essential sites. Five of these were within 0.5 map units of the unc-22 gene and four of these were uncovered by sDf2. These four lethal mutations and their times of blocking development are: let-59(s49) blocking in the L-1, let-56(s46) blocking in the L-4, let-52(s42) blocking in the L-1 and let-60(s59) which blocks in either the L-2 or L-3. The mutations s42, s49 and s59 are considered the canonical alleles of their respective genes because when hemizygous these mutants did not block any earlier in development. On the other hand the allele s46 when hemizygous blocked in the L-3 which

means that the let-56 gene is probably an L-3 blocker and s46 is a weak allele of the gene. Many other lethal mutations failed to complement with sDf2. From their characterization and some selected complementation tests several of these can also be designated as essential genes. Both s172, which blocks in the egg when hemizygous, and s171, which is a sterile adult when hemizygous, probably characterize unique essential sites. The fact that s65 complements s46, when hemizygous blocks in the L-4 and shows signs of interaction with unc-22, suggests that it too is an essential site. The mutation s175 is an unusual mutation which blocks in many larval stages (possibly polyphasic) and, therefore, probably defines a further essential site. The mutations s168 and s174 complement and yet both block in the L-3. They, therefore, define two essential sites. It is possible that one of these may be allelic to let-56. In summary, my data show that within 0.5 map units either side of the unc-22 gene there are a minimum of 10 genes, 9 essential (1 egg, 2 L-1, 1 L-2, at least 2 L-3, 1 L-4, 1 adult and 1 variable) and one visible (unc-22).

From the range of effective lethal phases of these essential genes, it does not appear that this region

has a bias for genes specific to a particular developmental stage. The ratio of 'lethals to visibles' in this region is similar to that found for _D. melanogaster_. Workers who examined the X-chromosome in the fly found ratios of lethals to visibles varying from 30:1 to 5:1 (cited in Hadorn, 1961). Judd et al. (1972) in their study of the _zeste-white_ region found only two visible but 12 lethal sites in this small well defined region. In the alcohol dehydrogenase region on chromosome 2 of _Drosophila_ the ratio of lethals to visibles is about 3:1 (21 lethals, 8 visibles; O'Donnell et al., 1977; Woodruff and Ashburner, 1979). This excess of lethal sites over visible sites, therefore, does not seem to be either regionally limited within the _Drosophila_ genome, nor limited phylogenetically.

From an estimate of 10 genes per map unit, one can calculate that there are approximately 3,200 genes in the nematode. This agrees well with earlier estimates of 2,000 to 4,000 genes in the worm (Brenner, 1974; Baillie, unpublished results). This agreement must be treated as being fortuitous because I doubt that I 'saturated' this region for mutational sites. Work done in _Drosophila_ demonstrated how hard it is to saturate

even a small region for mutational sites (Hochman, 1971, 1976; Judd et al., 1972; O'Donnell et al., 1977; Woodruff and Ashburner, 1979). Part of the problem stems from the fact that different sites within a region have different forward mutation rates. For example locus 2 on chromosome 4 of Drosophila accounted for 20% of all lethal 4 mutations (Hochman, 1971), and zw1 on the X-chromosome accounted for 25% of all the lethal mutations which were spread over 12 lethal complementation groups between zeste (z) and white (w) (Judd et al., 1972), and finally, piccolo, a mutation near rosy on chromosome 3 of Drosophila accounted for 1/3 of all the lethal mutations in this area, although there were far more than 3 essential sites in the region (Dr. A. Hilliker, personal communication). Therefore, there is the possibility of essential sites existing in the unc-22 region with very low forward mutation rates, and with a sample size of only 3094 chromosomes I could very well have missed them.

The other part of the problem with calculating the number of genes in the organism from an estimate of the number of genes in a small region is that it is almost impossible to screen systematically for all possible types of essential, visible, biochemical, behavioural,

and fertility mutants that may map within a small genetic region. This problem has been amply illustrated in the *zeste-white* region of *Drosophila* where the clock mutants (*per*) (Konopka and Benzer, 1971) and other non-essential sequences (Young and Judd, 1979) were found after the initial study had supposedly 'saturated' the region for mutational sites. I have looked for recessive lethal, visible, female sterile, maternal lethal and grandchildless mutations in the *unc-22* region, but this hardly exhausts the possible genes that may map here. There are several mutations in the worm with subtle or conditional phenotypes which I would have missed in my mutation studies, for example, the dauerlarvae (*daf*) mutations (Riddle, 1976), the catecholamine (*cat*) mutations (Sulston et al., 1976), and the flourescent gut cell mutations (*flu*) (Babu and Brenner, 1974), to name only a few. To conclude then, my estimate of 10 genes in this one map unit interval around *unc-22* is probably an underestimate.

There is also a problem in trying to estimate the number of genes in *C. elegans* because of regional differences in gene density. Baillie and Rose (1979) estimated that a minimum of 15 lethal and 7 visible sites lie within one map unit surrounding *unc-15*. This

region is in the cluster on chromosome I. Meneely and Herman (1979) however, estimated that about 30 essential genes and two visible genes can be identified in a 7.5 map unit interval at the tip of the X-chromosome, or, about 4 lethal sites per map unit. As can be seen, there is a significant divergence in the number of essential sites per map unit in the three regions. Finally, there are micro-regional differences to consider. A cursory examination of the distribution of the lethals mapped around the _unc-22_ site shows there are a greater number of essential sites to the left of the _unc-22_ gene than there are to the right (characterization and mapping of some of the 'floating' lethals by Ms. Teresa Rogalski further substantiates this conclusion). Interestingly, this left-hand side contains the cluster of visibles.

With an extensive analysis of this group of lethals in progress, with a fine structure map of the central gene, _unc-22,_ and with the development of a method for isolating deficiencies in the region, a framework has been laid down for the future analysis of this region. There are few systems available that can provide so much genetic information for a defined region of the genome, information that is absolutely

essential if we are to understand the regulation of genes and how they interact in controlling development.

(v) Function of the unc-22 gene product and its interaction with the unc-54 gene: As pointed out earlier (Results IV) the role of the unc-22 gene in the life-history of *C. elegans* is unknown. The pharmacological, morphological and genetic evidence indicates that the unc-22 gene product is located within the muscle cells and has a function in muscle contraction. To summarize this evidence; it is known that isolated muscle preparations from unc-22 mutants will continue to twitch, and that neither nicotine, nor oubain (Dr. J. Lewis, personal communication), are capable of inhibiting twitching in unc-22 mutants. The only way I have found to stop the twitching in a worm homozygous for an unc-22 mutation is to deplete or alter the unc-54 gene product, myosin. Eliminating the twitch by depleting the unc-54 gene product can be partially circumvented by increasing the other body-wall myosin (done by using the sup-3 mutation). The problem, therefore, seems to lie somewhere between the muscle cell membrane and the myosin/actin contractile complex.

The electron microscopic (EM) studies of Dr. R. H. Waterston (personal communication) may offer a clue as to the intracellular location of the unc-22 gene product. On examining cross-sections of different unc-22 alleles he finds they vary in their degree of muscle disorganization, but the constant among the different alleles is that all display a similar disorder of the sarcoplasmic reticulum (SR), a closed compartment within the muscle cell. The SR is specialized for the binding and release of calcium (for reviews, see Martonosi, 1971; MacLennan and Holland, 1975), and in muscle cells, contraction is regulated by the intracellular calcium concentration (Weber and Winicur, 1961). It is currently thought that the SR stores calcium in relaxed muscle and releases it into the sarcoplasm upon depolarization of the cell membrane and the transverse tubular system (Weber, 1966). The calcium then interacts with the actomyosin complex to trigger contraction. The actual site of action of calcium may be on either myosin (Kendrick-Jones et al., 1970), the thin filament troponin (Ebashi et al., 1967), or both, (Lehman et al., 1972; Lehman and Svent-Gyorgyi, 1975). Which of these types of calcium regulation is employed depends on the organism and the muscle type, vertebrate and

arthropod muscles having thin filament regulation, molluscan muscle using thick filament regulation, and polychaetes, some insects and nematodes displaying thick and thin filament regulation (Lehman et al., 1972; Lehman and Svent-Gyorgyi, 1975; Harris et al., 1977). It can be seen from this, that a disorder in the SR that increased the level of calcium in the sarcoplasm could induce a muscle contraction.

The disorder induced by the unc-22 mutations is more complicated than a simple contraction. It is a continuous cycle of contraction and relaxation, and this cycle can be superimposed on muscles whether they are engaged in movement or not. This suggests that the twitching is involved in controlling muscle tone rather than in controlling movement *per se*. To postulate how such a cycle could be induced by an SR disorder it is necessary to know more about the organization of the SR. The SR maintains the low intracellular calcium concentration of resting muscle by means of an ATP-dependent calcium pump (Ebashi and Lipmann, 1962). The proteins of the rabbit skeletal muscle SR involved with calcium transport have been identified (MacLennan et al., 1972; MacLennan and Holland, 1975). These are, an ATPase of 102,000 molecular weight, an acidic

protein of 54,000 molecular weight, calsequestrin of 44,000 molecular weight, a set of three acidic proteins with molecular weights ranging between 20,000 and 30,000 and a proteolipid of molecular weight 6,000 to 12,000. The ATPase and proteolipid are intrinsic to the SR membrane, while the calsequestrin and 54,000 molecular weight component are thought to be on the inside of the SR and the acidic proteins are thought to be on the outside of the membrane in the sarcoplasm. It is thought that the acidic proteins transport calcium in the sarcoplasm to the SR membrane and concentrate it for active transpost into the SR by the ATPase and the proteolipid. It is then sequestered and held on the interior of the SR by calsequestrin and the 54,000 molecular weight protein (MacLennan et al., 1972)

If the <u>unc-22</u> mutation is a problem in the SR the most likely candidate is the SR ATPase. ATPase is in the expected molecular weight range. A mutation affecting it would probably show a disruption in the SR under the EM and ATPase has the functional properties of both an enzyme and a structural protein. This last qualification is important since alleles of <u>unc-22</u> are qualitatively different (from the <u>s74</u> suppression

studies) which suggests a possible enzymatic function, while the poor suppression of s32 by sup-5 suggests that the unc-22 gene product is required stoichiometrically (see argument in Waterston and Brenner, 1978), as does the isolation of a dominant mutation and the conditional dominance of the other unc-22 alleles.

