ESSENTIAL GENES IN A REGION OF CHROMOSOME I IN *CAENORHABDITIS ELEGANS*

By

ANN MARIE HOWELL

B.Sc., Simon Fraser University, 1983

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

GENETICS PROGRAMME

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

May 1989

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Medical Genetics

The University of British Columbia
Vancouver, Canada

Date May 31, 1989

ABSTRACT

This thesis describes the identification and characterization of essential genes in a small region of the genome of the nematode *Caenorhabditis elegans*. The region analyzed was defined by a 1.2 map unit deficiency of chromosome I, *hDf6*. In order to do this, a system for recovering and analyzing a large number of autosomal recessive lethal mutations was developed. This system used a free duplication of the left third of chromosome I, *sDp2*. Lethal mutations were maintained with two mutant alleles on the normal chromosomes and a wild-type allele on the duplication. More than 31,000 chromosomes mutagenized with ethyl methane sulfonate were screened using the *sDp2* system and 495 lethal mutations were recovered. Two translocations involving chromosome I (*hT1(I;V)* and *szT1(I;X)*) were also used to recover lethal mutations. It was discovered that heterozygotes for one of these translocations, *szT1(I;X)*, exhibited an increased frequency of recombination adjacent to the chromosome I breakpoint. This had not been observed previously with other translocations. The increase in recombination frequency may be the result of a disruption of the normal regulation of recombination in the region.

Recombination mapping was used to position 59 EMS-induced *sDp2*-recovered lethal mutations. Deficiency mapping, duplication mapping, and *inter se* complementation analyses were used to define 36 essential genes to the left of the *dpy-5* gene on chromosome I. Nineteen of these genes, defined by 54 lethal mutations, were uncovered by the deficiency *hDf6*. A small gamma radiation-induced deficiency, *hDf7*, was identified and found to uncover six of the essential genes in *hDf6*. Two duplications which have breakpoints in the *hDf6* region, *hDp3* and *hDp25*, were also used to position essential genes. The genes in *hDf6* were thus positioned into five regions based on the breakpoints of these three other chromosomal rearrangements.

The stage of lethal arrest was determined for the lethal mutations in *hDf6*. Mutations in genes in the left portion of *hDf6* arrest development at an earlier stage than do mutations in genes in the right portion. As a whole, the *hDf6* region seems to have a lower proportion of early arresting mutations than other comparably sized regions of the genome.

Ten of the 19 genes in *hDf6* were represented by more than one lethal allele. One gene, *let-354*, was found to be an extremely mutable target since it was represented by seventeen alleles. A truncated Poisson analysis of the allele distribution indicated that the 19 genes identified represented a maximum of 75% of the essential genes predicted to be in *hDf6*. Extrapolating from this region to the entire genome, an estimate of 4,000 essential genes was obtained.

The average forward mutation rate was determined to be 5×10^{-5} mutations per gene. It was estimated that 60,000 chromosomes mutagenized with 0.015 M EMS must be screened to identify mutationally most of the genes in any region of the genome. Thus, it would require the screening of another 30,000 mutagenized chromosomes to identify genetically almost every gene in the *hDf6* region of *C. elegans*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

"The ease of handling of the nematode coupled with its small genome size suggests it is feasible to look for mutants in all of the genes to try to discover how they participate in the development and functioning of a simple multicellular organism."

(Brenner, 1974).

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my research supervisor, Dr. Ann Rose, for her support, advice and encouragement. I would like to thank my supervisory committee for helpful discussions; Dr. D. L. Baillie, Dr. D. Holm and Dr. R. McMaster. I wish to give lots of thanks to all of my colleagues in the Rose lab, past and present, especially Terry Starr (we made it!), Kim McKim, Joe Babity and Ken Peters, and anyone who ever had to help with a lethal screen. Thanks also to the worm people at SFU, especially Raja Rosenbluth. I gratefully acknowledge the technical assistance of Ayesha Hassan, John Kam, Rohinish Kisun, Pearly Lee, and Kelly McNeil. I would like to thank my family, especially my parents, for their encouragement and faith in my abilities. I will always be grateful for the love and support of my friends who have stuck with me through it all; Rohinish, Kingsley, Lorri, Diane, and Rene. This research was financially supported by a University Graduate Fellowship and a Medical Research Council of Canada Studentship.

I. INTRODUCTION

One of the major problems in biology is the determination of how genes are organized in eukaryotic genomes. There are several possible explanations for the arrangements of genes. The linkage of some sets of genes may have a selective advantage to the organism, or the linkage may simply be an evolutionary artefact. The organization of genes may be involved in the regulation of expression required to allow development of a higher eukaryote. Little is understood concerning the coordinated regulation of large networks of genes involved in producing differentiated tissue types. The arrangement of genes along a chromosome may also be related to the ways chromatin is packaged during meiosis. An understanding of how these or other mechanisms are involved in organizing eukaryotic genomes will require the description of many different genomic regions.

A complete description of the organization of the genome of an organism would facilitate investigations of the relevance of the mechanisms mentioned above. A complete description would allow for an extensive study of networks of gene regulation. The genetic description would include the identification of all genes, their regulatory regions and sites affecting chromosome behaviour. One approach by which the genome could be described would be to identify mutationally all of the genes essential for development and fertility. In the genetically characterized regions of the *Drosophila melanogaster* genome, most of the genes identified were essential genes (for example, Hilliker *et al.*, 1980; Hochman, 1971; Judd, Shen and Kaufman, 1972; Lasko and Pardue, 1988; Leicht and Bonner, 1988; Marchant and Holm, 1988; Woodruff and Ashburner, 1979; Wright, 1987).

The small free-living soil nematode *Caenorhabditis elegans* is an excellent model system for the study of eukaryotic genome organization (Brenner, 1974). (A brief summary of *C. elegans* nomenclature can be found in Materials and Methods). A unique opportunity exists with *C. elegans* to describe genetically large portions of the genome. The worms are easy to grow and maintain in the laboratory and strains can be kept frozen in liquid nitrogen for many years. In

Drosophila melanogaster, the maintenance of large numbers of strains is difficult because they can not be recovered after freezing.

The generation time of *C. elegans* is three and one half days at 20 ^{O}C. A wild-type hermaphrodite will produce an average of 350 progeny by self-fertilization. Hermaphrodites have five pairs of autosomes and two X chromosomes. Males have one X chromosome and are produced spontaneously by X-chromosome nondisjunction (Hodgkin, Horvitz and Brenner, 1979) at a frequency of 0.1% at 20 ^{O}C (Rose and Baillie, 1979). One half of the progeny resulting from mating a male to a hermaphrodite are male. These features make *C. elegans* extremely amenable to genetic analysis. The genetic system is well developed; there were approximately 800 genes on the most recently published genetic map (Edgley and Riddle, 1987).

The organism is composed of highly differentiated tissue types; muscle, nervous system, intestine, cuticle, *etc.* There is a small number of cells in the adult (less than 1,000 in an adult hermaphrodite). Since the worms are transparent, cell divisions can be observed in living animals. The entire embryonic and post-embryonic cell lineages have been described (Sulston and Horvitz, 1977; Sulston *et al.*, 1983). The patterns of cell divisions are practically invariant between wild-type individuals. This allows a precise determination of the effects of mutations on development of the organism.

A physical map of the entire *C. elegans* genome is being constructed (Coulson *et al.*, 1986, 1988) using cosmid contigs (sets of overlapping cosmid clones) and clones in yeast artificial chromosome vectors. This map is near completion and soon cloned DNA from any region of interest will be available. In many regions of the genome, the physical and genetic maps are being aligned (for example, Greenwald *et al.*, 1987; Prasad, 1988; Starr *et al.*, 1989). If a complete genetic map of the *C. elegans* genome were also available, a completely colinear genomic map could be created. The purpose of the work described in this thesis was to identify essential genes in a small autosomal region in *Caenorhabditis elegans*.

A. Studies of Genome Organization

Because of their small size, bacteriophage genomes were the first to be completely described genetically (for example T4 reviewed by Mathews *et al.*, (1983), and lambda reviewed by Hershey, (1971)). This small size also permitted rapid molecular analysis. For example, the bacteriophage lambda genome of almost 50,000 base pairs has been completely sequenced (Hendrix *et al.*, 1983). It follows from these studies that an ideal organism for the study of genome organization requires a small genome and a powerful genetic system. *E. coli* is a good example. The entire genome has been cloned and restriction mapped (Kohara, Akiyama and Isono, 1987; Smith *et al.*, 1987) and approximately half of its genes have been identified (Bachmann, 1983). *Saccharomyces cerevisiae* also has a small genome suitable for cloning and a powerful genetic system, but progress towards identification of all of the genes is lagging behind *E. coli*.

In *E. coli*, *S. cerevisiae*, *D. melanogaster*, and *C. elegans*, investigations of genome organization have been carried out at several levels of resolution. Studies of individual genes have shown that there are some similarities and some differences between the structures and methods of regulation of gene expression of prokaryotic and eukaryotic genes. The lack of polycistronic messages in eukaryotes showed that genes were not grouped into operons as had been described by Jacob and Monod (1961) for *E. coli*. However, both prokaryotic and eukaryotic genes are transcriptionally regulated by *cis*-linked regulatory regions and *trans*-acting factors. The lac operon system in *E. coli* is a classic example of transcriptionally regulated gene expression (reviewed by Miller and Reznikoff (1978)). A regulatory element was identified genetically by mutations at the 5′ end of the *rosy* (xanthine dehydrogenase) gene in *D. melanogaster* which result in over-production or under-production of the *rosy* gene product (Chovnick *et al.*, 1976; McCarron *et al.*, 1979). Some genes in both eukaryotes and prokaryotes have more than one promoter to allow for more flexibility in regulation (reviewed by Schibler and Sierra, 1987). Many eukaryotic genes have been shown to be regulated by transcription factors which bind to specific DNA sequences known generally as enhancers (reviewed by Schaffner, Serfling and Jasin, 1985). Tissue-specific

regulation of some genes has been shown to be controlled by the binding of tissue-specific transcription factors; for example, a B-cell specific transcription factor binds to an enhancer in an immunoglobulin heavy chain gene (Banerji, Olson and Schaffner, 1983; Gillies *et al.*, 1983).

Eukaryotic mRNAs are capped and polyadenylated. The choice of poly A addition site can be a step in regulation of gene expression (reviewed by Friedman, Imperiale and Adhya, 1987). Eukaryotic genes contain introns, as first described for adenovirus 2 mRNAs by Berget *et al.* (1977) and the rabbit beta-globin by Jeffreys and Flavell (1977). The presence of introns makes it possible for one gene to produce different protein products by alternative selection of splice junctions (reviewed by Breitbart, Andreadis and Nadal-Ginard, 1987). Alternative splicing can also be used to control the on/off regulation of some genes (reviewed by Bingham *et al.*, 1988).

The organization of gene families has been examined in many different eukaryotic systems. Several patterns of organization have been observed. One of the simplest patterns is tandem repetition of identical duplicated genes or gene repeat units; for example, ribosomal RNA genes (Drosophila, Rissotta, Atwood and Spiegelman, 1966; yeast, Bell *et al.*, 1977; *C. elegans*, Sulston and Brenner, 1974; Files and Hirsh, 1981; Nelson and Honda, 1985) or histone genes (sea star, Howell *et al.* 1987; sea urchins and other organisms, Maxson, Cohn and Kedes, 1983). Gene families which arose from tandem gene duplications and subsequent divergence have been observed; for example, the human beta-globin gene cluster (Karlsson and Nienhuis, 1985), Drosophila cuticle protein genes (Snyder and Davidson, 1983), vitellogenin genes (Wahli, 1988), and the gene cluster around the dopa decarboxylase gene in *D. melanogaster* (Wright, 1987). Some gene families are dispersed apparently randomly throughout the genome although some small clusters are often seen; for example tRNA genes (Cortese *et al.*, 1978), *C. elegans* major sperm protein genes (Ward *et al.*, 1988), and *C. elegans* collagen genes (Cox *et al.*, 1985).

Most genes are present in a single copy in the genome. These are the type of genes expected to be identified in screens for recessive mutations. However, there are examples of genes which are present in more than one copy in every organism studied. For example, *C. elegans* has three genes encoding acetylcholinesterase; animals with mutations in any one of

these genes alone have no phenotype, but animals with mutations in all three genes are not viable (Johnson *et al.*, 1988). Genes which are reiterated are not expected to be identified in screens for recessive mutations. They can be identified by rare dominant mutations, for example, the dominant mutations of the actin gene cluster in *C. elegans* (Landel *et al.*, 1984; Waterston, Hirsh and Lane, 1984). If an individual member of a gene family has acquired a unique function, recessive mutations may have a phenotype. For example, the *dpy-13* gene in *C. elegans* is a member of the collagen gene family which has a unique function and therefore could be identified by recessive mutations (von Mende *et al.*, 1988).

B. Lethal Analysis in *Drosophila melanogaster*

Drosophila melanogaster has traditionally been the metazoan organism of choice for genetic analysis. Several regions of the *D. melanogaster* genome have been analyzed genetically. In many cases an attempt was made to define all of the essential genes in a small region. The first genetic saturation analysis was undertaken by Hochman (1971). He attempted to identify all of the essential genes on the smallest chromosome, chromosome 4, and identified 33 essential genes. He estimated that only three or four genes were not identified. Judd, Shen and Kaufman (1972) attempted to identify all of the essential genes in the *zeste-white* region of the *D. melanogaster* X chromosome. They probably identified all of the essential genes since they were all represented by more than one allele. Woodruff and Ashburner (1979) analysed the region around the *Adh* gene. Hilliker *et al.* (1980) analysed the region adjacent to the *rosy* gene. They predicted that the 20 complementation groups they had identified were nearly all of the essential genes in their region. Lasko and Pardue (1988) identified seventeen lethal complementation groups in the *vestigial* region. Leicht and Bonner (1988) undertook an analysis of a small region around a cluster of small heat shock polypeptide genes. They identified at least 80% of the essential genes in that region. Marchant and Holm (1988) predicted they identified all of the essential genes in the heterochromatin of chromosome 3.

In the *Drosophila melanogaster* experiments, most of the lethal mutations were recovered in precomplementation screens using a deficiency. The mutations were then placed over a balancer chromosome which suppressed recombination (for example, Judd, Shen and Kaufman, 1972; Hilliker *et al.*, 1980; Leicht and Bonner, 1988; Marchant and Holm, 1988). Some of the mutations recovered in deficiency screens were "haplo-specific lethal", meaning that they are homozygous viable but hemizygous inviable (over a deficiency). Nash and Janca (1983) found that one third of lethal mutations recovered in a screen with an X chromosome deficiency were of this type. They explained them as hypomorphic alleles with an activity below the threshold for survival with one dose, but near enough to that threshold such that an individual with two mutant copies is viable. A possible problem with some deficiency screens is that mutations may be recovered from elsewhere in the genome which are synthetically lethal with the deficiency. In the screen reported by Leicht and Bonner (1988), 30% of the mutations recovered did not fall in the deficiency they were screening against. They proposed that the presence of a Minute locus in the deficiency was responsible for much of the synthetic lethality.

C. Lethal Analysis in *Caenorhabditis elegans*

Several regions of the *C. elegans* genome have been studied with regard to the distribution of essential genes. Many groups have recovered recessive lethal mutations over small autosomal regions: the region around *unc-15 (I)* (Rose and Baillie, 1980); the region around *unc-54 (I)* (Anderson and Brenner, 1984); the region around *unc-22 (IV)* (Rogalski, Moerman and Baillie, 1982; Rogalski and Baillie, 1985; Clark *et al.*, 1988); and the region around *ama-1 (IV)* (Rogalski and Riddle, 1988). Sigurdson, Spanier and Herman (1984) have used an unanalyzed cross-over suppressor on chromosome II, *mnC1*, to recover lethals over a large autosomal region. The well characterized reciprocal translocation *eT1* (Rosenbluth and Baillie, 1981) has been used to balance lethal mutations over two large autosomal regions (Rosenbluth, Cuddeford and Baillie, 1983, 1985; Rosenbluth *et al.*, 1988). Meneely and Herman (1979, 1981) used a duplication of X-

chromosome material linked to chromosome V to recover and analyse X-linked lethal mutations. There are still many regions which need to be analyzed in detail before the genome will be well characterized.

D. Lethal Recovery and Balancing Systems

In *C. elegans*, translocations have been used successfully to recover and analyse large numbers of lethal mutations (Rosenbluth, Cuddeford and Baillie, 1983, 1985; Clark *et al.*, 1988; Rosenbluth *et al.*, 1988). They are useful as balancers because they absolutely suppress recombination over large genetic intervals (Herman, 1978; Rosenbluth and Baillie, 1981; Herman, Kari and Hartman, 1982; Ferguson and Horvitz, 1985; Rosenbluth, Cuddeford and Baillie, 1985). A disadvantage to this approach is that the ends of the region under study are not precisely defined since lethal mutations which are linked to, but outside of, the boundaries of cross-over suppression are recovered. Screening for lethal mutations using a duplication or a deficiency will very precisely define the boundaries of the region under study. Deficiencies, however, generally can not be used to study large regions. A deficiency (*sDf4*) of approximately 2.5 map units on chromosome I has low viability as a heterozygote and could not practically be used in large-scale lethal screening experiments.