There are problems with this model. The first problem concerns s32, a nullo allele of unc-22. If s32 lacks all the SR ATPase then why is s32 not rigidly paralyzed? But what if there were two SR ATPases, and the second ATPase could partially compensate for the absence of the first? The evidence in favour of the two enzyme interpretation is that mutations in two genes in the nematode can induce twitching, unc-22 and lev-11. Since two myosin heavy chains have been found within a single muscle cell (Epstein et al., 1974; MacLeod et al., 1977; Schachat et al., 1977; Mackenzie et al., 1978), this is not an untenable suggestion. It does, however, predict that an unc-22(s32);lev-11 worm should be rigidly paralyzed. There is another problem in assuming the primary disorder caused by the unc-22 mutations is increased calcium concentration in the sarcoplasm. The proposal cannot explain why worms

homozygous for an *unc-22* mutation can still continue to twitch in 1% nicotine, although being rigidly paralyzed. If twitching is the result of a slight leakage of calcium into the sarcoplasm, then the depolarization of the muscle cell outer membrane and the T-system by nicotine should flood the sarcoplasm with calcium and overwhelm the *unc-22* induced twitching of the actomyosin complex. Therefore, *unc-22* worms should appear rigidly paralyzed in nicotine. This is not the case and so I think it is necessary to reexamine exactly how a muscle can twitch.

There appears to prevail among investigators of *C. elegans* a tacit assumption that the *unc-22* mutations induce twitching by causing small contractions in the actomyosin complex. This is why the model of excess calcium triggering a contraction event appears attractive. But what if we turn this around and instead hypothesize that the *unc-22* mutations cause twitching by relaxing the myosin/actin interaction? In other words, instead of inducing a greater overlap between the myosin and actin molecules, the *unc-22* mutations cause a transient reduction in the overlap between the two. This interpretation agrees with the nicotine results and is not in disagreement with any of the

previous data I have cited. Some recent results obtained by Dr. J. Lewis (personal communication) lend credence to this proposal. He has found that *unc-22* and *lev-11* mutations act as suppresors of some of the dumpy genes. He also finds that 10^{-5} M levamisole produces a dumpy phenocopy (the same occurs with nictine) and that *lev-11* and *unc-22* suppress this drug-induced dumpiness. He concludes from these observations that several of the dumpy genes cause hypercontraction of the actomyosin complex. If *unc-22* mutations induce transient contractions, it is difficult to understand how they suppress a dumpy phenotype. On the other hand, if *unc-22* mutations cause relaxation of the contractile complex, this might partially relieve the dumpy phenotype.

Although much is known about contractile proteins in muscle, the precise way in which a muscle contracts is still poorly understood (Squire, 1975; Mannherz and Goody, 1976). This is due to our lack of understanding of the events occurring in the myosin head during the stages of ATP hydrolysis (Huxley, 1972). If the *unc-22* gene product has a role in stabilizing the actomyosin complex rather than in stimulating it, then one protein, if present in the nematode, should be

considered. This is the C-protein which has a molecular weight of 140,000 and binds very strongly to the rod portion of rabbit skeletal myosin at low ionic strength (Offer, 1972; Moos et al., 1975). The role of this protein in muscle structure and function is unknown but there has been speculation that it is part of the mechanism for controlling conformational changes in the thick filaments to allow for actin/myosin binding (Offer, 1972).

Admittedly, these speculations on the role of the unc-22 gene product in *C. elegans* are based on tenuous data, but the possibilities are intriguing and should be explored. Whether these mutations induce contraction or relaxation is unknown although, in my opinion, the present evidence favours the latter view. The biochemical characterization of the unc-22 gene product should conclusively decide which view is correct.

The discovery of a new class of mutations affecting myosin further suggests that the unc-22 gene product is intimately involved in the contractile process. The interaction of unc-54 and unc-22 is an example of indirect suppression. A case of indirect suppression has previously been reported for *C. elegans*

by Riddle and Brenner (1978) and, as pointed out by these authors, instances of indirect suppression via protein-protein interaction have a potential usefulness for the elucidation of complex cellular processes. The isolation of two classes of mutations at the unc-54 locus (birefringence positive and birefringence negative) is reminiscent of the complex loci found in *D. melanogaster* (Welshons, 1965; Judd, 1976). This pleiotropy is not surprising when one considers the mutilple functions encoded within the myosin molecule. It has been known for some time that the amino and carboxyl ends of the myosin molecule have qualitatively different functions (Young et al., 1968; Huxley, 1969; Lowey et al., 1969; Starr and Offer, 1973). The rod-like portion or carboxyl end is necessary for the assembly of the thick filaments (Huxley, 1969; Lowey et al., 1969), while the amino end contains the globular head. The head portion is involved in several biologically important functions, notably the hydrolysis of ATP and in interactions with actin (Young et al., 1968; Lowey et al., 1969; Starr and Offer, 1973, Mannherz and Goody, 1976). Since the new unc-54 mutations do not alter filament structure greatly but instead seem to have their primary effect on the process of contraction, it is possible that these new

mutations might be localized in the globular portion of the myosin molecule.

To test this possibility the s74 allele has been mapped intracistronically. A fine structure map of several birefringence negative unc-54 alleles has been constructed (Dr. R. H. Waterston, personal communication). At one end of this map is e675, an allele with an internal deletion in its myosin near the carboxyl terminus of the molecule (MacLeod et al., 1977). This shortened myosin still retains its ATPase activity (Eptein et al., 1974; Harris and Epstein, 1977). Another allele on this map is the allele e190 which is the allele relative to which I mapped s74. Because of the complications of reciprocal and non-reciprocal exchanges for flanking markers (Chovnick et al., 1971; Moerman and Baillie, 1979,) I am not certain if s74 is to the left or to the right of e190. However, it is separable from e190 and the distance is compatible with s74 being a mutation in the amino portion of the molecule. If this is correct then we know the direction of transcription for the unc-54 gene. From the low forward mutation rate, approximately 10^{-5}, for the induction of mutations of this type, as compared to the average forward mutation rate per gene,

5×10^{-4} (Brenner, 1974), I suspect that the other mutations of this class will be localized to the same region of the unc-54 gene as s74.

There are many ways in which these mutations could cause disruptions in contraction. It is the binding of ATP to the actomyosin complex that causes the dissociaton of actin and myosin. This is followed by the cleavage of the ATP by myosin and a new interaction at a different actin site can then take place (see reviews by Gergely, 1972 and Mannherz and Goody, 1976). It is thought that actin and the nucleotide bind to different sites on myosin but interact through structural changes in the myosin molecule. The stiffness of s74 worms might suggest that myosin has a decreased ability to relax actin. Could this be due to a decreased affinity of the myosin heavy chain for ATP? If so, then one can see how the genes unc-54 and unc-22 interact. Mutations in unc-22 decrease the stability of the actomyosin complex while these new myosin alleles do the opposite by making the myosin/actin complex more difficult to dissociate. This is but speculation and only the biochemical characterization of these mutations will elucidate their true role in muscle action.

(vi) The organization of a gene: Prior to 1950 the gene was thought to be an indivisible unit of heredity (despite Oliver's demonstration of intragenic recombination in *Drosophila*, 1940), the ultimate unit of mutation, and to be associated with a single primary specific function in metabolism or development (Pontecorvo, 1958). The decade that followed saw all three of these properties of a gene violated, such that we now know that it is a single nucleotide of DNA which possesses the first two of these properties and not the gene. The third property, specificity of function, has proved more difficult to determine in relation to a gene. This is because, although RNA is the primary transcript from the DNA, genes code for a variety of terminal products, from rRNA and tRNA to translational products which may function as polypeptide monomers, homopolymers, or as part of heteropolymers. Monomeric proteins add a further complication by being either monofunctional or multifunctional (Kirschner and Bisswanger, 1976). Furthermore, the gene must have coded within it information pertaining to temporal, spatial and quantitative transcriptional control. With this amount of information coded along its linear array of nucleotides, the specificity of function of a given

gene can be difficult to deduce. But this gene/function relationship must be elucidated if an understanding of the organization of a gene, and indeed, of the genome is to be achieved.

To investigate genomic organization I have used a 'classical' genetic approach. That is, I have isolated a series of different mutations, mapped them to specific regions on the chromosomes, and investigated their various interactions. To analyze a specific gene, unc-22, a number of spontaneous, EMS, formaldehyde and gamma-ray induced alleles have been isolated and positioned relative to one another within the locus. Several mutagens were used to generate an array of different disruptions within the locus. In light of what I have said about the complex nature of a gene, how does one initially define a genetic locus? The tool of genetics is allelism or lack of complementation. This test involves bringing together two different mutations into a cell to form a heteroallelic pair. If they fail to complement i.e. if they cannot compensate for each others disorder, they are considered to be mutations within the same functional unit.

All twitcher mutations isolated form two

complementation groups: lev-11 on chromosome I and unc-22 on chromosome IV. On several occassions the definition of a genetic locus as a single complementaton group has been challenged (Fincham, 1966). Several mutations have been found that phenotypically resemble others at a particular locus, map to the same region of the chromosome, fail to complement some of the alleles in a heteroallelic genome, but complement other alleles of the locus. This phenomena has been called inter-allelic complementation and was first described by Fincham (1959) and Brenner (1959) who suggested that it was due to a protein-protein interaction. This idea was later elaborated upon by Kapular and Birnstein (1963), and Crick and Orgel (1964), who proposed that the complementation was the result of a homologous correction being made by the correct region of one monomer on an incorrect region of an adjacent monomer in a multimeric protein. Since then heterologous correction has also been hypothesized (McGavin, 1968) wherein a mutant with an altered active site complements a mutant with an altered allosteric site.