Linked duplications have previously been used as balancers in *Drosophila melanogaster* (Judd, Shen and Kaufman, 1972) and in *C. elegans* (Meneely and Herman, 1979, 1981). A drawback to the previous use of a linked duplication in *C. elegans* was that lethal mutations on the other chromosome were also recovered. In the study by Meneely and Herman (1979), only 21 of 176 putative lethals were in the region of interest.

In order to carry out a genetic analysis of part of chromosome I in *C. elegans*, it was necessary to develop a system for the recovery and analysis of a large number of mutations in essential genes in an autosomal region. At the outset of this analysis, no translocations of chromosome I were available. A free duplication of the left third of chromosome I, *sDp2*, had

been described by Rose, Baillie and Curran (1984). Since *sDp2* did not recombine with the normal homologues, Rose, Baillie and Curran (1984) suggested that it may be useful for balancing lethal mutations. An advantage to using a free duplication such as *sDp2* is that all of the recovered lethal mutations are in a very precisely defined region on the linkage group of interest. The *sDp2* system was also amenable to recombination mapping and complementation analyses.

Subsequently, two chromosome I translocations became available, *szT1(I;X)* (Fodor and Deak, 1985) and *hT1(I;V)* (McKim, Howell and Rose, 1988). These translocations were used to recover and analyze lethal mutations in the same large region. In this thesis, I compare the relative merits of *sDp2, szT1(I;X)* and *hT1(I;V)* for rescuing lethals on chromosome I.

E. Analysis of the *hDf6* region

Dr. Rose's lab has been studying the genomic organization of the left half of chromosome I. I developed a system using *sDp2* to isolate and characterize mutations in this region of the genome. For further analysis, I chose a smaller region which I could describe in greater detail. The region, to the left of *dpy-5*, was defined by the boundaries of the deficiency *hDf6*, and had not previously been examined with respect to the distribution of essential genes.

This region and its environs were known to contain some interesting genes. Mutations in three genes to the left of the gene *dpy-5*, (*unc-38, unc-63* and *unc-74*) confer resistance to the drug levamisole (Brenner, 1974; Lewis *et al.*, 1980) and have been proposed to code for subunits of the acetylcholine receptor. One of these three genes, *unc-74*, is uncovered by *hDf6*. Mutants of another gene in *hDf6, unc-57*, have a strong kinker uncoordinated phenotype (Brenner, 1974). They usually contract when touched on the head, but this is strongly inhibited in double mutants with *unc-38, unc-63* and *unc-74* (Lewis *et al.*, 1980), perhaps implying some functional interaction between these genes. *unc-73* mutants are severely uncoordinated because the processes from the motor neurons are displaced (Hedgecock *et al.*, 1987). They are also defective in egg-laying because the hermaphrodite specific neurons do not migrate properly and form the proper connections with the vulval muscles (Desai *et al.*, 1988). Mutants in a gene involved in muscle

structure, *unc-89*, have disorganized thick filament assembly (Waterston, Thomson and Brenner, 1980). A gene which reduces X chromosome recombination and increases X chromosome nondisjunction, *him-1*, also maps to this region (Hodgkin, Horvitz and Brenner, 1979).

Through a collective lab effort, 495 EMS-induced *sDp2*-recovered lethals have been analyzed. I have positioned those within the deficiency *hDf6* by recombination mapping, deficiency mapping and duplication mapping. A second deficiency within *hDf6*, *hDf7*, was identified and mapped with respect to the essential genes. In all, nineteen essential genes were identified inside *hDf6*. The minimum number of essential genes in *hDf6* was estimated to be twenty-five based on a calculation using the truncated Poisson distribution. Thus, approximately three quarters of the essential genes in this 1.25 map unit region of the *C. elegans* genome have been identified.

The research described in this thesis was designed to examine several different questions relating to genes and genomic organization. The distribution of essential genes on chromosome I had not been described. It was not known whether the distribution of essential genes would follow the clustered distribution of visible genes on the chromosome (Brenner, 1974). It was not known how groups of genes were organized on chromosome I; for example, whether any apparent organization based on the developmental stage at which gene products are required could be discerned. The different mutabilities of genes had not previously been studied with such a large sample size. The analysis of essential genes in the *hDf6* region resulted in the acquisition of new information regarding these questions.

MATERIALS AND METHODS

A. *C. elegans* Nomenclature

The nomenclature in this thesis follows the uniform system adopted for *C. elegans* (Horvitz *et al.*, 1979), which is briefly summarized here. Gene names indicate the general phenotypic class; for example, *dpy* mutations cause the worms to be shorter and fatter than wild-type worms, *unc* mutations cause the worms to move in an uncoordinated fashion, and *let* mutations cause the worms to arrest development or to be sterile. Gene names are represented by three italicized lower case letters followed by a number. Mutant alleles are designated with one or two italicized lower case letters (signifying the lab of origin) followed by a number, and can be used alone or in parentheses after the gene name (for example, *let-354(h79)*). Phenotypes of mutant worms are indicated by using the appropriate gene name without italics and with the first letter in upper case (*e.g.* Unc-13s). Nematode strains are identified with two upper case letters (signifying the lab of origin) followed by a number. The designations for A. Rose's lab are KR for strains and *h* for mutations. Chromosomal rearrangements are abbreviated as follows; *Df* for deficiency, *Dp* for duplication, and *T* for translocation. The five autosomes are indicated in upper case Roman numerals, and the X chromosome is indicated as "X". When discussing the reciprocal translocations, the nomenclature has been adapted from Horvitz *et al.* (1979). When discussing the individual component chromosomes of a translocation, the adapted nomenclature describes the structure of the new chromosomes. For example, $szT1(I;X) = I^{L}X^{L}szT1 + I^{R}X^{R}szT1$ and $hT1(I;V) = I^{R}V^{L}hT1 + I^{L}V^{R}hT1$. The $I^{L}X^{L}$ terminology implies that the new chromosome carries the left portion of the X chromosome joined to the left portion of chromosome I. When describing genotypes of worms carrying duplications or translocations, the genotypes of translocation chromosomes (as in McKim, Howell and Rose, 1988) or duplication chromosomes (as in Rogalski, Bullerjahn and Riddle, 1988) are shown in brackets.

B. Nematode Culturing and Strains

Caenorhabditis elegans strains are usually maintained as self-fertilizing hermaphrodites (5AA; XX). Males (5AA; XO) are found among the self progeny and result from X-chromosome nondisjunction (Hodgkin, Horvitz and Brenner, 1979). At 20 ^{O}C they occur at a frequency of approximately 0.1% (Rose and Baillie, 1979). Wild-type and mutant strains were maintained and mated on petri plates containing nematode growth media (NGM), streaked with *Escherichia coli* OP50 (a uracil-requiring mutant) as described by Brenner (1974). The wild-type N2 strain and some mutant strains of *C. elegans* var. Bristol were obtained from D. Baillie, Simon Fraser University, Burnaby, Canada or the Caenorhabditis Genetics Center at the University of Missouri, Columbia. Strains used, with the exception of lethal-bearing strains, are listed in Table 1. Unless otherwise indicated, the *e61* allele of *dpy-5* was used.

In addition to the new lethal mutations isolated in this study, the following mutant genes and alleles were used (listed by chromosome):

I *bli-3(e579); bli-4(e937); dpy-5(e61); dpy-5(mn287); dpy-14(e188); fer-7(hc34); fog-1(q253ts); him-1(e879); let-354(ct42); lin-6(e1466); spe-11(hc90); sup-11(n403n682); unc-11(e47); unc-13(e450); unc-15(e73); unc-29(e403); unc-38(e264); unc-57(e406); unc-63(e384); unc-67(e713); unc-73(e936); unc-74(x12); unc-75(e950); unc-89(e1460)*

II *dpy-10(e128); unc-4(e120)*

III *sma-2(e502); unc-36(e251)*

IV *unc-22(s7)*

V *dpy-11(e224); sma-1(e30); unc-23(e25); unc-42(e270)*

X *dpy-8(e130); lon-2(e678); unc-3(e151)*

Figure 1 a) is a partial *C. elegans* genetic map showing the map positions of the markers used. Figure 1 b) is an expansion of the *unc-11 - unc-29* region of chromosome I.

Table 1. Strains used (not including lethal strains generated)

Strain	Genotype	Origin
AF1	*+; dpy-8 + unc-3/szT1(I;X)[+;+ lon-2 +]*	A. Fodor
BA34	*fer-7(hc34)*	S. Ward
BA662	*spe-11(hc90) dpy-5; sDp2*	D. Shakes
BW962	*+ let-354(ct42) dpy-5 +/unc-73 + + dpy-14*	P. Mains
BC62	*dpy-5 unc-75*	A. Rose
BC207	*dpy-5 unc-29*	A. Rose
BC279	*dpy-5 unc-13 unc-29*	A. Rose
BC363	*dpy-11 unc-23*	R. Rosenbluth
BC417	*dpy-5 unc-67*	R. Rosenbluth
BC655	*unc-36 sma-2*	R. Rosenbluth
BC700	*sDf4/bli-4 dpy-14*	A. Rose
BC1142	*unc-42 sma-1*	D. Nelson
JK?	*fog-1(q253ts); him-5*	K. Barton
KR182	*unc-57 dpy-5*	A. Rose
KR217	*unc-73 dpy-5*	A. Rose
KR235	*dpy-5 unc-13 +/dpy-5 + unc-15; sDp2*	A. Rose
KR236	*dpy-5 unc-13; sDp2*	A. Rose
KR366	*bli-3 dpy-5*	A. Rose
KR900	*dpy-5 unc-13; + unc-3/szT1[+ +;lon-2 +]*	A. M. Howell
KR1012	*dpy-5 unc-29 unc-75*	A. M. Howell
KR1015	*unc-11 unc-73*	A. M. Howell
KR1069	*sDf4; +/hT1[+; +]*	K. McKim
KR1071	*unc-11 dpy-5*	A. M. Howell

Table 1. (continued)

Strain	Genotype	Origin
KR1072	*unc-63 dpy-5*	A. M. Howell
KR1088	*him-1 dpy-5*	A. M. Howell
KR1106	*unc-11 dpy-5; hDp2*	K. McKim
KR1139	*him-1 dpy-5; sDp2*	A. M. Howell
KR1155	*unc-38 dpy-5(mn287)*	J. Babity
KR1238	*unc-11 dpy-5; hDp3*	K. McKim
KR1239	*unc-11 dpy-5; hDp4*	K. McKim
KR1241	*unc-11 dpy-5; hDp6*	K. McKim
KR1242	*unc-11 dpy-5; hDp20*	K. McKim
KR1250	*unc-11 dpy-5; hDp5*	K. McKim
KR1280	*dpy-5 dpy-14; hDp13*	K. McKim
KR1292	*sup-11 dpy-5; +/szT1[+ +; lon-2]*	A. M. Howell
KR1458	*unc-11 dpy-14; +/szT1[+ +; lon-2]*	K. McKim
KR1459	*dpy-5 unc-13 +; +/hT1[+ + unc-29; +]*	K. McKim
KR1469	*dpy-5 dpy-14; hDp21*	K. McKim
KR1548	*dpy-5 dpy-14; hDp22*	K. McKim
KR1664	*dpy-5 dpy-14; hDp23*	K. McKim
KR1669	*dpy-5 dpy-14; hDp25*	K. McKim
KR1670	*dpy-5 dpy-14; hDp24*	K. McKim
KR1730	*dpy-5 dpy-14; hDp34*	K. McKim
KR1736	*hDf6 dpy-5 unc-13; unc-3;* $I^{L}X^{L}$*szT1*	K. McKim
KR1737	*hDf6 dpy-5 unc-13; hDp31*	A. M. Howell
KR1773	*dpy-5 dpy-14; hDp31*	K. McKim
KR1782	*unc-89 dpy-5*	K. McKim

Table 1. (continued)

Strain	Genotype	Origin
KR1783	*dpy-5 dpy-14; hDp32*	K. McKim
MT1403	*sup-11(n403n682) + dpy-5/ + unc-11 +*	J. Levin
MT1442	*lin-6 dpy-5; +/szT1[+ +; lon-2]*	R. Horvitz
SP74	*dpy-10 unc-4*	R. Herman
ZZ1012	*unc-74 dpy-5*	J. Lewis

The strain names indicate the lab of origin. AF = A. Fodor's lab, Hungary; BA = S. Ward's lab, Baltimore, MD; BC = D. Baillie's lab, Burnaby, B.C.; BW = B. Wood's lab, Boulder, CO; JK = J. Kimble's lab, Madison, WI; KR = A. Rose's lab, Vancouver, B.C.; MT = R. Horvitz's lab, Boston, MA; SP = R. Herman's lab, St. Paul, MN; ZZ = J. Lewis' lab, Columbia, MO.

Figure 1 a). Partial genetic map of *C. elegans* showing the positions of the markers used. The region of chromosome I marked with asterisks is expanded in Figure 1 b).

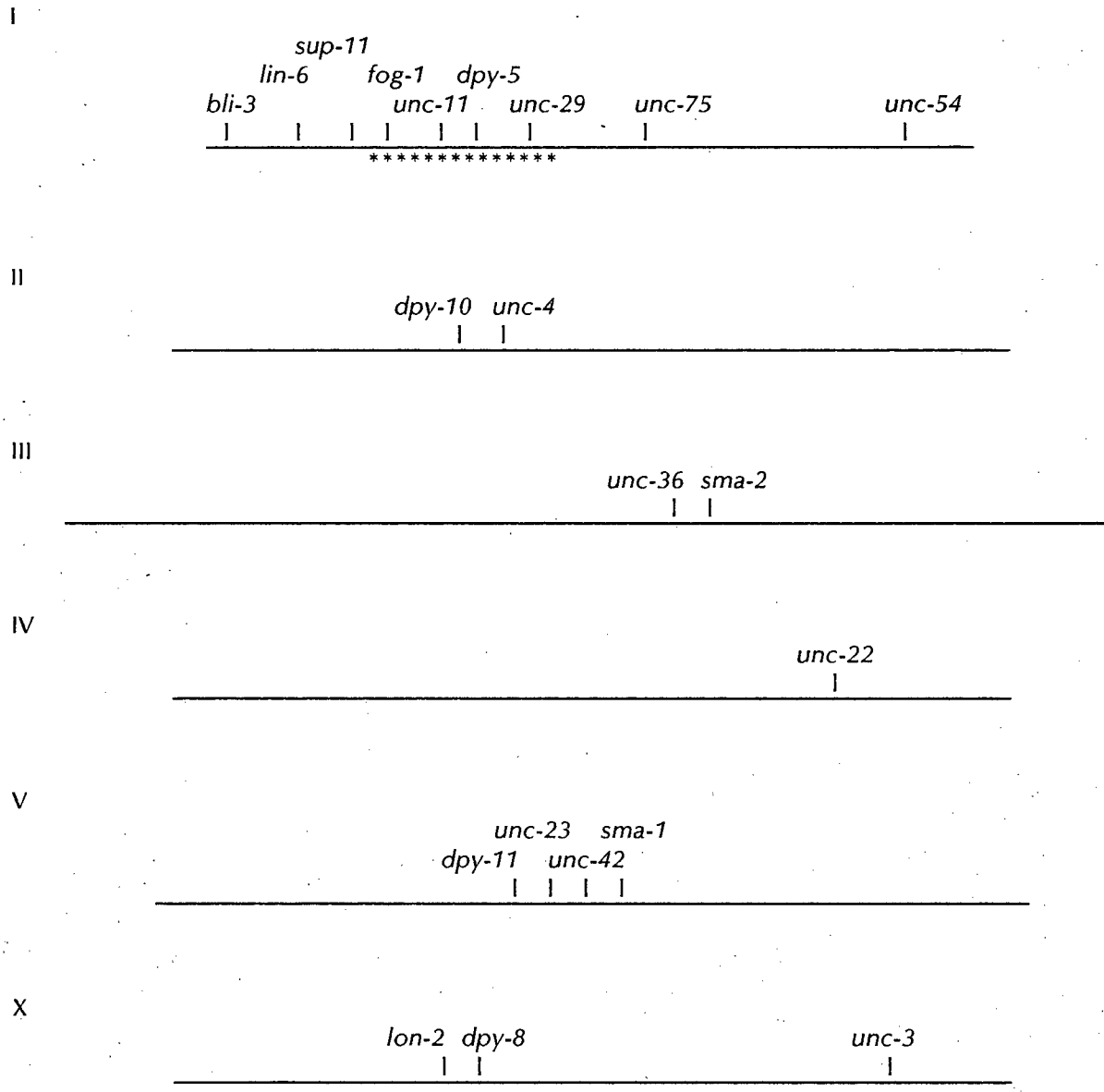
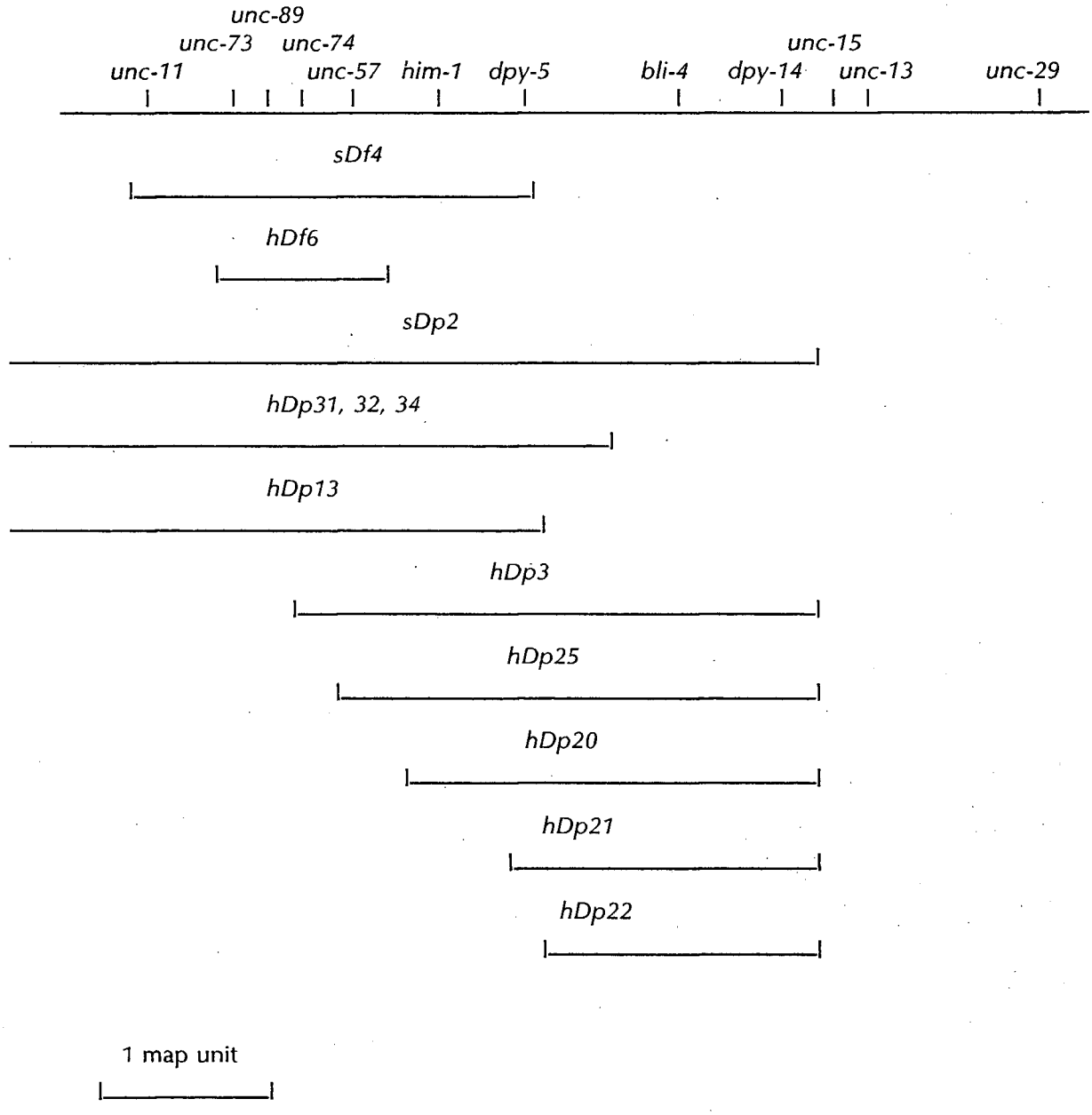


Figure 1 b). Expansion of *unc-11* - *unc-29* region of chromosome I showing extents of deficiencies and duplications.



C. Chromosomal Rearrangements Used

The translocation *szT1(I;X)* was initially isolated by Fodor and Deak (1985) as a dominant X-chromosome crossover suppressor. It was induced on a *lon-2(e678)(X)* chromosome using 7000 R of X-radiation. Their analysis showed that *szT1* consists of two abnormal chromosomes derived from the normal chromosome I and X chromosome and that the homozygotes arrest as embryos. They also showed that heterozygous hermaphrodites produce Lon-2 male self-progeny at a frequency of 0.08 - 0.12 and recombination was reduced in the *dpy-7* - *unc-3* (*X*) interval from 19.2 map units to 0.3 map units.

hT1(I;V) was isolated by K. McKim in a screen for gamma-induced mutations causing pseudolinkage of markers on chromosomes I and V. It consists of the two translocation chromosomes $I^{L}V^{R}hT1$ and $I^{R}V^{L}hT1$. Recombination is completely suppressed along the left half of chromosome I in translocation heterozygotes (from *let-362* to *let-88*) (McKim, Howell and Rose, 1988).

hT2(I;III) was isolated by K. Peters in a screen for gamma-induced mutations causing pseudolinkage of markers on chromosomes I and III. Recombination is completely suppressed along the left two thirds of chromosome I (at least as far as *unc-75)* in translocation heterozygotes (K. Peters and K. McKim, unpublished results).

The deficiency, *sDf4* (Figure 1 b), which deletes the *unc-11 dpy-5* interval (Rose, 1980), was generated by treating wild-type male sperm with 0.07% formaldehyde as described by Rose and Baillie (1980). Strains heterozygous for *sDf4* have no obvious visible phenotype, but have reduced progeny numbers. It has been maintained in strains heterozygous for *bli-4 dpy-14* or the translocation *hT1(I;V)* by selecting phenotypically wild-type heterozygotes each generation.

The deficiency, *hDf6* (Figure 1 b), which was isolated as a gamma-induced lethal mutation (*h545*) balanced over *szT1*, was identified by K. McKim. K. McKim showed that *h545* failed to complement *unc-57* and *unc-74* but not *unc-11*. It was later named *hDf6*. *hDf6* was maintained as *hDf6 dpy-5 unc-13 (I); unc-3 (X);* $I^{L}X^{L}$*szT1*, or as *hDf6 dpy-5 unc-13; hDp31*.

sDp2, a free duplication of the left third of chromosome I, carries the wild-type alleles of *dpy-5* and *dpy-14* but not of the *unc-15* or the *unc-13* loci (Rose, Baillie and Curran, 1984). Duplications with the *h* allele designation were generated and mapped with respect to visible markers by K. McKim (McKim and Rose, 1988; K. McKim, unpublished results). *sDp2*, *hDp13*, *hDp31*, *hDp32* and *hDp34* carry wild-type alleles for the farthest left known markers on chromosome I. The extents of all duplications used are shown in Figure 1 b.

D. Lethal Screening Using *sDp2*

In order to screen for lethal mutations of genes on the left third of chromosome I, the duplication *sDp2* was used. A strain was constructed in which each chromosome I homologue was differentially marked. A single hermaphrodite of the genotype, *dpy-5 + unc-13/ dpy-5 unc-15 +; sDp2[+]*, was used to establish the strain KR235.

Each of the segregating genotypes from KR235 was represented by a unique phenotype (Figure 2). Dpy-5 worms are approximately one-half the length of wild-type worms and are fatter. The two types of Uncs and Dpy Uncs present in these tests were phenotypically distinquishable from each other. Unc-13 animals are extremely uncoordinated and contract when touched, while Unc-15 worms have an extremely uncoordinated, limp phenotype. Diploid progeny were either Dpy (*dpy-5 + unc-13 / dpy-5 unc-15 +*) or Dpy Unc (*dpy-5 unc-13* or *dpy-5 unc-15*). Duplication-carrying progeny were either Unc (*dpy-5 unc-13/ dpy-5 unc-13; sDp2[+]* or *dpy-5 unc-15/ dpy-5 unc-15; sDp2[+]*) or "wild type" (*dpy-5 unc-13 +/ dpy-5 + unc-15; sDp2[+]*) ("wild type" = duplication-carrying non-Dpy, non-Unc, non-Dpy Unc). This strain was maintained by selecting the "wild-type" hermaphrodites and has been used for screening for chromosome I lethals.

Lethal mutations in genes present in the *sDp2* region were isolated and rescued by the wild-type allele on *sDp2*. Individuals of the KR235 strain were treated either with 12, 15 or 17 mMethylmethane sulfonate (EMS) for four hours or with 1500 R (13 R/sec)

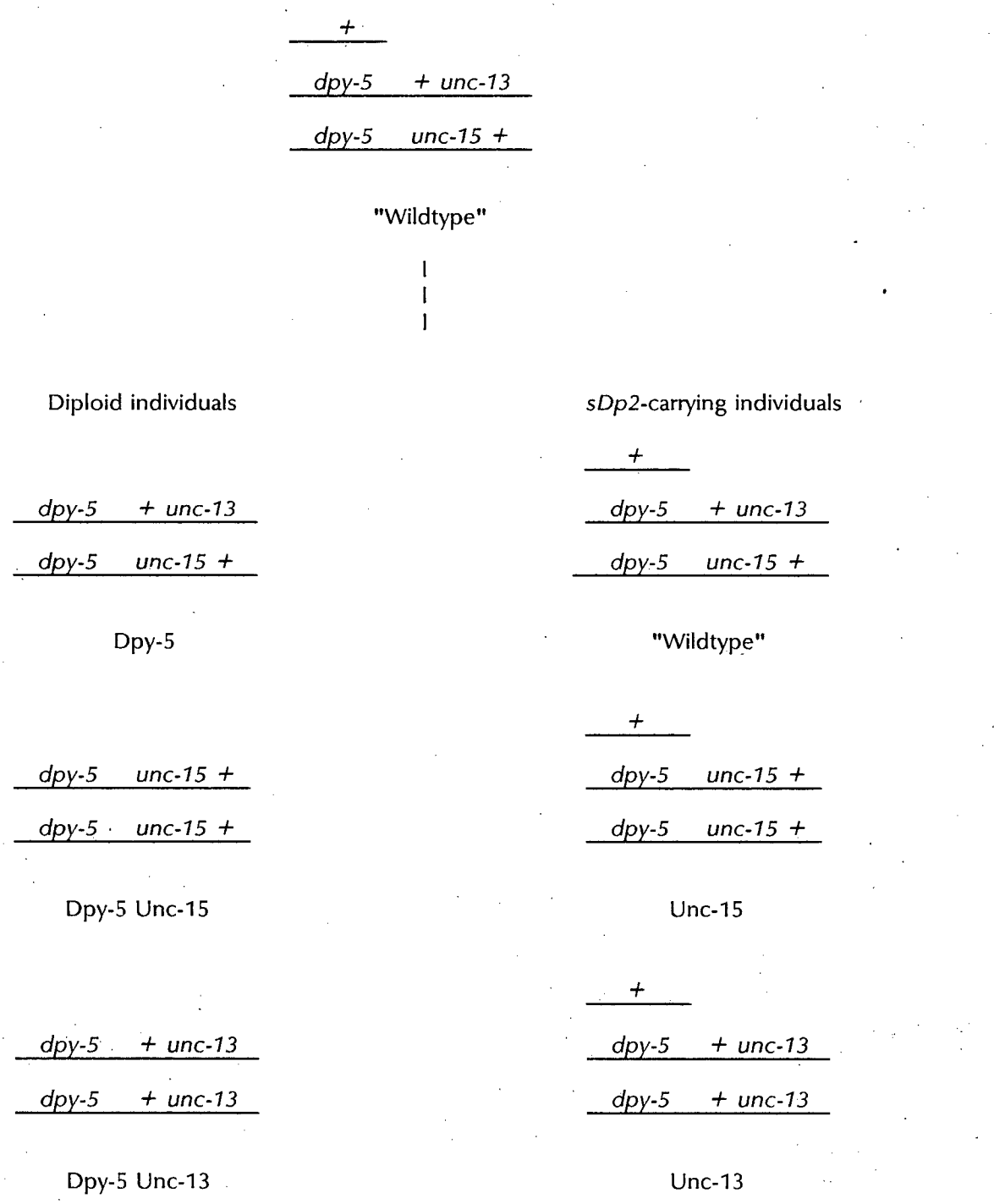


Figure 2. Segregation from the *sDp2* screening strain (KR235, *dpy-5 + unc-13 / dpy-5 unc-15 +; sDp2*). Lethal mutations induced on the *dpy-5 unc-13* marked chromosome are identified by the absence of fertile Dpy-5 Unc-13 progeny and are maintained by recovery of their Unc-13 siblings. Individuals which receive two copies of *sDp2* develop extremely slowly and are usually sterile.

of gamma radiation (Cobalt 60) as recommended by Rosenbluth, Cuddeford and Baillie (1983). Gravid "wild types" were individually placed on 10 x 60 mm culture plates after treatment. Five days later gravid "wild-type" F1s were placed individually on culture plates. Their progeny were screened for the absence of fertile Dpy-5 Unc-13 individuals. If fewer than 2 fertile Dpy-5 Unc-13s were observed, and if Unc-13s were present, a single Unc-13 was transferred to a fresh culture plate in order to confirm the existence of a lethal. A generation later the offspring of each Unc-13 strain were examined. Three types of strains were found: 1) both Uncs and fertile Dpy-5 Unc-13s were present (no lethal mutation); 2) no fertile Unc-13 could be recovered (a lethal not rescued by *sDp2*), and; 3) the desired cases, Unc-13s and developmentally arrested Dpy-5 Unc-13s were present. Thus, lethal mutations were rescued by the presence of a wild-type allele provided by *sDp2*. Lethal mutations rescued in this way were maintained in strains with the genotype *(let-x dpy-5) unc-13/ (let-x dpy-5) unc-13; sDp2[+ +]*. The parentheses indicate that the *let-* gene could map either to the left or the right of *dpy-5*. These strains have an Unc-13 phenotype and segregate a single fertile phenotype (only Unc-13s).

E. Recombination Mapping Using *sDp2* Lethals

Recombination mapping was done using procedures recommended by Rose and Baillie (1979). All recombination experiments were carried out at 20°C, and all of the progeny of a given hermaphrodite were scored. The total number of progeny was calculated as 4/3(wild types plus one recombinant class); the number of recombinants was calculated as two times one recombinant class. The recombination fraction, R, was calculated as (number of recombinants)/ (total progeny). The frequency of recombination, *p*, was calculated as $1 - (1-2R)^{1/2}$ (Brenner, 1974). Confidence limits of 95% were calculated using Poisson statistics according to Crow and Gardner (1959).

In order to generate the appropriate heterozygous individuals, N2 (wild-type) males were crossed to Unc-13 hermaphrodites from each of the lethal-bearing strains. Wild-type

heterozygotes which do not carry the duplication were required for mapping. Since the non-duplication carrying progeny were the first to reach adulthood, they were easily selected. Occasionally "wild types" carrying *sDp2* were accidentally set up. These were easily identified because they segregated approximately 12% Unc-13 progeny whereas the heterozygotes lacking the duplication produced less than 1% Unc-13 progeny, the result of recombination events in the *dpy-5 unc-13* interval. The self progeny of appropriate out-cross hermaphrodites (*+ + +/ (let-x dpy-5) unc-13*) were scored. For those lethal mutations to the left of *dpy-5*, fertile Dpy-5 Unc-13 recombinants were recovered in a frequency proportional to the distance to the lethal. Unc-13 recombinants were recovered at the frequency expected for the *dpy-5 unc-13* interval. For those lethal mutations to the right of *dpy-5*, Dpy-5 and Unc-13 recombinants were recovered. In this way, both right-left positioning relative to *dpy-5* and two-factor recombination frequencies were obtained for each of the lethal mutations.

F. Complementation Testing Using *sDp2* Lethals

1. Deficiencies

Lethal mutations were tested for complementation to the two deficiencies, *sDf4* and *hDf6*. N2 (wild type) males were crossed to Unc-13 hermaphrodites from the lethal-bearing strains. *let-x dpy-5 unc-13/ + + +* progeny males were mated to the deficiency strains. The presence of fertile Dpy-5s (for *sDf4*) or Dpy-5 Unc-13s (for *hDf6*) among the outcross progeny indicated complementation. A minimum of 30 wild-type outcross males were scored to ensure that the absence of the *let-x/Df* heterozygote was not due to reduced viability.

2. Duplications

Lethal bearing strains (*i.e. dpy-5 let-x unc-13; sDp2(I;f)*) were crossed to *unc-11 dpy-14; 0/szT1(I;X)*[*+;lon-2*] males. The resulting male progeny (*let-x dpy-5 + unc-13/ + + dpy-14 +*) were then crossed to a) *dpy-5 dpy-14; hDpz(I;f)* or b) *unc-11 dpy-5; hDpz(I;f)* hermaphrodites.

Wild-type hermaphrodite progeny from cross a) were *let-x dpy-5 + unc-13/ + dpy-5 dpy-14 +; hDpz* or *+ dpy-5 dpy-14 /unc-11 + dpy-14; hDpz* if *hDpz* was *dpy-14(+)*. These two possibilites were distinguished by examining their progeny. Upon examining the progeny of the former, Dpy-5 progeny were observed. Wild-type hermaphrodite progeny from cross b) were *+ let-x dpy-5 unc-13/ unc-11 + dpy-5 +; hDpz*. In both cases, fertile Unc-13 progeny were observed in the next generation if the duplication carried *let-x(+)*. In some cases where the duplication did not actually carry *let-x(+)*, a few fertile Unc-13s were observed. These were recombinants which had lost the lethal mutation on one homologue. They were easily distinguished because they segregated fertile Dpy-5 Unc-13s.

3. Non-lethal mutations

Lethal mutations were tested for complementation to non-lethal (visible) mutations. Visible mutations (m) linked to *dpy-5* were crossed to N2 (wild-type) males. *m dpy-5/ + +* progeny males were mated to Unc-13 hermaphrodites from different lethal-bearing strains. If non-M Dpy-5 progeny were observed, the visible mutation complemented the lethal mutation. If M Dpy-5 progeny were observed, the visible mutation failed to complement the lethal mutation. If no Dpy-5 progeny were observed after a successful mating, the visible mutation failed to complement the lethal mutation.