Interallelic complementation has been demonstrated only in multimeric proteins and this is why the above

models are based on interactions between defective subunits. In my studies on the unc-22 mutations I have not found any instances of interallelic complementation. Does this mean that the unc-22 gene product functions as a monomer or possibly as part of a heteropolymer? It is difficult to arrive at conclusions based on negative data, and in this case I think there are several reasons for exercising caution. From the studies I have done on gene dosage at the unc-22 site (Table 15) I believe the expression of the phenotype of this locus is quite sensitive to inactivation of its gene product. Reducing the active component by 1/3 is enough to show a slight effect and depleting it by 2/3 gives a visible twitcher (It should be pointed out that this study was done with s7, which from the suppression studies involving s74, see Table 20, is known to be one of the least severe of the unc-22 alleles). Further, the suppression of s32 by sup-5 does not provide enough of the wild-type unc-22 gene product to inhibit the visible twitching. The suppressor restores about 10% of the protein product of unc-15 and unc-54 (Waterston and Brenner, 1978) and presumably is as efficient in restoring the unc-22 gene product.

These observations suggest the unc-22 wild-type

protein activity must reach a relatively high threshold to restore a wild-type phenotype to a worm. Studies on complementation in other systems demonstrate that complementing alleles do not restore the enzymatic activity of a protein to the level of the wild-type protein. Garen and Garen (1963) studied twenty-one complementing pairs of alleles of the alkaline phosphatase gene in E. coli and found that the enzymatic activity ranged from 1 - 65% of the wild-type activity and that all but one pair had enzymatic activities equal to or less than 40% that of the wild-type. An even better example of how a low level of enzymatic activity arising from a complementing heterozygote can restore a wild-type phenotype is illustrated by the rosy locus which codes for the enzyme xanthine dehydrogenase (XDH) in D. melanogaster. Complementing alleles at this locus exhibit normal or near-normal eye-colour phenotypes, but the restored XDH activity levels are extremely low, from less than 1 to 16% (Chovnick et al., 1977).

If a pair of unc-22 alleles complemented to give 16% of wild-type protein activity, I would not have recorded it because the worm would still have twitched. Perhaps a more sensitive assay for complementing

alleles might be to examine a heteroallelic worm under birefringence optics and look for improved muscle fibre organization. This approach has not been tried but since there are worms that twitch even with normal muscle organization, birefringence could prove to be a more sensitive assay for complementation. Another point to consider is this, in genes where complementation exists, mutations are not equally distributed among the complementation groups. In rosy (Chovnick et al., 1977) there are sixty-six mutations distributed among eight complementation groups but fifty-six of these mutations are found in group I. Each of the other groups has very few alleles. This indicates that it may be quite difficult to find complementing alleles at the unc-22 locus, even if they exist.

The unc-22 locus is, at present, defined as a single complementation unit. In a sense one could argue that the alleles mapped within the locus form a multiple allelic series because the severity of the phenotype caused by each allele can be determined by using the mutations, sup-5(e1464) and unc-54(s74). With the aid of these two mutations I have been able to separate the alleles into at least four groups (see results III). Presumably the first three groups contain

hypomorphic alleles while the fourth group contains amorphic alleles. As mentioned earlier I believe that all four groups are part of the structural element of the gene i.e. they are in the portion of the gene that codes for the polypeptide.

Where then is the regulatory element of the <u>unc-22</u> gene? If the current views of eucaryotic gene organization are correct, the <u>unc-22</u> gene is a bipartite complex with a structural element and an adjacent but separate regulatory element. This view of gene organization is largely developed from work on procaryotes (Jacob and Monod, 1961; for a recent review see volume edited by Miller and Reznikoff, 1978) and as yet there is but meager evidence in eucaryotes in support of it. Hynes (1975, 1979) has found in <u>Aspergillus</u> three control variants of the acetamidase <u>(ambS)</u> gene which map adjacent to it. Two of these variants increase and one appears to decrease the amount of the enzyme. Sherman et al. (1978) have determined the structural gene and an adjacent regulatory region for the iso-2-cytochrome c <u>(CYC7)</u> gene in yeast. Their regulatory mutant has a twenty-fold increase in cytochrome-c levels. In higher eucaryotes the strongest evidence for a regulatory site

adjacent to a structural region is at the *rosy* locus in *D. melanogaster* (Chovnick et al., 1976; 1977 a,b; McCarron et al., 1979). Both a site for underproduction and a site causing overproduction of the enzyme XDH have been found adjacent to the structural element for XDH. A presumptive control mutation adjacent to the structural element for alcohol dehydrogenase (ADH) has also been reported (Thompson Jr. et al., 1977). Another possible control variant in *Drosophila* is the *ocelliless* mutation which has been shown to underproduce the major chorion proteins c36 and c38 which map near it (Spradling et al., 1979). In all of these examples the control elements are reported to be cis-acting.

The problem in identifying the regulatory region in the *unc-22* gene is that there is no general genetic means available for the recovery of regulatory mutants. The control mutants of Chovnick et al. (1976, 1977a) and Thompson Jr. et al. (1977) were isolated from natural populations of *Drosophila*, and the two *ambS* overproducers of Hynes (1979) arose spontaneously. Only the *ambS* underproducer and the *CYC7* overproducer were induced and these were isolated using the mutagen nitrous acid (Hynes, 1979; Sherman et al., 1978).

Recently Lifschytz and Green (1979) addressed themselves to this problem. In their paper they argue that several overproducing mutations may already be available for genetic study in the form of dominant mutations. They cite the _Beadex (Bx)_ mutations as a possible example of hypermorphic or overproducing mutants which control an adjacent structural element. They further suggest that _Beadex_ controls an adjacent element which is marked by the recessive mutation _held up (hdp)_. If this idea is correct, then the dominant mutation _unc-22(m52)_ could be a control variant. This is highly unlikely because _m52_ does not have the phenotype expected of a dominant overproducer at this locus. It is difficult to reconcile an overproducer possessing the same phenotype as the mutation _s32_, which does not produce any of the gene product. Instead, I expect a mutant overproducer to impart a stiff phenotype to the worm, similar to the phenotype of a worm homozygous for _unc-54(s74)_, but mapping at the _unc-22_ locus. Hence, probably the mutation _m52_ is a neomorphic mutation i.e. it is a mutation in which an altered gene product is produced, leading to a new dominant phenotype (Muller, 1932).

To date there are no documented control variants at the *unc-22* site. I have considered a number of schemes for inducing regulatory mutations within the locus, but the most promising protocol has been suggested to me by Dr. J. Lewis (personal communication). His idea is that one could isolate overproducers by mutagenizing *sup-5(e1464);unc-22(s32)* worms and then examining their F1 progeny for worms that are wild-type in appearance. There are several advantages in using this approach. The screen can be done in the F1 and there is a chance of isolating an overproducer at either the *unc-22* or the *sup-5* locus. Perhaps the most important aspect of this scheme is that one would not be trying to produce an abnormal amount of the twitcher protein, but would only be trying to return it to the wild-type level. This should exclude the possibility of the overproducer being lethal. Since it is known that the *sup-5* mutation only restores about 10% of the active protein this screen should allow for the selection of a control variant that produces at least ten times the wild-type level of the gene product. The mutagen of choice for this experiment would be either formaldehyde or nitrous acid. Formaldehyde induces deletions (Moerman and Baillie, in press; also see results II), and also has

been found to induce unc-22 mutations, which may be small internal deletions. Such a mutagen could be useful for the removal of a whole sequence of nucleotides involved in control. Nitrous acid should also be tried since the only two eucaryote control variants isolated were induced with this mutagen.

Tightly linked cis-acting regulatory elements are only one form of transcriptional regulation. Regulatory regions that control the expression of a genetic locus can also map some distance from it. There are two examples in Drosophila which are particularly illustrative of this form of control. The first case is the trans-acting control of the expression of the amylase structural gene (amy) exhibited by a gene called map which lies about two map units away from amy (Abraham and Doane, 1978). Alleles of the map gene seem to have the ability to control the tissue specific expression of the amylase genes in the mid-gut of the fly. Different alleles of the map gene show a different distribution of the enzyme amylase in the posterior mid-gut. The other example of regulatory interaction between loci is the zeste (z) - white (w) interaction (Gans, 1953; Jack and Judd, 1979). These two genes map approximately 0.5 map units apart. The current model of

Jack and Judd (1979) proposes that the _zeste_ gene product is a repressor of the _white_ gene, but is not an active suppressor unless it complexes with an RNA produced by part of the _white_ locus itself. The types of gene interaction shown in these two examples from _Drosophila_ have not been demonstrated in the nematode. The interaction of _s65_ and _unc-22_ is a possible candidate for trans-acting regulation. I believe, however, that this is most probably an example of post-translational interaction.

The current view of a eucaryotic gene from genetic analysis is that it is a region of the chromosome where a number of mutations will map. These mutations can be separated to form a linear array and may exhibit diverse phenotypes as well as complex patterns of interaction. Further, the locus is viewed as a bipartite structure having adjacent regulatory and structural elements. These elements themselves may interact with other areas of the genome at either a transcriptional, translational or post-translational level. How does this data accumulated from a genetic analysis of the gene compare to data from a molecular analysis of the gene? This is a difficult question to answer because, at present, there is not a single gene

in higher eucaryotes that has been the subject of both a sophisticated genetic and molecular analysis.