4. Lethal mutations

Heterozygous males generated by crossing *sDp2* lethal strains to N2 (wild type) males were used for complementation testing. These males were mated to Unc-13 hermaphrodites from different lethal-bearing strains. The *sDp2*-carrying males are slower at developing and less effective at mating than males lacking the duplication (Rose, Baillie and Curran, 1984). The out-cross progeny were scored. The presence of Dpy-5 Unc-13 males and fertile Dpy-5 Unc-13 hermaphrodites indicated complementation. For lethals tightly linked to *dpy-5*, the presence of fertile Dpy-5 Unc-13 progeny was diagnostic of complementation. For lethals which mapped ten

or more map units to the left of *dpy-5*, out-cross progeny were scored to ensure that Dpy-5 Unc-13 offspring were present in excess of the number expected from recombination between the lethal and *dpy-5*.

G. Recombination Mapping in *szT1* Heterozygotes

Recombination frequencies between pairs of markers were determined by scoring the progeny of *cis*-heterozygous hermaphrodites under the conditions described by Rose and Baillie (1979). When the interval being examined spanned the translocation breakpoint, one of the markers crossed onto the translocation chromosome. When the four types of gametes from a *dpy-5 unc-z/ szT1(I;X)* heterozygote occurred with equal frequencies, the recovery of the Unc-z recombinant was expected at a frequency twice that of the reciprocal Dpy-5 recombinant when p was small (described in McKim, Howell and Rose, 1988). In these situations recombination frequency was calculated as;

$$p = [(4A + 2W) - ((4A + 2W)^2 - 16A(4A + W))^{1/2}]/2(4A + W)$$

where A is the number of recombinant Dpy-5 progeny and W is the number of wild types.

H. Lethal Screening Using *szT1*

Spontaneous Lon-2 males from the strain AF1, *+; dpy-8 + unc-3/szT1(I;X)[+;+ lon-2 +]*, were crossed to *dpy-5 unc-13; unc-3* hermaphrodites to construct the strain KR900, *dpy-5 unc-13; + unc-3/ szT1(I;X)[+ +; lon-2 +]*. Hermaphrodites from this strain were treated with 1500 rads of gamma radiation (Cobalt 60) as recommended by Rosenbluth, Cuddeford and Baillie (1985) and the progeny of individual F1 wild types were examined for the absence of Dpy-5 Unc-13 Unc-3 offspring. In this way strains carrying lethal mutations induced in the cross-over suppressed regions of either chromosome I or the X chromosome were isolated. Strains which failed to produce Unc-3 male progeny after crossing to *dpy-5 unc-13/ + +* males were assumed to carry

an X-linked lethal mutation and were not analyzed further. The lethals were mapped with respect to the *dpy-5* and *unc-13* markers (after replacing *szT1* with a normal chromosome) and tested for complementation with visible and lethal mutations in the region.

I. Lethal Screening Using *hT1*

Young adult *dpy-5 unc-13 +; +/ hT1(I;V)[+ + unc-29;+]* hermaphrodites were treated with EMS in accordance with Brenner (1974), except that the dose used was only 0.017 M as recommended by Rosenbluth, Cuddeford and Baillie (1983). They were plated individually and allowed to self. The F1 progeny were checked to ensure segregation of fertile Dpy-5 Unc-13s (no lethal in the previous generation). Their wild type siblings were plated individually and allowed to self. The progeny of 3,000 F1s were screened for the absence of fertile Dpy-5 Unc-13s by A. Rose, K. McKim and myself. Strains were discarded if even one fertile Dpy-5 Unc-13 individual was seen since these would most likely carry lethal mutations just outside the boundaries of cross-over suppression. If no fertile Dpy-5 Unc-13s were seen, the strain was assumed to be carrying a new lethal mutation on the balanced regions of chromosome I or V and was maintained for further analysis.

J. Mapping *hT1*-recovered Lethals to Chromosome I and Balancing Over *szT1*

The lethal bearing strains described above could have had a lethal mutation on chromosome I or V. In order to assign each lethal mutation to the correct linkage group, the *hT1* chromosomes were replaced with a normal chromosome V and the translocated I and X chromosomes from *szT1(I;X)*. This eliminated the pseudolinkage of chromosome I and V in the lethal bearing strains. Lethal strains (*let-x dpy-5 unc-13 +; +/ hT1(I;V)[+ + + unc-29; +]* or *dpy-5 unc-13 +; let-x/ hT1(I;V)[+ + unc-29; +]*) were mated to Lon-2 males of the genotype *hT2(I;III) [dpy-5 +; +; 0]/ szT1(I;X)[+ unc-29;+; lon-2]*. Wild-type outcross hermaphrodites were

allowed to self. The segregation of Lon-2 males indicated the presence of *szT1*. The segregation of many fertile Dpy-5 Unc-13s indicated that the lethal mutation was on chromosome V. The presence of one or a few fertile Dpy-5 Unc-13s indicated that the lethal was on chromosome I but just outside of the boundary of cross-over suppression. If no fertile Dpy-5 Unc-13 individuals were observed, the lethal mutation was assumed to be in the cross-over suppressed region on chromosome I. The *szT1*-balanced strains were used for any further analyses.

III. RESULTS

A. Recovery of Lethal Mutations

The major goal of this research was to identify essential genes in the region of the deficiency *hDf6* on chromosome I. In order to do so, it was necessary to develop a usable system for recovering and analyzing a large number of lethal mutations in an autosomal region of *Caenorhabditis elegans*. Three different methods were adopted for recovering mutations in essential genes (described in detail in Materials and Methods). The free duplication, *sDp2,* and two translocations, *szT1(I;X)* and *hT1(I;V),* were used to rescue lethal mutations. Figure 3 shows the regions of the genome from which lethal mutations would be recovered using the three screening methods. Note that only with *sDp2,* all lethal mutations recovered were on chromosome I.

1. *sDp2*

In order to recover lethal mutations in the *sDp2* region, a strain carrying the duplication and two differentially marked chromosome I's was used. This strain, KR235, had the genotype *dpy-5 + unc-13/ dpy-5 unc-15 +; sDp2 [+].* After EMS mutagenesis, F1 progeny carrying a lethal mutation were identified by the absence of Dpy-5 Unc-13 F2 progeny. An Unc-13 individual was isolated from each of these F1's in order to maintain mutations induced on the *dpy-5 unc-13* chromosome within the *sDp2* region. These lethal strains had the genotype, *(let-x dpy-5) unc-13/ (let-x dpy-5) unc-13; sDp2 [+ +].* Since the duplication does not carry the wild-type allele of *unc-13,* these strains had an Unc-13 phenotype and segregated Unc-13 and developmentally arrested Dpy-5 Unc-13 animals. Because a lethal mutation is present on both normal homologues, only those mutations having a wild-type allele on the duplication were recovered in this way.

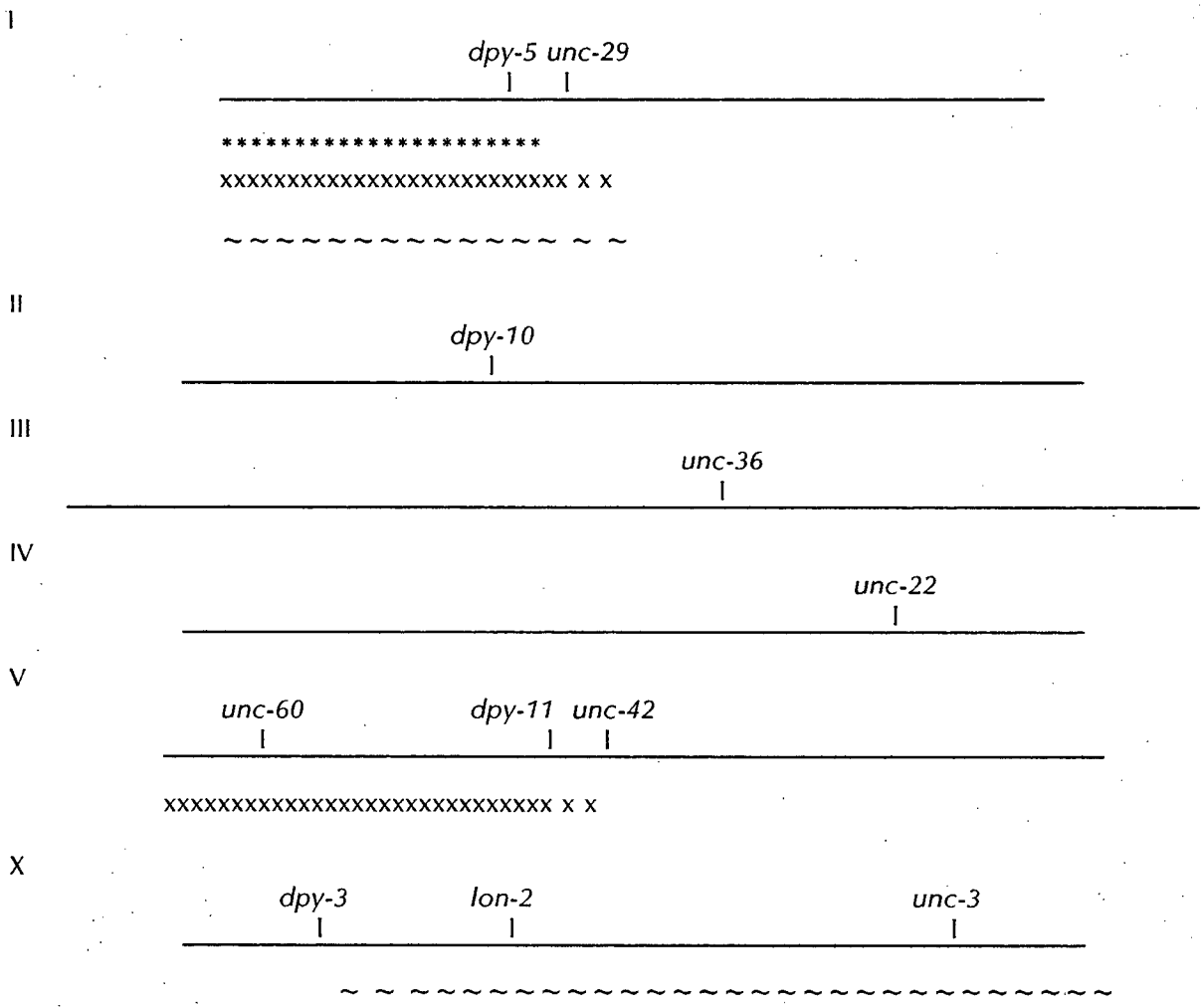


Figure 3. Map of the *C. elegans* genome showing extents of balanced regions. Regions of the genome from which lethal mutations will be recovered using the three screens described are indicated as follows:

******	Region of the genome screened using *sDp2(I;f)*
xxxxx	Region of the genome screened using *hT1(I;V)*
~~	Region of the genome screened using *szT1(I;X)*.

Several screens have been done in this laboratory (Table 2). A total of 552 lethal mutations have been recovered from 31,606 mutagenized chromosomes. The cumulative induction frequency was 1.7%. Fourteen strains did not grow upon thawing. In this study, 495 EMS-induced lethal bearing strains were analysed.

2. *szT1(I;X)*

Spontaneous Lon males from the strain AF1, *+; dpy-8 + unc-3/szT1(I;X)[+;+ lon-2 +]*, were crossed to *dpy-5 unc-13; unc-3* hermaphrodites to construct the strain KR900, *dpy-5 unc-13; + unc-3/ szT1(I;X)[+ +; lon-2 +]*. The segregation from this strain was examined as a control prior to mutagenesis. KR900 hermaphrodites segregated wild-type hermaphrodites (1009), Lon males (71), Dpy-5 Unc-13 Unc-3s (222 hermaphrodites and 19 males) and Unc-3s (50 hermaphrodites and 17 males). The absence of Dpy-5s (non-Unc-3s) and the low frequency of Unc-3s confirmed Fodor and Deak's (1985) findings that the progeny of *szT1* heterozygotes show a high degree of pseudolinkage between *dpy-5* and *unc-3*.

Lethal mutations would be recovered on the cross-over suppressed regions of both chromosome I and the X chromosome. The normal X chromosome was marked with *unc-3* to make it easier to identify X-linked lethal mutations. The strain was designed so that the lethal mutations recovered from chromosome I would be linked to *dpy-5* and *unc-13*, as in the *sDp2* strains. In this way, recombination mapping would give both the right-left orientation of the lethal with respect to the markers and the relative distance. The right-left positioning would be determined in the same manner as for the *sDp2*-recovered lethals except that lethals could be recovered to the right of *unc-13*. These would give fertile Dpy-5 and Dpy-5 Unc-13 recombinants, but not Unc-13s.

The screening strain (KR900) was treated with 1500 rads of gamma radiation and strains that segregated no fertile Dpy-5 Unc-13 Unc-3s were recovered. These strains could have carried lethal mutations in the cross-over suppressed regions of chromosome I or the X chromosome.

Table 2. Recovery of EMS-induced lethal mutations using *sDp2*

Date	EMS Dose	Screened	Lethals	Induction	Participants
01/1984	12 mM	1650	26	1.6	GG, AR
02/1985	12 mM	1933	32	1.7	AMH, JSK, AR
06/1985	17 mM	9356	134	1.4	DLB, LH, JSK, KMcN, AR, BR
01/1986	12 mM	2130	29	1.4	AMH, LH, AR, TS
01/1986	15 mM	8347	160	1.9	JB, AMH, LH, NM, AR, TS
01/1988	15 mM	3057	73	2.6	JB, JMcD, AMH, KMcK, AR, MZ
01/1988	15 mM	2663	51	2.1	AMH, KMcK, KP, AR, TS
01/1988	15 mM	2470	56	2.3	AMH, AR, MZ
TOTAL		31606	552	1.7	

JB = Joseph Babity, DLB = David Baillie, JMcD = Jennifer McDowall, GG = Grant Gilmour, AMH = Ann Marie Howell, LH = Linda Harris, JSK = Jong Sun Kim, KMcK = Kim McKim, KMcN = Kelly McNeil, NM = Nasrin Mawji, KP = Ken Peters, AR = Ann Rose, BR = Bruce Rattray, TS = Terry Starr, MZ = Monique Zetka

They were mated to *dpy-5 unc-13/ + +* males. Strains which gave no Unc-3 or Dpy-5 Unc-13 Unc-3 males but did give fertile Dpy-5 Unc-13 hermaphrodites after successful mating were assumed to carry an X-linked lethal mutation, and were discarded. Some strains gave wild-type and Dpy-5 Unc-13 males but no Dpy-5 Unc-13 hermaphrodites or Lon-2 or Unc-3 males after crossing. These did not behave like chromosome I or X-chromosome lethal strains and were not further characterized. Strains which gave Unc-3 and Dpy-5 Unc-13 Unc-3 males and fertile Dpy-5 Unc-13 hermaphrodites after crossing were assumed to carry a lethal mutation linked to *dpy-5 unc-13*. Four of these (*h546, h556, h563, h564*) mapped to the right of *dpy-5* and four (*h549, h550, h559, h565*) mapped to the left of *dpy-5*.

3. *hT1(I;V)*

Phenotypically wild-type hermaphrodites of the genotype *dpy-5 unc-13 +; +/ hT1(I;V)[+ + unc-29; +]* were treated with EMS. Approximately 450 putative lethal mutations were recovered, from 3,000 screened chromosomes, of which 111 were analysed. In twelve strains the lethal mutation was lost due to recombination (*i.e.* they were outside of the boundaries of cross-over suppression of *hT1*). Ninety-nine strains were crossed to Lon-2 males of the genotype *hT2(I;III)[dpy-5 +; +; 0]/szT1(I;X)[+ unc-29; +; lon-2]*. In this way it was possible to determine whether the lethal mutation in each strain was on chromosome I or chromosome V. If the lethal were on chromosome V, the resulting *szT1* strain would have the genotype *dpy-5 unc-13; let-x/+; +/ szT1[+ +; lon-2]* and would segregate many fertile Dpy-5 Unc-13s. The $I^{L}V^{R}$*hT1* chromosome in the screening strain was marked with *unc-29*, as was the $I^{L}X^{L}$*szT1* chromosome in the strain used to rebalance the lethals. This was done so that the *hT1/szT1* heterozygotes would be Unc-29 and therefore easily distinguishable from the desired wild-type *let-x dpy-5 unc-13/szT1* heterozygotes. The strain used to rebalance the lethals over *szT1* also carried the translocation *hT2(I;III)* marked with an induced *dpy-5* allele. This was intended to keep the *unc-29* on the $I^{L}X^{L}$*szT1* chromosome from crossing off. This was not completely effective, however, since two strains were created which contained an unmarked *szT1* chromosome in *trans* to *hT1*.

Thirty-eight strains carrying lethal mutations on chromosome V were discarded. In thirteen strains the lethal mutation was on chromosome I, but outside of the cross-over suppressed region of *szT1* on chromosome I. Two strains appeared to be chromosome I lethals when balanced over *szT1*, but were found to be heterozygous for *hT1* and *szT1* which had lost the *unc-29* marker. Forty-six strains were recovered which carried chromosome I lethal mutations in the cross-over suppressed region of *szT1*.