Despite this problem our view of the molecular architecture of genes has been altered dramatically with the advent of restriction enzymes, rapid DNA sequencing techniques and large 'cosmid' cloning vehicles (Sinshimer, 1977; Maxam and Gilbert, 1977; Sanger and Coulson, 1978; Collins and Hohn, 1978). The most startling discovery has come from restriction enzyme mapping and sequencing studies of several eucaryotic genes (including genes for tRNA, rRNA, globin, ovalbumin, immunoglobulin and ovomucoid) which reveals that structural information in these genes, although colinear, is not continuous. The coding sequences are interrupted by non-coding segments (Brack and Tonegawa, 1977; Breathnach et al., 1977; Glover and Hogness, 1977; Goodman et al., 1977; Jeffrey and Flavell, 1977; Mandel et al., 1978; Tilghman et al., 1978a; Tonegawa et al., 1978; Valenzuela et al., 1978; Catterall et al., 1979). The size of the non-coding intervening sequences ranges from about ten nucleotides to over one thousand nucleotides, and the number of intervening sequences ranges from one (in a yeast tRNA) to as many as seven (in chicken ovalbumin). The coding

and non-coding sequences are transcribed into one large RNA precursor. This has been demonstrated by Tilghman et al. (1978) who discovered that the 15s mouse beta-globin precursor contains the intervening sequences. It is believed that the discovery of intervening sequences partially explains the role of heterogenous nuclear RNA (Darnell, 1979). The processing (splicing) of the precursor RNA to mRNA is presumably a nuclear event. Blanchard et al. (1978) have shown that an <u>adenovirus</u> precursor message can be converted into a specific message in isolated nuclei and 'splicing factors' have been described by O'Farrell et al. (1978) and Knapp et al. (1978).

The first question is, why genes in pieces? There has been considerable speculation on this point (Doolittle, 1978; Gilbert, 1978; Blake, 1979; Crick, 1979). One thing that is clear, much of the excess DNA (cDNA paradox) of higher eucaryotes can now be accounted for as part of the intervening sequences within genes (Gilbert, 1978). Recent evidence suggests the intervening sequences act as spacers between the functional units of a protein. Sakano et al. (1979) examined a mouse immunoglobulin gamma heavy chain and found the three protein domains (CH1, CH2 and CH3) and

the hinge region encoded within separate DNA fragments. If intervening sequences really do separate protein functional groups then mosaic genes are, as described by Gilbert (1978) and Blake (1979), the frozen history of proteins. Kornberg (1976) has stated that enzymes have at least three 'faces', a catalytic face, a regulatory face or allosteric face, and a social face. It is this last aspect of a protein we know the least about. The large number of intervening sequences in some genes, for example, those coding for ovalbumin and ovomucoid, suggests these proteins are composed of many functional units. Which of the above 'faces' corresponds to which unit remains to be determined. In this regard, it is interesting to note that serum albumin is considered a multifunctional protein (Kirschner and Biswanger, 1976).

There are at least two methods of generating a mosaic gene (Crick, 1979 discusses others). The first method is by the tandem duplication of a functional unit as well as some of the sequences surrounding it, and then the subsequent divergence of the second unit, in time, to a new functon (Bridges, 1935; Lewis, 1951). This method is not satisfactory for explaining mosaic genes where the functional units have extremely diverse

roles. A tandem duplication hypothesis is more useful in explaining the clustering of gene families that has been observed in eucaryotes. Lawn et al. (1978) found that the human gamma- and beta-globin genes are adjacent, and Royal et al. (1979) found three genes (ovalbumin, X and Y) clustered which are under hormonal control in the chick oviduct. In this ovalbumin cluster there are extensive topological similarities in the distribution of the intervening sequences indicating a similar origin of all three genes from a single 'primordial' gene.

A second method of generating a mosaic gene is by bringing functionally different genes together and then fusing them (Gilbert, 1978, Tonegawa et al., 1978). Moving pieces of DNA about in the genome is not a problem. There is a considerable body of evidence supporting the concept of the genome as a much less stable, or static structure than originally considered (McClintock, 1957; Potter et al., 1979; Strobel et al., 1979; also see volume edited by Bukhari et al., 1977). This does not mean wholesale rearrangements occur during development. This is clearly not the case (Potter and Thomas, Jr., 1977; Emmons et al., 1979). I imply only that, given an evolutionary time span,

functionally unique sequences can be brought together. The fusion of the adjacent genes can be accomplished by either an unequal cross-over, a deletion or even a series of frameshift mutations. The last method of fusing two genes has been demonstrated in the histidine operon of Salmonella (Yourno et al., 1970).

Any of the above processes for promoting gene fusion would tend to leave gaps between the units owing to the problem of fitting them together precisely without losing part of one of the functional units. These gaps would be the intervening sequences. Presumably, procaryotic proteins have evolved through a similar shuffling of functional units, so why do they not have mosaic genes? There are three possible explanations for this discrepancy. Firstly, procaryotes have had much longer to evolve than eucaryotes and, therefore, may have had time to remove the intervening sequences. Second, it appears that non-eucaryotes are under evolutionary pressure to limit their genome size and instead increase the coding efficiency of the existing nucleotides. The phage phi-X 174 is an example, having two different proteins using the same DNA sequence but in different reading frames from one another (Sanger et al., 1977) The third possibility is

that, since phages that infect eucaryotes are the only non-eucaryotes with split genes (see for example Broker et al., 1977) then it is possible that the splicing enzymes for processing the RNA may have arisen with the eucaryotes. Procaryotes might never have had this shuffling mechanism. Finally, one could take quite a different view and argue that the operon of procaryotes is analagous to the mosaic gene of eucaryotes, the difference being that the functional protein units are not bound in procaryotes. To demonstrate the difficulty in making this distinction, Fink (1966), based on genetic data, at first proposed that the HIS-4 complex in yeast was an operon. It was only after isolating the protein complex that he realized it was a single multifunctional unit (Calvo and Fink, 1971).

Now that the molecular organization of a few select genes is known it seems pertinent to try and collate this information with what is known about the genetic organization of a gene. The realization that many genes in eucaryotes are split adds a new interpretation to terms such as complementation, multiple alleles and complex loci. I believe it also adds a new relevance to the term step-allelism. The step-allele, or sub-gene model was invoked by A.S.

Serebrovsky and N. P. Dubinin to explain complementation results that they observed in the *achaete-scute* complex (which affects the bristle pattern of the thorax) of *Drosophila* (data cited in Carlson, 1966; see also Garcia-Bellido, 1979). They constructed a model of the gene which divided it into a number of sub-genes, each of which was a separate functional unit. The problem with their model was the notion that each sub-gene was specific for a separate scutellar bristle. This, of course, was not the case and because of the numerous exceptions to the model it was eventually discarded.

Our new understanding of the gene as a mosaic structure gives the model a new validity. If one views the sub-genes as controlling different functional parts of a protein, complex complementation patterns, and multiple allelic series at loci will come as no surprise. Genes such as *rudimentary (r)* in *Drosophila* (Carlson, 1971) and *unc-54* in the nematode, whose gene products are known, support such a model. *Rudimentary*, a complex locus, codes for three different enzymes of the pyrimidine biosynthetic pathway (Norby, 1970; Jarry and Falk, 1974; Rawls and Fristrom, 1975) and *unc-54*, as stated earlier, codes for myosin, a complex protein.

Genetic studies of _rudimentary_ reveal a complex complementation map (Carlson, 1971), and my own studies, involving _unc-54_ (see Results III), demonstrate that this gene has multiple alleles. The sub-gene concept also allows one to view the gene itself as an evolving structure. Certain mutations within a gene might reveal something of its evolution by generating 'atavic proteins' and possibly even atavic phenotypes. Perhaps the _bithorax_ series in _D. melanogaster_ (Lewis, 1963) and _displaced vulva_ in _C. elegans_ are examples of such mutations.

The discovery of the mosaic nature of genes casts doubt on the model of a gene as a bipartite structure with separate regulatory and structural elements. If evolution is indeed parsimonious it is difficult to accept the idea that large intervening sequences do not have a function in gene expression. As stated earlier, the limited amount of evidence on gene regulation in eucaryotes suggests that transcriptional control sites are outside the limits of the structural element. Transcription of a mRNA precursor is but a small part of regulation. The RNA must still be processed and the message transferred through the nuclear membrane. There has to be a code within the RNA to do this. A

role for the intervening sequences in transcriptional regulation might also be visualized if, for instance, the intervening sequences of a gene being transcribed acted as inducers of other genes. This would be a means of coordinately regulating the transcription of several messages.

Little is known about the intervening sequences, or 'introns' (Gilbert, 1978). The few introns examined appear to have 'unique' sequences, rather than moderately or highly repetitive sequences (Robertson et al., 1979; also references in Crick, 1979). Data from several sources indicate the introns are not as conserved in evolution as are the coding sequences. A comparison of rabbit and mouse beta-globin genes demonstrates that the protein coding parts of the gene are quite similar but that the introns, although related, are considerably different (van den Berg et al., 1978). A similar comparison between the two beta-globin genes of mouse shows that the coding region and some of the sequences of the intron adjacent to it are conserved. The rest of the intron is not (Tiemeier et al., 1978). A study of the ovalbumin gene in chicken has demonstrated allelic differences in one of the intervening sequences of the gene in a chicken

population (Lai et al., 1979). These results indicate that introns are subject to evolutionary drift which suggests they might not have a function.

A study of deletion mutants in simian virus 40 (Lai and Khoury, 1979) does not agree with this interpretation. These workers isolated a series of deletions in the region of the DNA of sv40 coding for the late viral proteins. The genes in this region are of two types, split genes and overlapping genes. A characterization of the deletions has been done by heteroduplex mapping and some cover only a part of the coding element, while others include part of the coding sequence, a splice junction and part of the intron. An examination of the products of these deletion mutants reveals that deletions which include only a part of the coding sequence are capable of producing a stable mRNA. Deletions which span a splice junction and part of an intron, however, cannot produce a stable RNA product. The RNA is transcribed, but is turned over very quickly within the nucleus. From these results they conclude that the splice junctions and/or the intervening sequences are important for mRNA biosynthesis. Unfortunately, Lai and Khoury did not have a crucial deletion mutant, one that only removed the intron and

not the splice junctions or the coding sequences. Therefore, it could be argued that only part of the intron adjacent to the coding sequence, that part found to be conserved in evolution, is necessary for RNA processing.