The 111 strains analysed represented approximately one quarter of those recovered, and since they were chosen randomly they should be representative of one quarter of the chromosomes screened (750). This estimate is subject to any unknown bias that may have been placed on the lethals chosen for analysis. Forty-six lethal mutations in the *szT1*-balanced region of chromosome I were recovered giving an induction frequency of 6.1% (46/750).

B. Characterization of *szT1*

The reciprocal translocation *szT1(I;X)* was isolated and originally characterized by Fodor and Deak (1985). It was recovered as an X-ray induced, dominant cross-over suppressor on the X chromosome. It caused pseudolinkage of markers on chromosome I (*unc-11, unc-38, dpy-5* and *unc-15*) and the X chromosome (*unc-3* and *unc-58*). In order to determine whether *szT1* would be a useful balancer for lethal mutations on chromosome I, its recombinational properties were further characterized.

szT1 does suppress recombination on the left half of chromosome I. An interesting and novel observation was an increase in recombination adjacent to the chromosome I breakpoint in *szT1* heterozygotes.

1. Recombination suppression

Fodor and Deak (1985) observed reduced recombination in *szT1(I;X)* heterozygotes on the X chromosome, but did not investigate recombination suppression on chromosome I. Recombination frequencies were measured between chromosome I markers in controls and *szT1* heterozygotes in order to determine the extent and severity of crossover suppression. No recombination was observed from *let-362* (near the left end of chromosome I) to *dpy-5* (Table 3). No recombination events occurred between *dpy-5* and *unc-13* in three different strains tested (Table 3).

2. Recombination enhancement

Recombination frequencies were examined in regions of chromosome I to the right of the *szT1(I;X)* breakpoint. Recombination between *unc-13* and *unc-29* in *szT1* heterozygotes was increased from 1.2 map units to 2.5 map units (Table 3). Since K. McKim observed no recombination in the *unc-13 - let-88* interval, all of the increase must have occurred between *let-88* and *unc-29*. Normal recombination in this interval is less than 0.8 map units. By the same

Table 3. Recombination suppression and enhancement in *szT1(I;X)* heterozygotes

Genotype	Wts	Recombinants	pX100 (95% C.I.)
let-362 dpy-5 unc-13/+ + +[a]	1406	149 Dpy-5 Unc-13	15.4 (13.0 - 17.9)
		20 Unc-13	1.9 (1.2 - 2.9)
let-362 dpy-5 unc-13;+/szT1	817	0	0.0 (0 - 0.2)
unc-11 dpy-5/ + +[b]	2463	33 Dpy-5	2.1 (1.8 - 3.3)
		37 Unc-11	
unc-11 dpy-5;+/szT1	722	0	0.0 (0 - 0.6)
dpy-5 unc-13 unc-29/+ + +	2678	34 Dpy-5[c]	1.9 (1.3 - 2.6)
		21 Unc-29[d]	1.2 (0.8 - 1.8)
dpy-5 unc-13;+/szT1	929	0	0.0 (0 - 0.5)
dpy-5 unc-13 unc-29;+/szT1	680	17 Unc-29[d]	2.5 (1.5 - 3.8)
dpy-5 unc-29;+/szT1	707	9 Dpy-5	2.5 (1.1 - 4.7)
		14 Unc-29	
dpy-5 unc-29 unc-75/+ + +	1767	38 Unc-29 Unc-75[e]	3.1 (2.1 - 4.1)
		70 Unc-75[f]	5.8 (4.5 - 7.2)
dpy-5 unc-29 unc-75;+/szT1	1769	26 Dpy-5[e]	2.6 (1.7 - 3.7)
		66 Unc-29 Unc-75	
		234 Unc-75[f]	12.9 (11.2 - 14.6)
		100 Dpy-5 Unc-29	
dpy-5 unc-75;+/szT1	581	45 Dpy-5	14.6 (10.5 - 19.0)
		91 Unc-75	

Table 3. (continued)

Genotype	Wts	Recombinants	pX100 (95% C.I.)
2000 rads gamma radiation			
dpy-5 unc-13 unc-29/+ + +	2336	45 Dpy-5[c]	3.0 (2.4 - 3.7)
		51 Unc-13 Unc-29	
		28 Unc-29[d]	1.8 (1.2 - 2.5)

[a] From Table 7

[b] From Table 5

The intervals assayed were

[c] *dpy-5 - unc-13*

[d] *unc-13 - unc-29*

[e] *dpy-5 - unc-29*

[f] *unc-29 - unc-75*

reasoning, all recombination between *dpy-5* and *unc-29* in the *szT1* heterozygotes (Table 3) must have taken place between *let-88* and *unc-29*. In the three different strains tested, the same level of about 2.5 map units was measured. K. McKim mapped the *szT1* breakpoint on chromosome I to this region. Thus this interval, immediately adjacent to the *szT1* breakpoint on chromosome I, increased in size three-fold in *szT1* heterozygotes (from <0.8 map units to 2.5 map units). Recombination was also enhanced in the next interval to the right. The *unc-29* - *unc-75* interval increased in size two-fold from 5.8 map units to 12.9 map units (Table 3).

In order to determine if the *szT1*-enhanced region is one that can be expanded by radiation, *dpy-5 unc-13 unc-29*/+ + + hermaphrodites were treated with 2000 rads of gamma radiation and the resulting recombination frequency was determined. In these experiments, the *dpy-5* - *unc-13* interval increased from 1.9 map units to 3.0 map units, an increase slightly less than that reported by Kim and Rose (1987) (1.6 map units to 3.9 map units). The *unc-13* - *unc-29* interval, which spans the position of the szT1 breakpoint, increased by 50% from 1.2 map units to 1.8 map units (Table 3??). The increase appears to be significant in comparison to the control. The increase indicates that the unc-13 - unc-29 interval is expandable by gamma radiation.

3. Lack of any interchromosomal effect

Fodor and Deak (1985) reported increases in recombination on other chromosomes in their *szT1* strains. They tested an interval on each of chromosomes II, III and IV where they found increased recombination in *szT1* heterozygotes as compared to their controls. I measured recombination in four intervals on three autosomes and found no significant increases in recombination frequency (Table 4). Fodor and Deak (1985) reported that the *dpy-10* - *unc-4* interval on chromosome II increased from 2.5 map units to 5 map units in *szT1* heterozygotes. I found no significant increase in that interval. It appears that *szT1* does not affect recombination on autosomes other than chromosome I. It is possible that there was another mutation in the strain which Fodor and Deak were using which caused the apparent increases in recombination frequencies.

Table 4. Recombination on other chromosomes in szT1 heterozygotes

Genotype	Wts	Recombinants	pX100(C.I.)
dpy-10 unc-4/+ + (II)	2484	47 Unc-4	2.8 (2.1 - 3.7)
dpy-10 unc-4/+ +; +/szT1	1163	25 Unc-4	3.2 (2.2 - 4.5)
unc-36 sma-2/+ + (III)	1693	4 Unc-36	0.4 (0.09 - 0.9)
unc-36 sma-2/+ +; +/szT1	731	1 Unc-36	0.2 (0.01 - 1.0)
unc-42 sma-1/+ + (V)	1070	8 Unc-42	1.1 (0.4 - 2.1)
unc-42 sma-1/+ +; +/szT1	695	4 Unc-42	0.9 (0.2 - 2.2)
dpy-11 unc-23/+ + (V)	1186	19 Dpy-11	2.4 (1.4 - 3.6)
dpy-11 unc-23/+ +; +/szT1	659	14 Dpy-11	3.2 (1.8 - 5.2)

C. Recombination Mapping

A genetic characterization of the *hDf6* region should include determination of its size and its position on the chromosome with respect to other genes in the vicinity. Visible and lethal mutations inside of *hDf6* and to both sides of it were positioned by recombination mapping. As well as positioning *hDf6*, the distribution of lethal mutations recovered by *sDp2* could be derived from the mapping data. It was also important to test some of the initially recovered EMS-induced *sDp2*-recovered lethal mutations to ensure that they behaved predictably as single point mutations before undertaking large scale lethal screens.

1. Non-lethal mutations

Previous to this study, several non-lethal mutations had been placed on the genetic map to the left of *dpy-5* on chromosome I (Edgley and Riddle, 1987). However, some of these were not precisely positioned. In order to confirm the published positions of some of these genes, they were mapped by determining their recombination distance from *dpy-5* (or *unc-11*). The data for the distances between *dpy-5* and *unc-11, unc-38, unc-57, unc-63* and *unc-74* are presented in Table 5 along with the distance between *unc-11* and *unc-73*.

Three-factor positioning of three genes was also carried out (Table 6). The positioning of *him-1* between *unc-57* and *dpy-5* was confirmed. *unc-73* had not previously been positioned with respect to *unc-11*. It is now positioned between *unc-11* and *dpy-5*. *fer-7* failed to separate from *dpy-5* in 28 recombinants tested. Therefore it is tightly linked to, but not identified as being to the right or left of *dpy-5*.

2. Lethal mutations

a. EMS-induced lethal mutations

In order to characterize the distribution of an initial set of lethals, 59 EMS-induced *sDp2*-recovered lethal mutations were mapped. Two-factor and three-factor data are reported in Table

Table 5. Two-factor mapping data for non-lethal mutations

Genotype	Wild-types	Double Mutants	Recombinants		p (95% C.I.)
unc-11 dpy-5	2463	755	33 Dpy-5	37 Unc-11	2.1 (1.8 - 3.3)
unc-38 dpy-5	1951	568	3 Dpy-5	3 Unc-38	0.2 (0.1 - 0.5)
unc-57 dpy-5	3703	1098	21 Dpy-5	26 Unc-57	1.0 (0.7 - 1.2)
unc-63 dpy-5	3586	1157	7 Dpy-5	7 Unc-63	0.3 (0.2 - 0.5)
unc-74 dpy-5	2053	631	23 Dpy-5	31 Unc-74	2.0 (1.5 - 2.5)
unc-11 unc-73	1072	298	7 Unc-11	ND	1.0 (0.5 - 1.9)

ND = Not determined

Table 6. Three-factor positioning data for non-lethal mutations

Genotype of Heterozygote	Recombinant Chromosome	(#)	Result
unc-57 + dpy-5	*unc-57 + +*	(6)	*unc-57* (11/23) *him-1* (12/23) *dpy-5*
+ him-1 +	*unc-57 him-1 +*	(4)	
	+ + dpy-5	(7)	
	+ him-1 dpy-5	(6)	
unc-11 + dpy-5	*unc-11 + +*	(7)	*unc-11* (5/12) *unc-73* (7/12) *dpy-5*
+ unc-73 +	*unc-11 unc-73 +*	(5)	
unc-11 (dpy-5 +)	*unc-11 (fer-7 +)*	(13)	*unc-11* (28/28) (*dpy-5 fer-7*)
+ (+ fer-7)	*+ + dpy-5*	(15)	

7. The recombination frequency for the *dpy-5 unc-13* interval in a non-lethal strain is given in the first line of the table. These data were obtained from the strain KR236 (*dpy-5 unc-13; sDp2 [+]*). Unc-13 hermaphrodites from KR236 were crossed to N2 males and the recombination frequency in the resulting heterozygotes calculated from the Unc-13 recombinant class. In a similar manner, recombination frequencies were obtained for each of the lethal strains (identified in Table 7 by the allele carried). The criteria for assignment of gene names is described in section III.D.2 of this thesis. There were no anomalies in map position of alleles, *i.e.* all alleles of a gene mapped close to each other. For the lethal mutations which mapped to the left of *dpy-5* (Section B of Table 7), Dpy-5 Unc-13 and Unc-13 recombinants were expected. This was the case for 30 of the EMS-induced mutations. The distance the lethal maps from *dpy-5* was calculated from the Dpy-5 Unc-13 recombinant class. The lethals have been ordered by their distance from *dpy-5*, with the farthest left at the top of Table 7. Dpy-5 recombinants were not expected for this group of lethals since the cross-over chromosome (*let-x dpy-5 +*) would carry the lethal and would not survive if fertilized by a *let-x dpy-5 unc-13* chromosome. In a few cases one cross-over chromosome (*let-x dpy-5 +*) was fertilized by another cross-over chromosome (*+ dpy-5 unc-13*). Twenty-one Dpy-5 recombinants of this type were observed. A second possible type of Dpy-5 recombinant could be the result of a double cross-over event (*+ dpy-5 +/let-x dpy-5 unc-13*). None of the Dpy-5 recombinants listed in Section B of Table 7 were of the latter type, presumably the result of positive chromosomal interference.

Section C shows the data for lethals mapping to the right of *dpy-5*. Gene names were assigned based on complementation analysis done by A. Rose. In these cases the distance to *dpy-5* was calculated from the Dpy-5 recombinant class. Lethals are ordered by their distance from *dpy-5*, with the closest to *dpy-5* at the top of the table. The distance from the lethal to *unc-13* was also calculated from the Unc-13 class (data not shown) and no anomalies were observed. Section D shows the data for the lethals that were inseparable from *dpy-5*.

Table 7. Two-factor mapping data for EMS-induced lethals

Gene	Allele[a]	N[b]	Progeny[c]	DpyUnc	Dpy	Unc	p[d] (95% C. I.)
A: Control							
dpy-5 unc-13/+ +		30	6435	1422	52	51	0.016 (0.012 - 0.021)
B: EMS-induced lethals to the left of *dpy-5*:							
let-362	*h86*	9	2099	148	1	20	0.154 (0.130 - 0.179)
	h93	9	2427	164	0	22	0.146 (0.122 - 0.171)
lin-6	*h92*	8	1551	72	3	5	0.102 (0.079 - 0.129)
	h99	8	2031	106	2	9	0.113 (0.092 - 0.136)
let-365	*h108*	10	2663	56	2	20	0.044 (0.034 - 0.057)
	h129	10	2273	61	0	15	0.055 (0.042 - 0.070)
N. A.	*h197*	8	1719	40	1	19	0.049 (0.034 - 0.066)
let-360	*h96*	10	1716	39	0	10	0.046 (0.033 - 0.063)
let-357	*h89*	8	1681	24	1	14	0.030 (0.020 - 0.044)
	h132	10	2065	39	0	22	0.038 (0.027 - 0.051)
N. A.	*h192*	9	1791	21	2	11	0.026 (0.017 - 0.039)
let-356	*h83*	9	1356	15	1	16	0.024 (0.014 - 0.038)
let-351	*h43*	7	1041	12	0	16	0.023 (0.013 - 0.040)
let-366	*h112*	9	1647	15	1	20	0.020 (0.012 - 0.031)
let-368	*h121*	10	1899	19	0	12	0.020 (0.012 - 0.031)
let-372	*h126*	16	3413	31	4	24	0.018 (0.014 - 0.028)
let-369	*h125*	8	868	7	0	4	0.016 (0.008 - 0.032)
let-354	*h79*	12	2577	16	1	24	0.013 (0.007 - 0.021)
	h90	10	2068	14	1	15	0.015 (0.008 - 0.022)
	h267	8	2125	13	0	17	0.012 (0.006 - 0.020)

Table 7. (continued)

Gene	Allele[a]	N[b]	Progeny[c]	DpyUnc	Dpy	Unc	p[d] (95% C. I.)
let-353	*h46*	6	872	4	0	12	0.009 (0.003 - 0.044)
him-1	*h134*	9	1719	4	0	18	0.005 (0.002 - 0.011)
let-361	*h97*	10	2263	5	0	19	0.004 (0.002 - 0.010)
	h113	8	1441	0	0	11	0 (0 - 0.004)
	h116	6	1131	5	1	8	0.011 (0.005 - 0.023)
let-364	*h104*	10	2337	3	0	26	0.003 (0.001 - 0.007)
let-363	*h98*	8	1893	0	0	10	0 (0 - 0.003)
	h111	10	1883	3	0	14	0.003 (0.001 - 0.009)
	h114	10	2367	3	0	17	0.002 (0.001 - 0.007)
	h131	9	2343	1	0	26	0.001 (0.0001- 0.005)
let-371	*h123*	16	2367	2	0	16	0.002 (0.0003- 0.006)
N. A.	*h198*	8	1696	2	0	15	0.002 (0.0004- 0.008)
let-352	*h45*	6	1360	1	0	12	0.001 (0.0001- 0.005)
let-359	*h94*	16	2891	1	0	23	0.001 (0.0001- 0.004)
C: EMS-induced lethals to the right of *dpy-5*:							
let-376	*h130*	34	5768	0	1	44	0.0003(0.0001-0.002)
let-377	*h110*	10	2088	0	2	16	0.002 (0.003 - 0.006)
let-378	*h124*	10	2156	0	2	14	0.002 (0.003 - 0.006)
let-388	*h88*	8	2051	0	4	14	0.004 (0.001 - 0.009)
let-379	*h127*	9	2043	0	4	10	0.004 (0.001 - 0.009)
let-380	*h80*	9	1380	0	5	4	0.007 (0.003 - 0.016)
let-384	*h84*	9	1447	0	5	8	0.007 (0.003 - 0.016)
let-387	*h87*	8	1937	0	8	10	0.008 (0.003 - 0.016)

Table 7. (continued)

Gene	Allele[a]	N[b]	Progeny[c]	DpyUnc	Dpy	Unc	p[d] (95% C. I.)
bli-4	*h42*	19	4116	0	22	21	0.011 (0.007 - 0.016)
let-382	*h82*	11	2345	0	13	2	0.011 (0.006 - 0.018)
let-391	*h91*	10	2332	0	13	15	0.011 (0.006 - 0.018)
let-381	*h107*	10	2429	0	14	14	0.012 (0.007 - 0.019)
let-383	*h115*	10	1972	0	12	4	0.012 (0.007 - 0.021)
let-386	*h117*	10	2176	0	13	9	0.012 (0.006 - 0.020)
let-392	*h120*	8	1525	0	11	8	0.014 (0.007 - 0.025)
	h122	10	2093	0	12	3	0.012 (0.006 - 0.020)
let-385	*h85*	9	2349	0	14	1	0.012 (0.007 - 0.020)
	h109	10	2145	0	25	8	0.023 (0.016 - 0.034)
	h135	8	1840	0	14	5	0.015 (0.009 - 0.025)
let-389	*h106*	10	2096	0	14	4	0.013 (0.008 - 0.022)
let-390	*h44*	5	887	0	7	2	0.016 (0.007 - 0.032)
D: EMS-induced lethals inseparable from *dpy-5*:							
let-355	*h81*	31	7532	0	0	52	0 (0 - 0.001)
let-367	*h119*	26	5356	0	0	35	0 (0 - 0.001)
let-370	*h128*	26	5267	0	0	45	0 (0 - 0.001)
N. A.	*h194*	9	1353	0	0	15	0 (0 - 0.005)

[a] Lethal strain outcrossed to N2 males
[b] Number of heterozygotes
[c] Calculated from wild types and recombinant progeny
[d] Frequency of recombination with *dpy-5*
N. A. = Not assigned

Figure 4. Distribution of *sDp2*-recovered essential genes. This histogram is based on the data presented in Table 7.

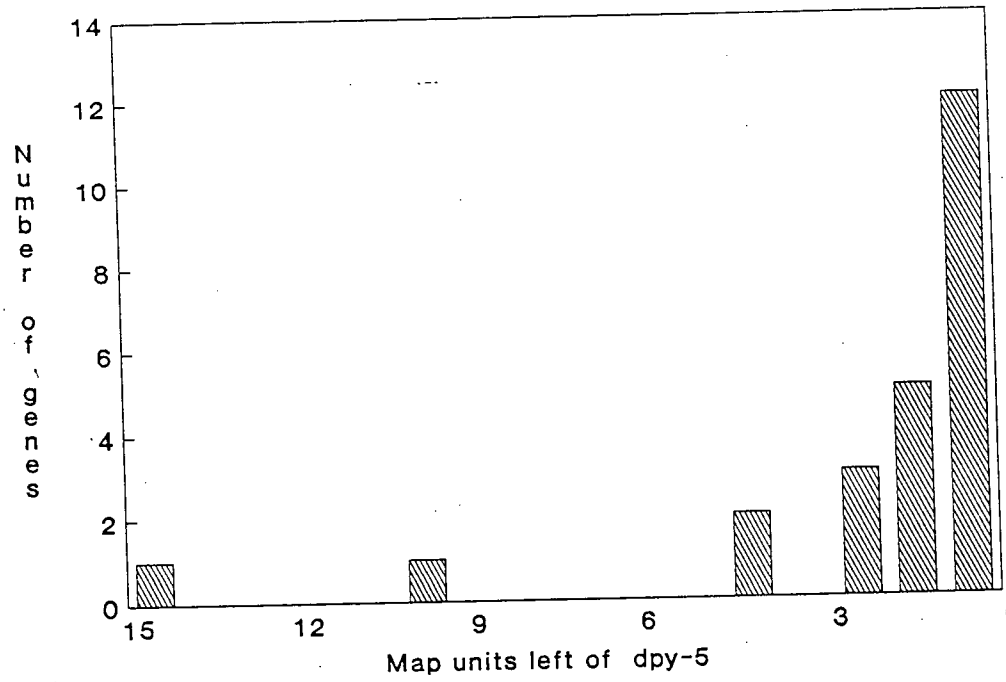


Figure 4 shows the distribution of essential genes inseparable from or to the left of *dpy-5*. The histogram displays the relative essential gene density per map unit for the left third of chromosome I. Genes on *C. elegans* autosomes form one large cluster per chromosome, leaving most of the rest of chromosome with a low gene density per map unit (Brenner, 1974). This distribution of essential genes shown in Figure 4 is very similar to the distribution of non-essential genes previously identified (Edgley and Riddle, 1987). Similarities in distributions of essential and non-essential genes on the other autosomes have been reported by others (Clark *et al.*, 1988; Rosenbluth *et al.*, 1988; Sigurdson, Spanier and Herman, 1984).

b. Gamma-induced lethal mutations

Gamma-induced lethal mutations were mapped in the same manner as the EMS-induced lethals. The results for eighteen lethal mutations are given in Table 8. The data in Table 8 is arranged similarly to that in Table 7. Another strain, carrying the mutation *h330*, gave fertile Unc-11 and Dpy-5 Unc-11 recombinants (1313 wild types, 11 Dpy-5 Unc-11s, 11 Dpy-5 Unc-13s, 28 Unc-11s, 15 Unc-13s; p = 0.021 for the *unc-11(h330) - dpy-5* distance). *h330* failed to complement *unc-11(e47)*. Worms homozygous for the *unc-11(h330) dpy-5 unc-13* chromosome arrested development at a late larval stage. It thus appeared that this viable *unc-11* allele produced a synthetic lethal in combination with these markers. Since the *unc-11(h330) dpy-5* recombinants were viable, the synthetic lethality was probably due to *unc-13*. I attempted to construct an *unc-11(e47) unc-13* double mutant strain to see if the *e47* allele would behave in the same way. Hermaphrodites with the genotype *unc-11(e47) + unc-13/unc-11(e47) dpy-5 +* segregated Unc-11s, Dpy-5 Unc-11s and late larval arrested Unc-13s (Unc-13s are quite paralyzed and would mask the Unc-11 phenotype). Thus, both alleles of *unc-11* resulted in synthetic lethality with *unc-13*.

Table 8. Two-factor mapping data for gamma-induced lethals

Gene	Allele	N	Progeny	DpyUnc	Dpy	Unc	p (95% C. I.)
A: Gamma-induced mutations to the left of *dpy-5*							
	h75	11	2293	174	1	17	0.166 (0.141 - 0.195)
	h331	9	1797	18	0	13	0.020 (0.012 - 0.031)
hDf7	*h54*	6	1079	8	0	9	0.015 (0.006 - 0.028)
let-373	*h70*	10	1729	13	0	17	0.015 (0.008 - 0.025)
let-354	*h72*	9	1551	6	0	25	0.008 (0.003 - 0.017)
	h327	9	276	1	0	3	0.007 (0.0003- 0.024)
let-361	*h323*	8	1235	3	0	8	0.005 (0.001 - 0.011)
let-363	*h60*	6	1487	3	0	16	0.004 (0.001 - 0.011)
	h57	9	2191	2	0	16	0.002 (0.0002- 0.006)
	h71	10	1663	2	0	5	0.002 (0.0003- 0.008)
him-1	*h55*	9	2096	1	0	18	0.001 (0.0001- 0.005)
	h324	11	2761	1	0	14	0.001 (0.0001- 0.002)
B: Gamma-induced mutations to the right of *dpy-5*							
	h76	4	1539	0	8	0	0.010 (0.004 - 0.020)
	h59	6	348	0	2	2	0.012 (0.002 - 0.039)
C: Gamma-induced mutations inseparable from *dpy-5*							
	h56	10	517	0	0	8	0 (0 - 0.012)
	h58	17	3664	0	0	9	0 (0 - 0.002)
	h167	7	1693	0	0	0	0 (0 - 0.004)
	h171	10	2197	0	0	0	0 (0 - 0.003)

D. Complementation Analysis of Mutations Left of *dpy-5*

Essential genes were defined on the basis of extensive complementation analysis of mutations to the left of or inseparable from *dpy-5*. Several methods were used to divide the lethal mutations into groups for *inter se* complementation analysis. Mutations which had been positioned by recombination mapping or duplication mapping to the right of *dpy-5*, or which complemented *sDf4*, were not analysed. The remaining lethal mutations were tested for complementation with *hDf6*. The duplication *hDp3* and the deficiency *hDf7* were used to divide the *hDf6* lethals into three groups for *inter se* complementation analysis. The lethal mutations from Table 7 which mapped to the left of (or inseparable from) *dpy-5* were also subjected to *inter se* complementation analysis. Genes were assigned on the basis of EMS-induced mutations. Genes were not assigned using gamma-induced mutations because these could be small deficiencies.

1. Deficiencies

The set of EMS-induced *sDp2*-recovered lethal mutations fell into three major categories: 1) 158 positioned with respect to *dpy-5* by recombination mapping ; 2) 195 positioned to the right of *dpy-5* by complementation to *hDp13* (A. Rose and J. McDowall, unpublished results); and, 3) 142 that had no prior positioning.

The 85 lethal mutations from category one which mapped within 5 map units to the left of *dpy-5* were tested for complementation with *sDf4*. In order to obtain some positional information for the unmapped lethal mutations (category 3), 90 were tested for complementation with *sDf4*. Strains heterozygous for *sDf4* grow very slowly (even after back-crossing) and show low viability. A control cross was scored in order to determine the proper criteria for assessing complementation with *sDf4*. Heterozygous *+ +/ dpy-5 unc-13* males were generated by crossing N2 males to Unc-13 hermaphrodites from the KR236 strain. These males were mated to *sDf4/ bli-4 dpy-14* hermaphrodites and the outcross progeny were scored. Sixteen Dpy-5 and 208

Table 9. Deficiency mapping of EMS-induced *sDp2*-rescued lethals

Position	Number		In *hDf6*	Out *hDf6*
in *sDf4*	93		24	69
in *hDp13*	[a]124		21	103
not tested with *sDf4* or *hDp13*	52		9	43
not in *sDf4*	82		not tested	
not in *hDp13*	[a]71		not tested	
positioned right of *dpy-5*,[b]	73		not tested	
far left of *dpy-5*, or allelic to non-*hDf6* gene	495	TOTAL		

a = Data from A. Rose and J. McDowall

b = Data from JB, JMcD, AMH, LH, JSK, RK, KMcK, KMcN, DP, KP, BR, and TS. Initials are as listed for Table 2 with the addition of RK = Rohinish Kisun, DP = David Pilgrim.

wild-type males were observed giving a control segregation ratio of 1:13 (predicted 1:3). Comparable numbers of Dpy-5 and wild-type hermaphrodites were also present. The Dpy-5 hermaphrodites grew slowly and gave few progeny. A similar experiment was carried out with *sDf4* balanced over *hT1*. In this case the control segregation ratio was 1:6. In complementation tests with the lethal-carrying strains, more than 50 wild-type males were scored in order to ensure that apparent allelism was not the result of *sDf4*-heterozygote inviability. Of the 175 lethal mutations tested, 93 failed to complement *sDf4* (Table 9).

Appropriate *sDp2*-recovered lethals were tested for complementation with *hDf6* (Table 9). Since both *hDf6* breakpoints are inside of *sDf4*, it was not necessary to test any lethal mutations outside of *sDf4* for complementation with *hDf6*. Since the right breakpoint of *hDf6* is to the left of *dpy-5*, it was not necessary to test any lethal mutations outside of *hDp13* for complementation with *hDf6*. A total of 54 EMS-induced *sDp2*-recovered lethal mutations failed to complement *hDf6*.

The *hT1*-recovered lethal mutations in the *szT1*-balanced strains were also tested for complementation with *hDf6*. Lon males from two of the 46 strains (*h984* and *h1003*) failed to mate after several attempts and were not positioned. The remainder were tested regardless of their position in order to determine if there were mutations which could be rescued in a translocation heterozygote that would not be rescued by *sDp2*. Four of 44 tested failed to complement *hDf6*.

The gamma-induced mutations recovered using *sDp2* which mapped to the left of *dpy-5* were tested with *hDf6*. Four of these (*h54, h70, h72* and *h327*) failed to complement *hDf6*.

The gamma-induced lethal mutations recovered with *szT1* which mapped to the left of *dpy-5* were tested with *hDf6*. One, *h549*, failed to complement *hDf6*.

2. Lethal mutations

The lethal mutations in Sections B and D from Table 7 (except *h192, h194, h197* and *h198*) and all other lethal mutations which failed to complement *hDf6* were subjected to *inter se*

complementation analysis. The lethals were divided into three groups for complementation analysis. Group 1 mapped to the region from the left end of chromosome I to the left breakpoint of *sDf4*. Group 2 included lethal mutations in *sDf4* but not in *hDf6* (the three mutations in section D of Table 7 were in *sDf4*). Group 2 was further divided into two groups: 2a) included lethal mutations to the left of *hDf6*; and 2b) included lethal mutations to the right of *hDf6*. Group 3 included all the mutations in *hDf6* and was further divided into three subgroups: 3a) contained lethal mutations complemented by *hDp3* (see Section III.D.4); 3b) examined lethal mutations in *hDf7* (see below); and, 3c) included lethal mutations outside both *hDp3* and *hDf7*. Complementation tables indicating the direction of each cross can be found in Appendix I.

The nine lethal mutations in group 1 define five complementation groups, four of which are represented by two alleles. Studies on *h92* and *h99* were published previously as alleles of *let-358* (Howell *et al.*, 1987). It was later determined that *let-358* is the same gene as *lin-6*, previously described by Sulston and Horvitz (1981) who found that *lin-6* is required for DNA synthesis and that mutations of this gene cause the absence of most postembryonic cell divisions. Since it was published first under the name *lin-6*, that name takes precedence and the *let-358* designation will no longer be used.

The 18 lethals in Group 2 define 13 complementation groups; three to the left of *hDf6* and ten to the right. *let-361* is represented by three alleles, and *let-363* is represented by four alleles. It was reported by Howell *et al.* (1987) that *h234* was an allele of *let-372*, a gene to the left of *hDf6*. It was later shown that *h234* fails to complement *hDf6*, indicating the previous result was incorrect. *h234* now defines the essential gene, *let-373*.

The 54 EMS-induced *sDp2*-rescued lethal mutations in *hDf6* (Group 3) define nineteen complementation groups. The mapping of these genes with respect to duplication and deficiency breakpoints are reported in Tables 10 and 14, and shown in Figure 5. Ten genes are represented by more than one allele (Table 10). Table 11 compares the observed distribution of genes with multiple alleles to that predicted by the truncated Poisson formula (Beyer, 1976). This will be described in the Discussion.

The four *hT1*-recovered lethal mutations in *hDf6* were complementation tested with the *sDp2*-recovered lethals. All four of them were allelic to complementation groups defined by the *sDp2*-recovered lethals (Table 12).

The gamma-induced *szT1*-recovered lethal mutation in *hDf6*, *h549*, is an allele of *let-354*.

The four gamma-induced *sDp2*-recovered lethal mutations in *hDf6* were tested for complementation with each other and the EMS-defined complementation groups in *hDf6*. Three failed to complement single complementation groups: *h70* is an allele of *let-373; h72* is an allele of *let-354;* and *h327* is an allele of *let-504*. *h54* failed to complement alleles of six of the complementation groups in *hDf6* (Table 10). It was renamed *hDf7*. *hDf7* complements lethal mutations outside of *hDf6*. *hDf7* complements all of the visible mutations in *hDf6* (see below). One gene uncovered by *hDf7, let-507*, is complemented by *hDp3* (see below). This positions *hDf7* to the right of *unc-89*, since *unc-89* is to the left of *hDp3*.

3. Non-lethal mutations

Some visible mutations are alleles of essential genes; for example *bli-4* (Peters and Rose, 1988), *rol-3* (Rosenbluth *et al.*, 1988), and *unc-70* (Park and Horvitz, 1986) are known to have both lethal and visible alleles. It was thus appropriate to determine whether any of the visible mutations positioned by others to the left of *dpy-5* on the genetic map (Edgley and Riddle, 1987) were allelic with essential genes identified in this study. The visible mutations were first tested for complementation with deficiencies. They were then tested for complementation with lethal mutations in the same region.

unc-38, unc-63 and *unc-67* fail to complement *sDf4* but are outside of *hDf6*. *unc-73* and *unc-89* fail to complement *hDf6*. No visible mutation failed to complement *hDf7*.

sup-11 and *fog-1* are to the left of the left breakpoint of *sDf4*. No lethal alleles of these genes were found, even though the null phenotype of *sup-11* is known to be lethal (Greenwald and Horvitz, 1982). *unc-57* and *unc-73* complemented all of the essential complementation groups in *hDf6*. Mutations in *spe-11* result in male sperm-rescuable self-sterile hermaphrodites

Table 10. Duplication and deficiency mapping of essential genes in *hDf6*

Gene	Allele	*hDp3*	*hDf7*
let-356	*h83*	OUT	
	h501		OUT
	h679	OUT	
	h871		
let-366	*h112*		OUT
	h265	OUT	
	h411		
	h422		
	h505		OUT
	h852		
let-373	*h234*	OUT	OUT
	h573		OUT
let-501	*h714*	OUT	OUT
	h498		OUT
let-509	*h521*	OUT	OUT
	h522		OUT
	h867	OUT	OUT
let-510	*h740*	OUT	OUT
let-511	*h755*	OUT	OUT
let-353	*h46*	OUT	IN
let-503	*h313*		
	h418	OUT	IN
let-504	*h448*	OUT	IN
	h844		

Table 10. (continued)

Gene	Allele	*hDp3*	*hDf7*
let-505	*h426*	OUT	IN
let-506	*h300*	OUT	IN
let-507	*h439*	IN	IN
let-354	*h79*	IN	OUT
	h90		
	h201		
	h267		
	h370		
	h390		
	h441		
	h482		
	h504		
	h508	IN	
	h693		
	h803		
	h809		
	h819		
	h841	IN	
	h863		
	h866		
let-374	*h251*	IN	OUT

Table 10. (continued)

Gene	Allele	*hDp3*	*hDf7*
let-502	*h392*		
	h509		OUT
	h732		
	h783		
	h835	IN	OUT
let-508	*h452*	IN	OUT
let-351	*h43*	IN	OUT
let-375	*h259*	IN	OUT
	h391		

For *hDp3*, IN indicates the lethal mutation is covered by the duplication and OUT indicates the lethal mutation is not covered by the duplication.