These results demonstrate unequivocally that a host of new mutations would be exposed by a combined genetic and molecular study of a eucaryote gene. The aforementioned results predict a sub-class of null mutants that are due to RNA instability. Depending on how complicated the splice junction recognition sequence is, it may be possible to generate a splicing signal within the coding sequence to generate a peptide with an internal deletion (unc-54(e675)?). This would be unlikely if the splicing sequence is very complex. Finally, a role for introns in intragenic or intergenic transcriptional regulation can be firmly established only when regulatory mutations are found to map within these sequences. These speculations can be confirmed by combining the approach that I have used in this thesis to analyze the unc-22 region with an equally sophisticated molecular study of the region. Neither approach alone can hope to answer all questions concerning the organization of the gene.

To conclude, in our understanding of the gene one feels much like the turn-of-the-century biologist must have felt when confronted with chromosomes on the one hand, and Mendelian genetics on the other. The two must complement, but how? It took the work of Bridges (1916) and others, on non-disjunction in *Drosophila*, to bring the two together into the chromosome theory of inheritance. We are at a similar impasse in our understanding of the gene and its relationship to a DNA sequence. How can we describe the molecular nature of the gene? Only with the characterization of transcriptional control mutations can this question be answered.

Recently Francis Crick (1979) has stated, ''...I have deliberately used the word 'gene' in a loose sense since at this time any precise definition would be premature.'' One wonders how Richard Goldschmidt would view this turn of events.

REFERENCES:

Abraham, I. and W. W. Doane, 1978 Genetic regulation of tissue specific expression of Amylase structural genes in Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S. A. 75: 4446-4450.

Adams, R. L. P., R. H. Burdon, A. M. Campbell and R. M. S. Smellie, 1976 Davidson's The biochemistry of the nucleic acids. 8 th edition. Academic Press. New York.

Albers, R. W. 1972 Molecular biology of neural receptors. pg. 67-79. In Basic neurochemistry. (eds.) R. W. Albers, G. J. Siegel, R. Katzman and B. W. Agranoff. Little, Brown.

Amati, P. and M. Messelson, 1965 Localized negative interference in bacteriophage lambda. Genetics 51: 369-379.

Axel, R., H. Cedar and G. Felsenfeld, 1973 Synthesis of globin ribonucleic acid from duck-reticulocyte chromatin in vitro. Proc. Natl. Acad. Sci. U.S.A. 70: 2029-2032.

Babu, P. and S. Brenner, 1974 Biochemical genetics of Caenorhabditis elegans. Mol. Gen. Genet. 135: 39-44.

Baillie, D. L. and A. Rose, 1979 Analysis of lethals in a small region on the first chromosome of C. elegans. Genetics, Abstract.

Ballantyne, G. H. and A. Chovnick, 1971 Gene conversion in higher organisms: Non-reciprocal recombination events at the rosy cistron in Drosophila melanogaster. Genet. Res. 17: 139-149.

Beadle, G. W., 1932 A possible influence of the spindle fiber on crossing-over in Drosophila. Proc. Natl. Acad. Sci. U. S.A. 18: 160-165.

Beckwith, J. R., 1978 Lac: The genetic system. pg. 11-30. In The Operon (eds.) J. H. Miller and W. Reznikoff. Cold Spring Harbor Laboratory.

Benzer, S., 1959 On the topology of the genetic fine structure. Proc. Natl. Acad. Sci. U.S.A. 45: 1607-1620.

Benzer, S., 1961 On the topology of the genetic fine structure. Proc. Natl. Acad. Sci. U.S.A. 47: 403-415.

Blake, C. C. F., 1979 Exons encode protein functional units. Nature 277: 598

Brack, C. and S. Tonegawa, 1977 Variable and constant parts of the immunoglobulin light chain gene of a mouse myeloma cell are 1250 nontranslatable bases apart. Proc. Natl. Acad. Sci. U.S.A. 74: 5652-5656.

Breathnack, R., J. L. Mandel and P. Chambon, 1977 Ovalbumin gene is split in chicken DNA. Nature 270: 314-319.

Brenner, S., 1959 The mechanism of gene action. p34 In: Symposium on Biochemistry of Human Genetics eds. G. E. W. Wolstenholme and C. M. O'connor. Churchill, London.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics 77: 71-94.

Bridges, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics 1: 1-52: 107-163.

Bridges, C. B., 1935 Salivary chromosome maps. J. Hered. 26: 60-64.

Britten, R. J. and E. H. Davidson, 1969 Gene regulation for higher cells: a theory. Science 165: 349-357.

Broker, T. R., L. T. Chow, A. R. Dunn, R. E. Gelinas, J.A. Hassell, D. F. Klessig, J. B. Lewis, R. J. Roberts and B. S. Zain, 1977 *Adenovirus-2* messengers - An example of baroque molecular architecture. Cold Spring Harb. Symp. Quant. Biol. 42: 531-553.

Brown, I. R. and R. B. Church, 1972 Transcription of nonrepeated DNA during mouse and rabbit development. Dev. Biol. 29: 73-84.

Bukhari, A. I., J. A. Shapiro and S. L. Adhya, 1977 DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory.

Byerley, L., R. C. Cassada and R. L. Russell, 1976 The life cycle of the nematode *Caenorhabditis elegans*. I Wild type growth and reproduction. Devel. Biol. 51: 23-33.

Calvo, J.M. and G. R. Fink, 1971 Regulation of biosynthetic pathways in bacteria and fungi. Ann. Rev. Biochem. 40: 943-968.

Carlson, E. A., 1966 The gene: A critical history. W. B. Saunders Company.

Carlson, P. S., 1971 A genetic analysis of the _rudimentary_ locus of _Drosophila melanogaster_. Genet. Res. Camb. 17: 53-81.

Catcheside, D. G., 1977 _The genetics of recombination_. University Park Press.

Catterall, J. F., J. P. Stein, E. C. Lai, S. L. C. Woo, A. Dugaiczyk, M.L. Mace, A. R. Means and B. W. O'Malley, 1978 The chick ovomucoid gene contain s at least six intervening sequences. Nature 275: 510-513.

Chovnick, A., A. Schalet, R. P. Kernaghan and J. Talsma, 1962 The resolving power of genetic fine structure analysis in higher organisms as exemplified by _Drosophila_. Am. Nat. 96: 281-296.

Chovnick, A., G. H. Ballantyne and D. G. Holm, 1971 Studies on gene conversion and its relationship to linked exchange in _Drosophila melanogaster_. Genetics 69: 179-209.

Chovnick, A., W. Gelbart, M. McCarron, B. Osmond, E. P. M. Candido and D. L. Baillie, 1976 Organization of the _rosy_ locus in _Drosophila melanogaster_: Evidence for a control element adjacent to the xanthine dehydrogenase structural element. Genetics 84: 233-55.

Chovnick, A., W. Gelbart and M. McCarron, 1977a Organization of the _rosy_ locus in _Drosophila melanogaster_. Cell 11: 1-10.

Chovnick, A., M. McCarron, A. Hilliker, J. O'Donnell, W. Gelbart and S. Clark, 1977b Gene organization in _Drosophila_. Cold Spring Harb. Symp. Quant. Biol. 42: 1011-1021.

Collins, J. and B. Holm, 1978 Cosmids: A type of plasmid gene-cloning vector that is packageable _in vitro_ in bacteriophage _lambda_ heads. Proc. Natl. Acad. Sci. U.S.A. 75: 4242-4246.

Coulondre, C. and J. H. Miller, 1977 Genetic studies of the _lac_ suppressor. IV. Mutagenic specificity in the _lacI_ gene of _Escherichia coli_. J. Mol. Biol. 117: 577-606.

Coulondre, C., J. H. Miller, P. J. Farabaugh and W. Gilbert, 1978 Molecular basis of base substitution hotspots in _Escherichia coli_. Nature 274: 775-780.

Crick, F. H. C., 1971 General model for the chromosomes of higher organisms. Nature 234: 25-27.

Crick, F. H. C., 1979 Split genes and RNA splicing. Science 204: 264-271.

Crick, F. H. C. and L. E. Orgel, 1964 The theory of interallelic complementation. J. Mol. Biol. 8: 161-65.

Darnell, Jr., J. E., 1979 Transcription units for mRNA production in eucaryotic cells and their viruses. Prog. Nucleic Acid Research and Mol. Biol. 22: 327-353.

Davidson, E. H., 1976 Gene activity in early development. Academic Press, N. Y.

Debell, J., J. S. del Castillo and U. Sanchez, 1963 Electrophysiology of the somatic muscles of Ascaris lumbricoides. J. Cell Comp. Physiol. 62: 159-165.

Deppe, E., E. Schierenberg, T. Cole, C. Krieg, D. Schmitt, B. Yoder and G. von Ehrenstein, 1978 Cell lineages of the embryo of the nematode Caenorhabditis elegans. Proc. Natl. Acad. SCi. U.S.A. 75: 376-380.

Doolittle, W. F., 1978 Genes in pieces: Were they ever together. Nature 272: 581-582.

Dunn, L. C., 1917 Nucleus and Cytoplasm as vehicles of heredity. Am. Nat. 51: 286-300.

Dunn, L.C., 1965 A short history of genetics. McGraw-Hill.

Ebashi, S. and F. Lipman, 1962 Adenosine triphosphate linked concentration of calcium ions in a particular fraction of rabbit muscle. J. Cell Biol. 14: 389-400.

Ebashi, S., F. Ebashi and A. Kodoma, 1967 Troponin as the Ca^{++} receptive protein in the contractile system. J. Biochem. 62: 137-138.

Emmons, S.W, M.R. Klass and D. Hirsh, 1979 Analysis of the constancy of DNA sequences during development and evolution of the nematode Caenorhabditis elegans. Proc. Natl. Acad. Sci. U.S.A. 76: 1333-1337.

Epstein, H. F., R. H. Waterston and S. Brenner, 1974 A mutant affecting the heavy chain of myosin in Caenorhabditis elegans. J. Mol. Biol. 90: 291-300.

Fincham, J. R. S., 1959 On the nature of glutamic dehydrogenase produced by inter-allelic complementation at the am locus of Neurospora crassa. J. Gen. Microbiol. 21: 600-611.

Fincham, J. R. S., 1966 Genetic complementation. W. A. Benjamin, Inc., N.Y.

Fink, G. R., 1966 A cluster of genes controlling three enzymes in histidine biosynthesis in Saccharomyces cerevisiae. Genetics 53: 445-459.

Fink, G. and G. A. Styles, 1974 Gene conversion of deletions in the HIS4 region of yeast. Genetics 77: 231-244.

Fogel, S. and R. K. Mortimer, 1969 Informatioal transfer in meiotic gene conversion. Proc. Natl. Acad. Sci. U.S.A. 62: 96-103.

Fristrom, J. W. and J. M. Rawls, 1975 A complex genetic locus that controls the first three steps of pyrimidine biosynthesis in Drosophila. Nature 255: 738-740.

Gans, M. 1953 Etude genetique et physiologique du mutant z de Drosophila melanogaster. Bull. Biol. Fr. Belg. Suppl. 38: 1-90.

Garcia-Bellido, A., 1979 Genetic analysis of the achaete-scute system of Drosophila. Genetics 91: 491-520.

Garen, A. and S. Garen, 1963 Complementation in vivo between structural mutants of alkaline phosphatase from E. coli. J. Mol. Biol. 7: 13-22.

Gelbart, W. M. and A. Chovnick, 1979 Spontaneous unequal exchange in the rosy region of Drosophila melanogaster. Genetics (in press).

Georgiev, G. P., 1969 On the structural organization of operon and the regulation of RNA synthesis in animal cells. J. Theoret. Biol. 25: 473-490.

Gergely, J., 1972 Muscle. pg. 387-406. In Basic neurochemistry. (eds.) R. W. Albers, G. S. Siegel, R. Katzman and B. W. Agranoff. Little, Brown Co. Boston

Gilbert, W., 1978 Why genes in pieces? Nature 271: 501.

Gilmour, R. S., P. R. Harrison, J. D. Windass, N. A. Affara and J. Paul, 1973 Globin messenger RNA synthesis and processing during haemoglobin induction in Friend cells. I. Evidence for transcriptional control in clone M2. Cell Differ. 3: 9-22.

Glover, D. M. and D. S. Hogness, 1977 A novel arrangement of the 18s and the 28s sequences in a repeating unit of Drosophila melanogaster rDNA. Cell 10: 167-176.

Goldschmidt, R., 1918 A preliminary report on some genetic experiments concerning evolution. Am. Nat. 52: 28-50.

Goldschmidt, R., 1938 Physiological genetics. McGraw-Hill. N. Y.

Goldschmidt, R., 1956 Portraits from memory. Recollections of a zoologist. University of Washington Press. Seattle.

Goodman, H. M., M. V. Olson and B. Hall, 1978 Nucleotide sequence of a mutant eukaryotic gene: The yeast tyrosine-inserting ochre suppressor SUP4-o. Proc. Natl. Acad. Sci. U.S.A. 74: 5453-5457.

Gould, S. J., 1977 Ontogeny and phylogeny. The Belknap press of Harvard University press. Cambridge, Mass.

Groudine, M. and H. Weintraud, 1975 Rous sarcoma virus activates embryonic globin genes in chicken fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 72: 4464-4468.

Grouse, L., M. D. Chilton annd B. J. McCarthy, 1972 Hybridization of ribonucleic acid with unique sequences of mouse deoxyribonucleic acid. Biochemistry 11: 798-805.

Gurdon, J. B., 1974 The control of gene expression in animal development. Harvard University press. Cambridge, Mass.

Hadorn, E., 1961 Developmental genetics and lethal factors. London: Methuen and Co. Ltd.

Harris, H. E. and H. F. Epstein, 1977 Myosin and paramyosin of C. elegans. Biochemistry and structural properties of wild-type and mutant proteins. Cell 10: 709-719.

Harris, H. E., Man-Yin W. Tso and H. F. Epstein, 1977 Actin and myosin linked calciun regulation in the nematode C. elegans. Biochemical and structural properties of native filaments and purified proteins. Biochemistry 16: 859-865.

Hastings, P. J., 1975 Some aspects of recombination in eukaryotic organisms. Ann. Rev. Genet. 9: 129-144.

Henderson, S. A., R. C. Woodruff and J,. N. Thompson, 1978 Spontaneous chromosome breakage at male meiosis associated with male recombination in Drosophila melanogaster. Genetics 88: 93-107.

Herman, R.K., 1978 Crossover suppressors and balanced recessive lethals in Caenorhabditis elegans. Genetics 88: 49-65.

Herman, R. K., D. G. Albertson and S. Brenner, 1976 Chromosome rearrangements in Caenorhabditis elegans. Genetics 83: 91-105.

Herskowitz, I. H. 1965 Genetics. Second edition. Little, Brown and Co. Boston.

Hiraizumi, Y., 1971 Spontaneous recombination in Drosophila melanogaster males. Proc. Natl. Acad. Sci. U.S.A. 68: 268-270.

Hiraizumi, Y., B. Slatko, C. Langley and A. Nill, 1973 Recombination in Drosophila melanogaster male. Genetics 73: 439-444.

Hirsh, D., D. Oppenheim and M. Klass, 1976 Development of the reproductive system of Caenorhabditis elegans. Develop. Biol. 49: 200-219.

Hochman, B., 1971 Analysis of chromosome 4 in Drosophila melanogaster. II. Ethyl methane-sulfonate induced lethals. Genetics 67: 235-252.

Hochman, B., 1976 The fourth chromosome of Drosophila melanogaster. pg. 903-928. In The genetics and biology of Drosophila. (eds.) M. Ashburner and E. Novitski. 1A. Academic press. N. Y.

Hunt, J. A., 1974 Rate of synthesis and half-life of globin messenger ribonucleic acid. Rate of synthesis of globin messenger ribonucleic acid calculated from data of cell haemoglobin content. Biochem. J. 138: 499-510.

Hurst, D. D., S. Fogel and R. K. Mortimer, 1972 Conversion-associated recombination in yeast. Proc. Natl. Acad. Sci. U.S.A. 69: 101-105.

Huxley, H. E., 1963 EM studies on the structure of natural and synthetic protein filaments from striated muscle. J. Mol. Biol. 7: 281-308.

Huxley, H. E., 1972 Muscle 1972: Progress and problems. Cold Spring Harbor Symp. Quant. Biol. 37: 689-693.

Hynes, M. J., 1975 A cis-dominant regulatory mutation affecting enzyme induction in the eucaryote Aspergillus nidulans. Nature 253: 210-212.

Hynes, M. J, 1979 Fine-structure mapping of the acetamidase gene and its controlling region in Aspergillus nidulans. Genetics 91: 381-92.

Jack, J. W. and B. H. Judd, 1979 Allelic pairing and gene regulation: A model for _zeste-white_ interaction in _Drosophila melanogaster_. Proc. Natl. Acad. Sci. U.S.A. 76: 1368-1372.

Jacob, F., 1973 _The logic of life_. (Betty Spillman, translator). First Vintage books edition. N.Y.

Jacob, F. and J. Monod, 1961 Genetic regulatory menchanisms in the synthesis of proteins. J. Mol. Biol. 3: 318-356.

Jeffreys, A. J. and R. A. Flavell, 1977 The rabbit B-globin gene contains a large insert in the coding sequence. Cell 12: 1097-1108.

Jarry, B. and D. Falk, 1974 Functional diversity within the _rudimentary_ locus of _Drosophila melanogaster_. Molec. Gen. Genet. 135: 113-122.

Judd, B. H. 1961 Formation of duplication-deficiency products by asymmetrical exchange within a complex locus of _Drosophila melanogaster_. Proc. Natl. Acad. Sci. U.S.A. 47: 545-550.

Judd, B. H. 1976, Genetic units of _Drosophila_ complex loci. pg. 767-799. In _The Genetics and Biology of Drosophila_. (eds.) M. Ashburner and E. Novitski. Academic press. N. Y.

Judd, B. H., M. W. Shen and T. C. Kaufman, 1972 The anatomy and function of a segment of the X-chromosome of _Drosophila melanogaster_. Genetics 71: 139-156.

Kapular, A. M. and H. Bernstein, 1963 A molecular model for an enzyme based on a correlation between the genetic and complementation map of the locus specifying the enzyme. J. Mol. Biol. 6: 443-451.

Kendrick-Jones, J., W. Lehman and A. G. Szent-Gyorgyi, 1970 Regulation in molluscan muscles. J. Mol. Biol. 54: 313-326.

Kimble, J. and D. Hirsh, 1979 The postembryonic cell lineage of the hermaphrodite and male gonads of _Caenorhabditis elegans_. Dev. Biol. 70: 396-417.

Kirschner, K. and H. Bisswanger, 1976 Multifunctional proteins. Ann. Rev. Biochem. 45: 143-166.

Knapp, G., J. S. Beckman, P. F. Johnson, S. A. Fuhrman and J. Abelson, 1978 Transcription and processing of intervening sequences in yeast tRNA genes. Cell 14: 221-236.

Konopka, R. J. and S. Benzer, 1971 Clock mutants of Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A. 68: 2112-2116.

Kornberg, A., 1976 For the love of enzymes. pg. 243-251 In: Reflections on biochemistry in honor of Severo Ochoa. Eds. A. Kornberg, L. Cornudella, B. L. Horecker and J. Oro. Pergamon press. London.

Lai, C. J. and G. Khoury, 1979 Deletion mutants of sv40 defective in biosynthesis of late viral mRNA. Proc. Natl. Acad. Sci. U.S.A. 76: 71-75.

Lai, E. C., S.L.C. Woo, A. Dugaiczyk and B. W. O'Malley, The ovalbumin gene: Alleles created by mutations in the intervening sequences of the natural gene. Cell 16: 201-211.