For *hDf7*, IN indicates the lethal mutation fails to complement the deficiency and OUT indicates the lethal mutation complements the deficiency.

Table 11. Distribution of alleles compared to truncated Poisson distribution

Number of alleles	Observed number	Expected number
0		6
1	9	9
2	5	6
3	1	3
4	1	1
5	1	0
6	1	0
17	1	0

Table 12. Duplication and deficiency mapping of *hT1*-recovered lethal mutations

Gene[a]	Allele	*hDp13*	*hDp3*	*hDf6*
	h880	IN	OUT	OUT
	h935	IN	OUT	OUT
	h988	IN	OUT	OUT
	h989	IN	OUT	OUT
	h1004	IN	OUT	OUT
let-504	*h888*	IN	OUT	IN
let-366	*h890*	IN	OUT	IN
let-354	*h934*	IN	IN	IN
let-508	*h995*	IN	IN	IN
	h883	IN	IN	OUT
	h887	IN	IN	OUT
	h889	IN	IN	OUT
	h983	IN	IN	OUT
	h936	IN	IN	OUT
	h937	IN	IN	OUT
	h985	IN	IN	OUT
	h987	IN	IN	OUT
	h996	IN	IN	OUT
	h1001	IN	IN	OUT
	h1006	IN	IN	OUT
	h881	IN		OUT
	h998	IN		OUT
	h1000	IN		OUT
	h878	OUT		OUT

Table 12. (continued)

Gene[a]	Allele	*hDp13*	*hDp3*	*hDf6*
	h879	OUT		OUT
	h882	OUT		OUT
	h884	OUT		OUT
	h885	OUT		OUT
	h886	OUT		OUT
	h931	OUT		OUT
	h933	OUT		OUT
	h938	OUT		OUT
	h940	OUT		OUT
	h986	OUT		OUT
	h990	OUT		OUT
	h991	OUT	IN	OUT
	h993	OUT		OUT
	h994	OUT		OUT
	h997	OUT	IN	OUT
	h999	OUT		OUT
	h1005	OUT	IN	OUT
	h1007	OUT	IN	OUT
	h932			OUT
	h1002			OUT

[a] - Gene names were assigned only to the four mutations which failed to complement *hDf6*.

(L'Hernault, Shakes and Ward, 1988). This gene maps close to the left of *dpy-5* but outside *hDf6* (L'Hernault, Shakes and Ward, 1988; K. McKim, unpublished results). It was tested with the appropriate lethal mutations from Table 7 and found to complement them all.

Three genes (*unc-38, unc-63* and *unc-74)* whose mutant alleles confer resistance to the drug levamisole map to this region (Lewis *et al.* 1980). Lethal mutations were not tested for complementation to these genes because known null alleles are not lethal (Lewis *et al.* 1980), and are not synthetic lethals with *unc-13. unc-89* was not tested for complementation with lethals because null alleles for this locus are not lethal.

a. *unc-11*

unc-11 fails to complement *sDf4* (Rose, 1980) but complements *hDf6. unc-11(e47)/sDf4* worms are fertile Dpy-5 Unc-11s. One of the *sDp2*-rescued lethal strains, KR776, gave sterile Dpy-5 Uncs when crossed to *sDf4* and sterile Dpy-5 Unc-13s when crossed to *hDf6.* The phenotype of the former Dpy Uncs resembled that of Dpy-5 Unc-11 (Unc-11s have a distinctive backward jerking phenotype). KR776 failed to complement *unc-11(e47)* for the uncoordinated phenotype but complemented the sterile phenotype. The fertile *unc-11* mutation (*h1008*) was separated from the sterile mutation in *hDf6* (*h452*) by recombination. Thus, what at first appeared to be a sterile *unc-11* mutation was in fact the result of two mutational events.

b. *him-1*

him-1 was originally described by Hodgkin, Horvitz and Brenner (1979). The single EMS-induced allele, *e879,* causes a high incidence of males (Him phenotype) in the self progeny of homozygous hermaphrodites, the result of increased X-chromosome nondisjunction. *e879* homozygotes produce about 20% male self progeny (Hodgkin, Horvitz and Brenner, 1979; Table 13). *e879* also causes a reduction in recombination frequency on the X chromosome (Hodgkin, Horvitz and Brenner, 1979). *him-1* maps outside *hDf6* but within *sDf4. e879/sDf4* heterozygotes produce significantly more males than *e879* homozygotes (Table 13) suggesting that *e879* is a

Table 13. X-chromosome nondisjunction in *him-1* strains

Genotype[a]	Herma-phrodites	Males	Frequency	95% C.I.
e879/e879	764	181	19.2%	(16.9 - 21.4)
e879/sDf4	514	200	28.0%	(25.1 - 30.7)
e879/h55	325	144	30.7%	(27.0 - 34.2)
e879/h134	499	176	26.1%	(23.1 - 28.9)
e879/ +	3236	9	0.3%	(0.1 - 0.5)
e879/e879; sDp2	2154	27	1.2%	(0.8 - 1.7)
h134/h134; sDp2	1039	12	1.1%	(0.6 - 1.9)
e879/ + ; sDp2	991	2	0.2%	(0.04 - 0.7)
+ / + ; sDp2	2480	3	0.1%	(0.03 - 0.3)
+ / + [b]	4663	5	0.1%	(0.04 - 0.3)

a - *e879, h55,* and *h134* are alleles of *him-1*. *e879* is on a chromosome marked with *dpy-5*. *h55* and *h134* are on chromosomes marked with *dpy-5* and *unc-13*. *sDf4* deletes *him-1* and *dpy-5*.

b - Data from Rose and Baillie (1979).

hypomorphic allele of *him-1* (Muller, 1932).

Two of the *sDp2*-recovered lethal mutations were found to be alleles of *him-1*. *him-1(h55)* was a gamma-ray induced mutation and *him-1(h134)* was an EMS-induced mutation. Both are recessive, late larval lethals. *e879/h55* and *e879/h134* heterozygotes produce frequencies of males similar to that of *e879/sDf4* (Table 13). They enhance the *e879* phenotype to the same degree as a deficiency, so they are likely amorphic alleles. Since these two alleles are recessive lethals and both are inviable over *sDf4*, analyses of effects on recombination and X-chromosome nondisjunction can not be done.

Hodgkin, Horvitz and Brenner (1979) reported that *him-1(e879)* had a dominant Him phenotype, producing 0.7 % males; this value was significantly different from the wild-type control frequency. I also observed a slight dominant Him phenotype for *e879* (Table 13). The dominant Him phenotype of *him-1* was more pronounced when in a ratio of two mutant copies to one wild-type copy (*i.e.* in *sDp2* strains). *e879/e879; sDp2* and *h134/h134; sDp2* gave approximately 1 % males (Table 13). The presence of *sDp2* was not responsible for this effect since it did not increase X-chromosome nondisjunction (Table 13, lines 8 and 9). It is possible that the wild-type allele of *him-1* on *sDp2* does not produce as much product as a wild-type allele on a normal chromosome, or the locus is dosage sensitive.

4. Duplications

A number of duplications of chromosome I were available which could be used to position essential genes (see Figure 1 in Materials and Methods). *hDp3* is a duplication which breaks in the *hDf6* region between *unc-74* and *unc-89*. *hDp3* was used to position the genes in *hDf6* (Table 10). Approximately one third (7/19) of the essential genes in *hDf6* are covered by *hDp3*.

Table 14 shows the results of complementation testing some of the essential genes to the left of *dpy-5* from Table 7 with some duplications. The table is arranged with essential genes farthest from *dpy-5* at the top, and duplications with breakpoints closest to *dpy-5* at the left. The

first three genes in Table 14 are in *sDf4* to the left of *hDf6*. The two-factor recombination mapping distances for these three genes (Table 7) indicated that they were to the left of *hDf6,* and the fact that they are not covered by *hDp3* confirms this positioning. The next nine genes are uncovered by *hDf6*. Lethals in *hDf6* which were only tested with *hDp3* are listed in Table 10. The last seven genes in Table 14 are between *dpy-5* and the right breakpoint of *hDf6*.

hDp22 includes *dpy-5* but has not been found to complement any genes to the left of *dpy-5*. *hDp25* separates into two groups those genes which are found both in *hDf6* and in *hDp3*. *hDp25* includes *let-351* but not *let-354* or *let-374* (Table 14). Figure 5 is a map of the *sDf4* region which is drawn according to the results presented in Tables 10 and 14.

The genetic distance from the left breakpoint of *hDf6* to the left end of the chromosome is at least thirteen map units. The distance from the right breakpoint of *hDf6* to *dpy-5* is approximately 0.75 map units. Forty-six lethal mutations in *hDp13* (but not in *hDf6*) were tested with *hDp3*. Twenty-four mapped to the right of *hDf6* and 22 were to the left of *hDf6*. The frequency of recovery of lethal mutations per map unit is fifteen-fold higher in the region close to *dpy-5* than in the region to the left of *hDf6*. If the relative mutability per gene is roughly equal across the chromosome, this would reflect a fifteen-fold difference in gene density in these two regions.

The *hT1*-recovered lethals balanced by *szT1* were tested with *hDp13* (Table 12). Twenty-three of the 42 tested mutations were complemented by *hDp13*, positioning them either to the left of *dpy-5* or close to the right of it. Using *hT1*, the frequency of induction of lethals in *hDp13* was 3.1% (23/750). Of the 19 mutations tested, twelve were covered by *hDp3* and seven were not.

One of the *hT1*-recovered lethals which failed to complement *hDf6* (*h995*) appeared not to be rescuable by *hDp13*. This was because the chromosome carried two new mutations. The *hDp13* strain was a sperm-rescuable sterile hermaphrodite which layed unfertilized oocytes. The Dpy-5 Unc-13 worms that were heterozygous for the lethal-bearing chromosome and *hDf6* arrested at a late larval stage, but did not produce oocytes. During a strain construction to

Table 14. Duplication mapping of *sDp2*-rescued lethal mutations

	Duplication (*hDp*)						
Gene	*22*	*21*	*20*	*25*	*2*	*3*	*5*
let-368					OUT	OUT	OUT
let-369						OUT	OUT
let-372						OUT	
let-353						OUT	OUT
let-356					OUT	OUT	
let-366					OUT	OUT	
let-373						OUT	OUT
let-507						IN	
let-354		OUT	OUT	OUT		IN	
let-374		OUT	OUT	OUT	IN	IN	IN
let-351			OUT	IN	IN		IN
let-375		OUT	OUT			IN	IN
him-1	OUT	OUT	IN			IN	
let-352	OUT	IN					
let-359	OUT	IN	IN			IN	
let-361	OUT	IN	IN				
let-363	OUT	IN	IN			IN	
let-364	OUT	IN					
let-371			IN			IN	

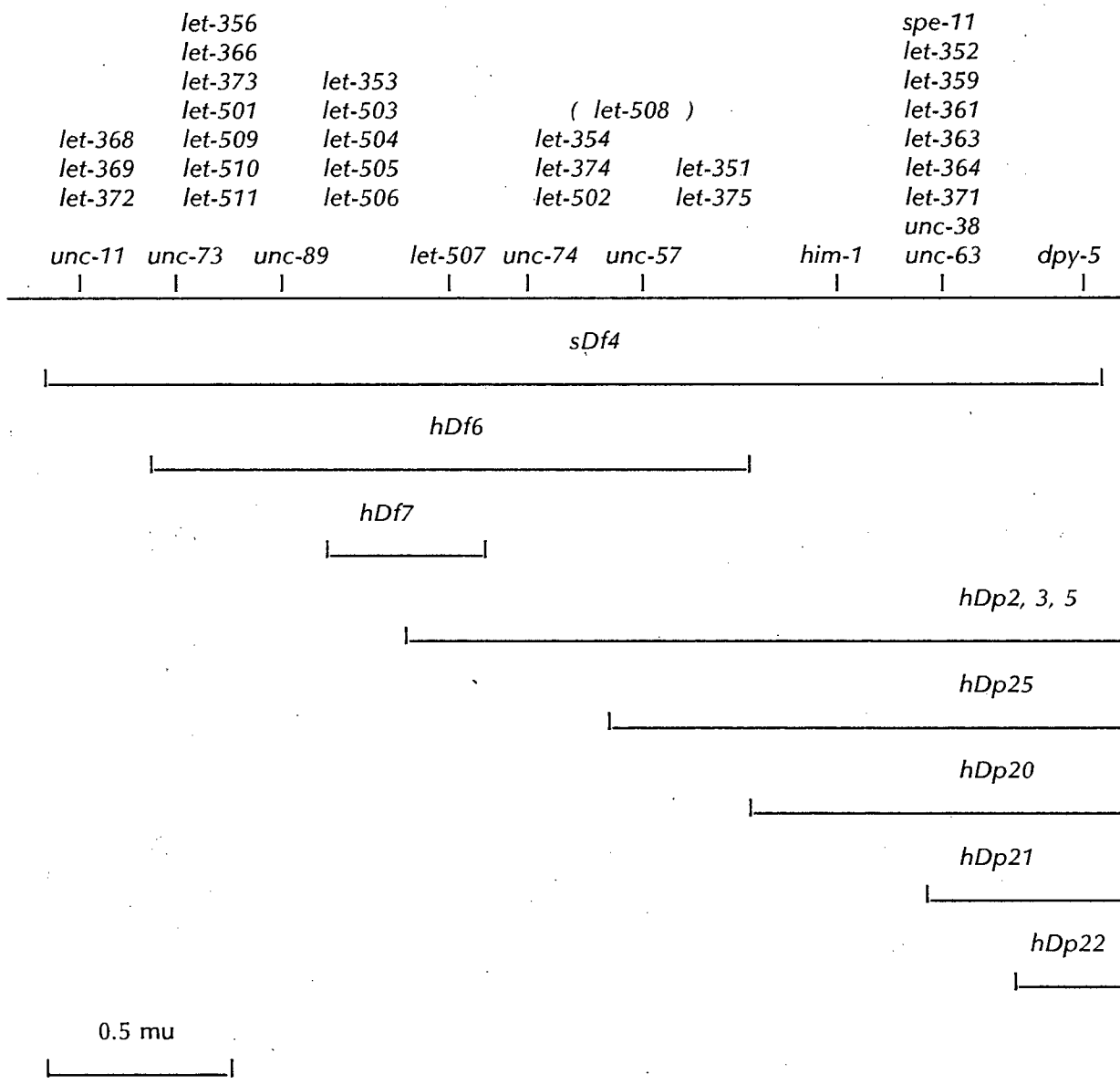


Figure 5. Genetic map of the *unc-11* - *dpy-5* region on chromosome I. Essential and visible genes are positioned based on deficiency (Sections III.D.1, III.D.2. and III.D.3) and duplication mapping (Section III.D.4 and K. McKim, unpublished results). The positions of the endpoints of *sDf4* are based on data of Rose (1980) and J. McDowall (unpublished results). *let-508* was not tested with *hDp25*.

rebalance the lethal-bearing chromosome under *sDp2*, the sperm-defective mutation was lost by recombination. This resulted in a chromosome with the *h995* mutation (in *hDf6*) which was rescuable by *hDp13*.

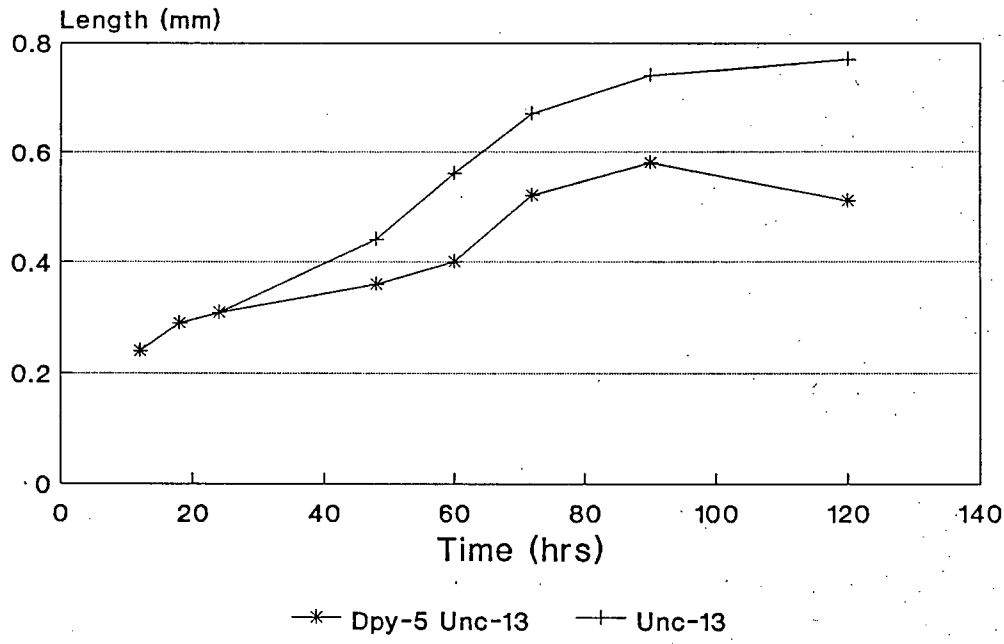
5. Stage of arrest of lethal mutations in *hDf6*

The stage of lethal arrest was examined for each of the mutations in *hDf6* (Table 15). For strains where arrested Dpy-5 Unc-13 individuals were easily visible, some were picked and allowed to sit for several days to ensure they developed as far as possible. Their lengths were measured and compared to a growth curve of Dpy-5 Unc-13s from KR236 (*dpy-5 unc-13; sDp2*) prepared by G. Duncan (see Fig. 6). For strains where arrested Dpy-5 Unc-13s were not easily visible, heterozygous hermaphrodites (*let-x dpy-5 unc-13/ + + +*) were allowed to lay eggs for about eight hours and then removed. Plates were checked the next day to see if all the eggs hatched. If all had hatched the plates were checked later to see when the Dpy-5 Unc-13s arrested development. Since no record was taken of larval moults, the arresting stages are classified as early, mid-larval, late larval or sterile adult.