Lawn, R. M., E. F. Fritsch, R. C. Parker, G. Blake and T. Maniatis, 1978 The isolation and characterization of linked g and b globin genes from a cloned library of human DNA. Cell 15: 1157-74.

Lefevre Jr., G., 1976 A photographic representative and interpretation of the polytene chromosomes of Drosophila melanogaster salivary chromossomes. pg. 32-66. In The genetics and biology of Drosophila. 1A. (eds.) M. Ashburner and E. Novitski. Academic press. N. Y.

Lehman, W., J. Kendrick-Jones and A. G. Szent-Gyorgyi, 1972 Myosin-linked regulatory systems: Comparative studies. Cold Spring Harbor Symp. Quant. Biol. 37: 319-330.

Lehman, W. and A. G. Svent-Gyorgi, 1975 Regulation of molluscan contraction. J. Gen. Physiol. 66: 1-30.

Lewis, E. B., 1951 Pseudoallelism and gene evolution. Cold Spring Harbor Symp. Quant. Biol. 16: 159-174.

Lewis, F. B. 1963 Genes and developmental pathways. Amer. Zool. 3: 33-56.

Lifschytz, E. and M. M. Green, 1979 Genetic identification of dominant overproducing mutations: The Beadex gene. Molec. gen. Genet. 171: 153-159.

Lissouba, P., J. Mousseau, G. Rizet and J. L. Rossignol, 1962 Fine structure of genes in the ascomycetes Ascobolus immersus. Adv. in Genet. 11: 343-380.

Lowey, S., H. S. Slayter, A. G. Weeds and H. Baker, 1969 Substructure of the myosin molecule. I. Subfragments of myosin by enyzymatic degradation. J. Mol. Biol. 42: 1-29.

McCarron, M. J., J. O'Donnell, A. Chovnick, B. S. Bhullar, J. Hewitt and E. P. M. Candido, 1979 Organization of the rosy locus in Drosophila melanogaster: Further evidence in support of a cis-acting control element adjacent to the xanthine dehydrogenase structural element. Genetics 91: 275-293.

McClintock, B., 1957 Controlling elements and the gene. Cold Spring Harbor Symp. Quant. Biol. 21: 197-216.

McGavin, S., 1968 Interallelic complementation and allostery. J. Mol. Biol. 37: 239-242.

Mackenzie, Jr., J. M., F. Schachat and H. F. Epstein, 1978 Immunocytochemical localization of two myosins within the same muscle cells in C. elegans. Cell 15: 413-419.

MacLennan, D. H., C. C. Yip, G. H. Iles and P. Seeman, 1972 Isolation of sarcoplasmic reticulum proteins. Cold Spring Harbor Symp. Quant. Biol. 37: 469-477.

MacLennan, D. H. and P. C. Holland, 1975 Calcium transport in sarcoplasmic reticulum. Ann. Rev. Biophys. Bioeng. 4: 377-404.

MacLeod, S., R. H. Waterston, R. M. Fishpool and S. Brenner, 1977 Identification of the structural gene for a myosin heavy chain in Caenorhabditis elegans. J. Mol. Biol. 114: 133-140.

MacLeod, A. R., R. H. Waterston and S. Brenner, 1977 An internal deletion mutant of a myosin heavy chain in Caenorhabditis elegans. Proc. Natl. Acad. Sci. U.S.A. 74: 5336-5340.

Mandel, J. L., R. Breathnach, P. Gerlinger, M. LeMeur, F. Gannon and P. Chambon, 1978 Organization of coding and intervening sequences in the chicken ovalbumin gene. Cell 14: 641-653.

Manney, T. R. and R. K. Mortimer, 1964 Allelic mapping in yeast by X-ray induced mitotic reversion. Science 143: 581-583.

Mannherz, H. G. and R. S. Goody, 1976 Proteins of the contractile system. Ann. Rev. Biochem. 45: 427-465.

Markert, C. L. annd H. Ursprung, 1971 Developmental genetics. Princeton-Hall Inc. N. J.

Martonosi, A., 1971 The structure and function of cytoplasmic reticulum membranes. Biomembranes 1: 191-256.

Maxam, A. M. and W. Gilbert, 1977 A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. 74: 560-564.

Meneely, P. M. and R. K. Herman, 1979 Lethals, steriles and deficiencies in a region of the X-chromosome of _Caenorhabditis elegans_. Genetics 92: 99-115.

Miller, J. H., 1978 The _lacI_ gene: Its role in _lac_ operon control and its use as a genetic system. pg. 31-88. In _The Operon_ (eds.) J. H. Miller and W. S. Reznikoff. Cold Spring Harbor Laboratory.

Miller, J. H. and W. S. Reznikoff (eds.) 1978 _The operon_. Cold Spring Harbor Laboratory.

Mirsky, A. E. and H. Ris. 1949 Variable and constant components of chromosomes. Nature 163: 666-667.

Mitchell, M. B., 1955 Aberrant recombination of pyridoxine mutants of _Neurospora_. Proc. Natl. Acad. Sci. U.S.A. 41: 215-220.

Moerman, D. G. and D. L. Baillie, 1979 Genetic organization in _Caenorhabditis elegans_: Fine-structue analysis of the _unc-22_ gene. Genetics 91: 95-103.

Moerman, D. G. and D. L. Baillie, 1979 Formaldehyde mutagenesis in _Caenorhabditis elegans_. In press.

Moore, C. W. and F. Sherman, 1974 Lack of correspondence between genetic and physical distances in the iso-1-cytochrome c gene of yeast. pg. 295-306. In _Mechanisms in recombination_. ed. R. F. Grell. Plenum press. N. Y.

Moore, C. And F. Sherman, 1975 Role of DNA sequences in genetic recombination in the iso-1-cytochrome c gene of yeast. I. Discrepancies between physical distances and genetic distances determined by five mapping techniques. Genetics 79: 397-418.

Moore, C. and F. Sherman, 1977 Role of DNA sequences in genetic recombination in the iso-1-cytochrome c gene of yeast. II. Comparisons of mutants altered at the same and nearby base pairs. Genetics 85: 1-22.

Moos, C., G. Offer, R. Starr, And P. Bennett, 1975 Interaction of C-protein with myosin, myosin rod and light meromyosin. J. Mol. Biol. 97: 1-9.

Morgan, T. H., 1914 No crossing over in the male of Drosophila of genes in the second and the third pairs of chromosomes. Biol. Bull. 26: 195-204.

Morgan, T. H., 1934 Embryology and genetics. Princeton University press, Princeton.

Muller, H. J., 1925 The regionally differential effect of X-rays on crossing over in autosomes of Drosophila. Genetics 10: 470-507.

Muller, H. J., 1935 A viable two-gene deficiency phenotypically resembling the corresponding hypomorphic mutations. J. Heredity. 26: 469-478.

Muller, H. J., 1950 Evidence of the precision of genetic adaptation. Harvey lecture series XLIII 165-229.

Muller, H. J. and A. A. Prokofyeka, 1935 The individual gene in relation to the chromomere and the chromosome. Proc. Natl. Acad. Sci. U.S.A. 21: 16-26.

Nigon, V. 1965 Development et reproduction des nematodes. In Traite de Zoologie, Tome IV. (ed.) P. P. Grasse. Masson et C., Paris.

Nigon, V. and E. C. Dougherty, 1950 A dwarf mutation in a nematode. J. Heredity 41: 103-109.

Nomura, M. and S. Benzer, 1961 The nature of the 'deletion' mutants in the rII region of phage T4. J. Mol. Biol. 3: 684-692.

Norby, S. 1970 A specific nutritional requirement for pyrimidines in rudimentary mutants of Drosophila melanogaster. Hereditas 66: 205-214.

Norkin, L. C., 1970 Marker-specific effects in genetic recombination. J. Mol. Biol. 51: 633-655.

O'Donnell, J., H. C. Mandel, M. Kraus and W. Sofer, 1977 Genetic and cytogenetic analysis of the adh region in Drosophila melanogaster. Genetics 86: 553-566.

O'Farrell, P. Z., B. Cordell, P. Valenzuela, W. J. Rutter and H. M. Goodman, 1978 Structure and processing of yeast tRNA containing intervening sequences. Nature 274: 438-445.

Offer, G., 1972 C-protein and the periodicity in the thick filaments of vertebrate skeletal muscle. Cold Spring Harbor Symp. Quant. Biol. 37: 87-93.

Oliver, C. P. 1940 A reversion to wild-type associated with crossing-over in Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A. 26: 452-454.

Palmiter, R. D., 1973 Rate of ovalbumin messenger ribonucleic acid synthesis in the oviduct of estrogen-primed chicks. J. Biol. Chem. 248: 8260-8270.

Parker, J. H. and F. Sherman, 1969 Fine-structure mapping and mutational studies of genes controlling yeast cytochrome c. Genetics 62: 9-22.

Plough, H. H., 1917 The effect of temperature on crossing over in Drosophila. J. Exptl. Zool. 24: 147-210.

Pontecorvo, G., 1958 Trends in genetic aanalysis. Columbia University press. New York and London.

Potter, S. S. and C. A. Thomas, 1977 The two-dimensional fractionation of Drosophila DNA. Cold Spring Harbor Symp. Quant. Biol. 42: 1023-1031.

Potter, S.S., W. J. Borein Jr., P. Dunsmuir and G. M. Rubin, 1979, Transposition of elements of the 412, copia and 297 dispersed repeated gene families in Drosophila. Cell 17: 415-27.

Rhoades, R. E., G. S. McKnight and R. T. Sshimke, 1973 Quantitative measurement of ovalbumin messenger ribonucleic acid activity. Localization in polysomes, induction by estrogen and effect of actinomycin D. J. Biol. Chem. 248: 2031-2039.

Riddle, D. L., 1977 A genetic pathway for dauer larva formation in Caenorhabditis elegans. Stadler Genet. Symp. 9: 101-119.

Riddle, D. L., 1978 The genetics of development and behavior in Caenorhabditis elegans. J. Nematology 10: 1-15.

Riddle, D. L. and S. Brenner, 1978 Indirect suppression in Caenorhabidits elegans. Genetics 89: 299-314.

Robertson, M. A., R. Straden, Y, Tanaka, J. F. Catterall, B. W. O'Malley and G. G. Brownlee, 1979 Sequence of three introns in the chick ovalbumin gene. Nature 278: 370-372.

Roman, H., 1956 Studies of gene mutations in _saccharomyces_. Cold Spring Harbor Symp. Quant. Biol. 21: 175-185.

Roman, H., 1963 In, _Methodology in basic genetics_. (ed) W. J. Burdette. San Francisco. Holden-Day.

Roman, H. and F. Jacob, 1958 A comparison of spontanecus and ultra-violet induced allelic recombination with reference to recombination of outside markers. Cold Spring Harbor Symp. Quant. Biol. 23: 155-160.

Ronen, A. and Y. Salts, 1971 Genetic distances separating adjacent base pairs in bateriophage _T4_. Virology 45: 496-502.

Royal, A., A. Garapin, B. Cami, F. Perrin, J. L. Mandel, M. LeMeur, F. Bregegegre, F. Gannon, J. P. LePeennec, P. Chambcn and P. Kourilsky, 1979 The ovalbumin gene region: Common features in the organization of three genes expressed in chicken oviduct under hormonal control. Nature 279: 125-132.

Rose, A. M. and D. L. Baillie, 1979 The effect of temperature and parental age on recombination and nondisjunction in _Caenorhabditis elegans_. Genetics 92: 409-418.

Rose, A. M. and D. L. Baillie, 1979 A mutation in _Caenorhabditis elegans_ that icreases recombination frequency more than threefold. Nature 281: 599-600.

Rudkin, G. T., 1965 The relative mutabilities of DNA in regions of the X-chromoscme of _Drosophila melanogaster_. Genetics 52: 665-681.

Sakano, H., J. H. Rogers, K. Huppi, C. Brack, A. Traunecker, R. Maki, R. Wall and S. Tonegawa, 1979 Domains and the hinge region of an immunoglobulin heavy chain are encoded in separate DNA segments. Nature 277: 627-633.

Sanger, F., G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddles, C. A. Hutchinson III, P. M. Slocombe and M. Smith, 1977 Nucleotide sequence of bacteriophage _phix 174_ DNA. Nature 265: 687-695.

Schachat, F. H., H. E. Harris and H. F. Epstein, 1977 Two homogeneous myosins in body-wall muscle of _C. elegans_. Cell 10: 721-728.

Schachat, F., R. L. Garcea, and H. F. Epstein, 1978 Myosins exist as homodimers of heavy chains: Demonstration with specific antibody purified by nematode mutant myosin affinity chromatography. Cell 15: 405-411.

Schachat, F., D. J. O'Connor and H. F. Epstein, 1978 The moderately repetitive DNA sequences of Caenorhabditis elegans do not show short period interspersion. Biochim Biophys. Acta. 520: 668-692.

Sherman, F. J., W. Stewart, C. Helms and J. A. Downie, 1978 Chromosome mapping of the CYC7 gene determining yeast iso-2-cytochrome c : Structural and regulatory regions. Proc. Natl. Acad. Sci. U.S.A. 75: 1437-1441.

Sinsheimer, R. L., 1977 Recombinant DNA. Ann. Rev. Biochem. 46: 415-438.

Slizynska, H., 1957 Cytological analysis of formaldehyde induced chromosomal changes in Drosophila melanogaster. Proc. Roy. Soc. Edinb. B, 66, 288-304.

Smith, P. D., V. G. Finnerty and A. Chovnick, 1970 Gene conversion in Drosophila: Non-reciprocal events at the maroon-like cistron. Nature 228: 442-444.

Sonnenblick, P. 1950 The early embryology of Drosophila melanogaster. pg. 62-167. In Biology of Drosophila. (ed.) M. Demerec. Hafner.

Spradling, A. C., G. L. Waring and A. P. Mahowald, 1979 Drosophila bearing the Ocelliless mutation underproduce two major chorion proteins both of which map near this gene. Cell 16: 609-616.

Squire, J. M., 1975 Muscle filament structure and muscle contraction. Ann Rev. Biophys. Bioeng. 4: 137-163.

Starr, J. and G. Offer, 1973 Polarity of the myosin molecule. J. Mol. Biol. 81: 17-31.

Stern, C., 1936 Somatic crossing over and segregation in Drosophila melanogaster. Genetics 21: 625-730.

Strobel, E., P. Dunsmuir and G. M. Rubin, 1979 Polymorphisms in the chromosomal locations of elements of the 412, copia and 297 dispersed repeated gene families. Cell 17: 429-39.

Sulston, J. E. and S. Brenner, 1974 The DNA of Caenorhabditis elegans. Genetics 77: 95-104.

Sulston, J. E., M. Dew and S. Brenner, 1975 Dopaminergic neurons in the nematode *Caenorhabditis elegans*. J. Comp. Neurol. 163: 215-226.

Sulston, J. E. and H. R. Horvitz, 1977 Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. Develop. Biol. 56: 110-156.

Thompson, P. E., 1964 Evidence on the basis on the centromere effect in the large autosomes of *Drosophila melanogaster*. Genetics 49: 761-769.

Thompson Jr., J. N., M. Ashburner and R. C. Woodruff, 1977 Presumptive control mutations for alcohol dehydrogenase in *Drosophila melanogaster*. Nature 270: 363.

Tiemeier, D. C., S. M. Tilghman, F. I. Polsky, J. G. Seidman, A. Leder, M. H. Edgell and P. Leder, 1978 A comparison of two cloned mouse B-globin genes and their surrounding and intervening sequences. Cell 14: 237-245.

Tilghman, S. M., D. C. Tiemeier, J. G. Seidman, B. M. Peterlin, M. Sullivan, J. Maizel and P. Leder, 1978 Intervening sequence of DNA identified in structural portion of a mouse B-globin gene. Proc. Natl. Acad. Sci. U.S.A. 75: 725-729.

Tilgman, S. M., P. J. Curtis, D. C. Tiemeier, P. Leder and C. Weisman, 1978 The intervening sequence of a mouse B-globin gene is transcribed within the 15s B-globin mRNA precursor. Proc. Natl. Acad. Sci. U.S.A. 75: 1309-1313.

Tonegawa, S., A. Maxam, R. Tizard, O. Bernard and W. Gilbert, 1978 Sequence of a mouse germ-line gene for a variable region of an immunoglobulin light chain. Proc. Natl. Acad. Sci. U.S.A. 75: 1485-1489.

Valenzuela, P., A. Vennegas, F. Weinberg, R. Bishop and W. J. Rutter, 1978 Structure of yeast phenylalanine-tRNA genes: An intervening DNA segment within the region coding for tRNA. Proc. Natl. Acad. Sci. U.S.A. 75: 190-194.

Van den Berg, J., A. von Ooyen, N. Martei, A. Schambrock, G. Grosveld, R. A. Flavell and C. Weisman, 1978 Comparison of cloned rabbit and mouse B-globin genes showing strong evolutionary divergence of two homologous pairs of introns. Nature 276: 37-44.

Ward, S. and J. Miwa, 1978 Characterization of a temperature sensitive fertilization-defective mutants of the nematode Caenorhabditis elegans. Genetics 88: 285-303.

Waterston, R. H., R. M. Fishpool and S. Brenner, 1977 Mutants affecting paramyosin in Caenorhabditis elegans. J. Mol. Biol. 117: 679-697.

Waterston, R. H. and S. Brenner, 1978 A suppressor mutation in the nematode acting on specific alleles of many genes. Nature 275: 715-719.

Watson, J. D., 1976 Molecular biology of the gene. 3 ed. W. A. Benjamin Inc.

Weber, A., 1966 Energized calcium transport and relaxing factors. In Current topics in Bioenergetics. vol. 1 (ed.) D. R. Sanadi. Academic. N. Y.

Weber, A. and S. Winicur, 1961 The role of calcium in the superprecipitation of actomyosin. J. Biol. Chem. 236: 3198-3202.

Weisman, A., 1904 The evolution theory, vol. 2, The biogenetic law. Edward Arnold. London.

Welshons, W.J., 1965 Analysis of a gene in Drosophila. Science 150: 1122-1129.

Welshons, W. J., 1974 The cytogenetic analysis of a fractured gene in Drosophila. Genetics 76: 775-794.

Welshons, W. J. and D. O. Keppy, 1975 Intragenic deletions and salivary band relationships in Drosophila. Genetics 80: 143-150.

White, J. G., E. Southgate, J. N. Thomson and S. Brenner, 1976 The structure of the ventral nerve cord of Caenorhabditis elegans. Phil. Trans. Roy. Soc. B, 275: 328-348.

Woodruff, R. C. and M. Ashburner, 1979 The genetics of a small autosomal region of Drosophila melanogaster containing the structural gene for alcohol dehydrogenase. II. Lethal mutations in the region. Genetics 92: 133-149.

Yanofsky, C., 1963 Amino acid replacements associated with mutation and recombination in the A gene and their relationship to in vitro coding data. Cold Spring Harbor Symp. Quant. Biol. 28: 581-588.

Yanofsky, C., B. C. Carlton, J. R. Guest, D. R. Helinski and U. Hennning, 1964 On the colinearity of gene structure and protein structure. Proc. Natl. Acad. Sci. U.S.A. 51: 266-272.

Young, M., M. H. Blanchard and D. S. Brown, 1968 Selective nonenzymatic cleavage of the myosin rod: Isolation of the coiled-coil a rope selection from the C-terminus. Proc. Natl. Acad. Sci. 61: 1087-94.

Young, M. W. and B. H. Judd, 1978 Nonessential sequences, genes, and the polytene chromosome bands of Drosophila melanogaster. Genetics 88: 723-742.

Yourno, J., T. Kohno and J. R. Roth, 1970 Enzyme evolution: Generation of a bifunctional enzyme by fusion of adjacent genes. Nature 228: 820-824.