Minor differences were seen between alleles of a given essential gene for which multiple alleles were recovered. For example, three alleles of *let-502* arrest at a mid-larval stage but the fourth arrests at a later larval stage. All 17 alleles of *let-354* appear to arrest development at the same mid-larval stage (probably L2).

None of the EMS-induced lethal mutations result in embryonic lethality. Very few (3/54) of the mutants arrest development at an early larval stage. The largest proportion (34/54 if *let-354* is included *let-354*, 17/34 if *let-354* is not included) arrest at a mid-larval stage, even if *let-354* alleles are not counted. Six of the sterile adult mutations are slightly leaky, meaning that some of the homozygous lethal Dpy-5 Unc-13 hermaphrodites produced a few progeny. Both alleles of *let-375* and all three alleles of *let-509* are leaky. The escaping progeny from *let-509* never grow past hatching, indicating that these mutations also have a maternal affect. Dpy-5 Unc-13 hermaphrodites from three of these leaky mutant strains (*let-375(h259), let-375(h391)* and

Figure 6. Growth curve for Unc-13s and Dpy-5 Unc-13s. The genotype of the Unc-13s was *dpy-5 unc-13; sDp2* (strain KR236) and the Dpy-5 Unc-13s were the progeny of KR236 Unc-13s.



T=0 is at egg lay.

Table 15. Stage of arrest of lethal mutations in *hDf6*

Gene	Allele	Stage of arrest
let-351	*h43*	mid-larval
let-353	*h46*	mid-larval
let-354	all (17)	mid-larval
let-356	all (4)	mid-larval
let-366	all (6)	mid-larval
let-373	both	early larval
let-374	*h251*	mid-larval
let-375	both	sterile adult (leaky)
let-501	*h714*	early larval
	h498	mid-larval
let-502	*h392*	mid-larval
	h509	sterile adult
	h732	mid-larval
	h783	late larval
	h835	mid-larval
let-503	both	sterile adult
let-504	*h448*	sterile adult
	h844	late larval
let-505	*h426*	late larval
let-506	*h300*	late larval

Table 15. (continued)

Gene	Allele	Stage of arrest
let-507	*h439*	sterile adult (leaky)
let-508	*h452*	late larval
let-509	*h521*	sterile adult (leaky)
	h522	sterile adult (leaky)
	h867	late larval (leaky)
let-510	*h740*	late larval
let-511	*h755*	early larval

let-507(h439)) also produced a few progeny after crossing to males. Since the number of out-cross progeny was not much more than self progeny, they are probably not sperm-defective mutations. They also did not exhibit the usual phenotype of sperm-defective mutants, that is they did not lay many unfertilized eggs. These three leaky mutations were not leaky over *hDf6* (and *hDf7* for *let-507*); *i.e.* over a deficiency their phenotype was more severe. This is in agreement with Muller's (1932) definition of a hypomorphic mutation, so they are probably hypomorphic mutations. None of the other sterile adult mutants gave any progeny after attempted mating to wild-type males.

An egg count was done for *hDf6 dpy-5 unc-13/ + + +* hermaphrodites. One quarter of the eggs should have been homozygotes for *hDf6*. From a total of 70 eggs laid, 17 did not hatch. It thus appears that *hDf6* is an embryonic lethal. A second smaller deficiency of this region, *hDf7*, is an early larval lethal. All eggs from *hDf7 dpy-5 unc-13/ + + +* hermaphrodites hatched but the Dpy-5 Unc-13s did not develop to the L2 stage. Thus, *hDf7* homozygotes seem incapable of any post-embryonic growth. The earliest blocking mutant identified in *hDf7* was mid-larval, most of the lethals blocked development at a late larval stage or produced sterile adults. The stage of arrest for homozygotes of either deficiency is earlier than that for homozygotes of any single mutation uncovered by them. It is possible that earlier blocking mutations are yet to be identified, or that the earlier block is the result of a cumulative affect of the loss of several gene products.

a. *let-354*

Seventeen alleles of *let-354* were identified among the 495 EMS-induced lethal mutations recovered by *sDp2*. One EMS-induced allele was recovered in the *hT1* screen. Two gamma-induced alleles were also recovered. *h72* was isolated from a screen using *sDp2*, and *h549* was isolated from a screen using *szT1*. In total, approximately 1/3 (20/61) of all mutations uncovered by *hDf6* were found to be alleles of *let-354*.

I used the recessive lethal mutation, *ct42*, recovered by P. Mains (personal communication) for complementation tests with lethal mutations in *hDf6*. I found it to be an allele of *let-354*. Because *ct42* also had a temperature-sensitive, dominant, maternal-effect lethal phenotype (P. Mains, personal communication), I tested ten *let-354* alleles for temperature-sensitive sterility. If any of them were similar to *ct42*, they would have been sterile at 25 ^{o}C. Late larval Unc-13 hermaphrodites from lethal strains (*i.e. let-354 dpy-5 unc-13; sDp2*) were incubated at 25 ^{o}C. Only one allele, *h482*, was sterile at 25 ^{o}C. Many eggs did not hatch and no larvae grew past hatching. None of the other nine alleles tested were sterile at this temperature. *hDf6 dpy-5 unc-13; hDp31* was also tested and found to be fertile at the restrictive temperature. Since *hDf6* must represent the null phenotype of *let-354*, the amorphic phenotype is not temperature sensitive. Furthermore, the null phenotype is recessive lethality, not a dominant, maternal-effect lethality.

The *ct42* allele of *let-354* can not be rescued by *sDp2*. Lon males with the genotype *let-354(ct42) dpy-5; 0/szT1[+ +;lon-2]* were mated to Dpy-14 Unc-29 hermaphrodites. The resulting wild-type males (*let-354(ct42) dpy-5 + +/ + + dpy-14 unc-29*) were mated to *dpy-5 unc-29; sDp2* (Unc-29) hermaphrodites. Except for recombinants, all wild-type hermaphrodite progeny from that cross were *let-354(ct42) dpy-5 + +/ + + dpy-14 unc-29; sDp2*. They were allowed to self at 16 ^{o}C because of the dominant temperature-sensitive sterility caused by *let-354(ct42)*. Thirty of their wild-type hermaphrodite progeny were picked. They were expected be of the parental genotype or *let-354(ct42) dpy-5/ let-354(ct42) dpy-5; sDp2* in a 2:1 ratio. Twenty-one were found to be of the parental genotype by progeny testing. The other nine laid fertilized eggs which did not hatch. *hDf6 dpy-5 unc-13; hDp31* were fertile at 16 ^{o}C, indicating this maternal-effect can not be the result of insufficient wild-type product but that the *ct42* allele is as an antimorph (Muller, 1932).

6. *sDp2* has a lethal mutation in the *hDf6* region

hDf6 can not be maintained in a homozygous state with *sDp2* in the same manner as the *sDp2*-rescued lethal strains. Two different strains were constructed in attempts to recover an *hDf6 dpy-5 unc-13; sDp2* strain. Both *hDf6 dpy-5 + unc-13/ + dpy-5 + unc-15; sDp2* and *hDf6 dpy-5 + unc-13/ + dpy-5 dpy-14 +; sDp2* hermaphrodites segregated sterile adult Unc-13 hermaphrodites. They did not lay oocytes and were not male sperm rescuable. Any fertile Unc-13s recovered were shown to be recombinants by progeny testing.

A possible explanation of this result was that *hDf6* could not be maintained in a 2:1 ratio of the deficiency chromosome to wild type. This was shown not to be the case by constructing two *hDf6/hDf6; $I^{L}X^{L}$szT1* strains. In this strain one of the translocated chromosomes of *szT1* (*$I^{L}X^{L}$szT1*) is being used as a duplication of the left half of chromosome I. *hDf6 dpy-5 unc-13; unc-3; $I^{L}X^{L}$szT1* was isolated as an aneuploid segregant from *hDf6 dpy-5 unc-13; unc-3/ szT1 [+ + +; + lon-2]*. *hDf6 dpy-5 unc-13; $I^{L}X^{L}$szT1* was constructed by crossing *hDf6 dpy-5 + unc-13/ + + dpy-14 +* males to *dpy-5 dpy-14; $I^{L}X^{L}$szT1* hermaphrodites and picking the appropriate segregant in the next generation. The viability of the two *hDf6/hDf6; $I^{L}X^{L}$szT1* strains showed that null alleles of all *hDf6* genes can be recovered in a 2:1 ratio of the deficiency chromosome to wild type.

$I^{L}X^{L}$szT1 covers more of chromosome I than does *sDp2*, so it was possible that the difference in ability to rescue *hDf6* was due to the difference in size. This was addressed by testing *hDf6* for complementation by three other duplications, *hDp31, hDp32* and *hDp34* (see Figure 1b). These three duplications could also rescue *hDf6* in Unc-13 strains (*i.e. hDf6 dpy-5 unc-13; hDpx*). Since these three duplications are shorter than *sDp2*, the inability of *sDp2* to rescue *hDf6* was not a function of its size. Thus, it is more than likely that *sDp2* carries a lethal mutation in the *hDf6* region. The mutation can not be in the *hDf7* region since it is rescuable by *sDp2*. No mutations were found in the *hT1* or *szT1* screens which failed to complement the lethal mutation on *sDp2*. It is not known whether the lesion on *sDp2* affects only one gene, or is a small deletion.

DISCUSSION

A. Conclusions of Research

I have undertaken an analysis of essential genes in a region of the *Caenorhabditis elegans* genome. The region was defined by the deficiency, *hDf6*, on chromosome I. In order to do this analysis, it was necessary to develop a system for identifying, maintaining and characterizing a large number of lethal mutations. The usefulness of a free duplication as a balancer for recessive lethal mutations in a large autosomal region in *C. elegans* was examined. In this thesis I report the development of a system for lethal recovery and analysis using *sDp2*, a free duplication of the left third of chromosome I. Using this system, 495 EMS-induced recessive lethal mutations have been analysed. The advantages of this system are discussed below. However, as a result of my analysis, it was shown that *sDp2* carries a lethal mutation in the *hDf6* region. *sDp2* could therefore not be used to recover alleles of every essential gene uncovered by *hDf6*. This analysis has demonstrated the feasibility of using a free duplication as a genetic balancer for autosomal lethal mutations.

The most common lethal balancing systems in *C. elegans* made use of translocations that suppress recombination (Clark *et al.*, 1988; Rosenbluth *et al.*, 1988). The first genetically well characterized translocation in *C. elegans* was *eT1(III;V)* (Rosenbluth and Baillie, 1981). It has been developed for use as a mutagen test system (Rosenbluth, Cuddeford and Baillie, 1983), used to study the effects of gamma mutagenesis in *C. elegans* (Rosenbluth, Cuddeford and Baillie, 1983), and to characterize a large number of essential genes on chromosome V (Rosenbluth *et al.*, 1988). In order to compare the usefulness of the duplication to translocations, two translocations involving chromosome I were used to screen for and maintain lethal mutations. One translocation, *szT1(I;X)*, has been extensively characterized by Fodor and Deak (1985) and McKim, Howell and Rose (1988). *szT1* was used for recovering and maintaining gamma-induced lethal mutations on chromosome I. *szT1* spontaneously generates X-chromosome fusion events at a

frequency of approximately 3%, resulting in strains which appear to be carrying a lethal mutation (McKim, Howell and Rose, 1988). Because many false lethal strains are recovered, *szT1* is not very good for lethal screening. A second translocation, *hT1(I;V)* (isolated by K. McKim) was shown to recover EMS-induced lethal mutations at a higher frequency than that of *sDp2*, suggesting that it may be a more efficient system for the recovery of lethal mutations. However, because the lethal mutations recovered using *hT1* are more difficult to map and analyse than *sDp2*-recovered lethals, the *sDp2* system was used for the bulk of the analysis.

Lethal mutations inseparable from or to the left of *dpy-5* were analysed and a saturation analysis of the small deficiency, *hDf6*, was begun. A combination of recombination mapping, deficiency mapping, duplication mapping and *inter se* complementation analysis led to the identification of 36 new essential genes (listed in Sections B and D of Table 7 and in Table 10) in the region from *dpy-5* to the left end of the chromosome. Nineteen of these are in *hDf6*. A maximum of three quarters of the essential genes in this deficiency have now been identified.

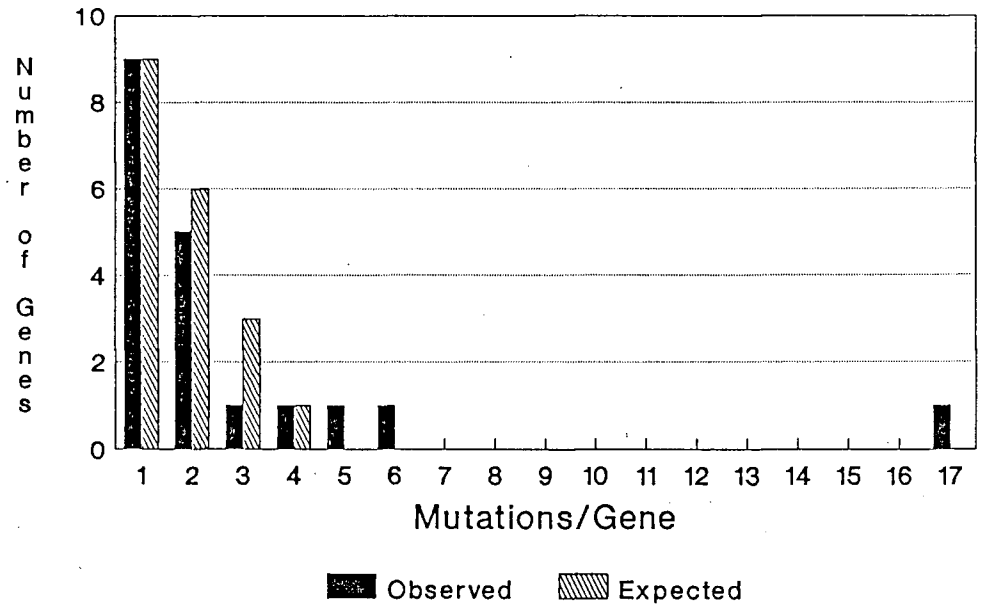
B. Estimate of the Number of Genes in *hDf6*

In an attempt to identify all of the genes in the small deficiency *hDf6*, nineteen essential genes were identified. These mutations were recovered by screening 31,606 mutagenized chromosomes. Ten of these genes were represented by more than one allele. An estimate of the number of genes yet to be identified can be gained from a statistical analysis of the allele frequencies. The Poisson distribution can be applied only if the following two assumptions are met; the probability of mutating is the same for each gene in the region, and the ability to detect mutants is the same for all genes (Lefevre and Watkins, 1986). The latter assumption holds if one considers only essential genes. The mutability of genes is far from being equal (Meneely and Herman, 1979; Hilliker, Chovnick and Clark, 1981; Lefevre and Watkins, 1986). The mutability of genes in *hDf6* is obviously not the same since *let-354* has seventeen alleles while others have none. The high mutation frequency indicates either that the gene is large or is extremely sensitive

to mutation. Two other frequent mutational targets in *C. elegans*, *unc-22* and *unc-54*, have been cloned and shown to be large genes (Moerman, Benian and Waterston, 1986 for *unc-22*; MacLeod, Karn and Brenner, 1981 for *unc-54*). Barrett (1980) analysed published data on lethal analysis in *Drosophila melanogaster* and showed that it did not fit well to a Poisson distribution, except when the sample sizes were small. Figure 7 shows a comparison of the observed distribution of mutations in *hDf6* to that predicted by the truncated Poisson calculation. It is obvious that the observed data does not fit the Poisson distribution exactly; there are more genes with multiple alleles than expected. The data may be interpreted as representing three classes of genes; the genes with one to three alleles are genes with average mutability, the genes with four to six alleles are a different class of genes with much higher mutability, and *let-354* with 17 alleles is a rare extremely mutable gene. Meneely and Herman (1979) proposed the use of a truncated Poisson formula which decreases the emphasis on highly mutable genes [$f = (1-e^{-m}-me^{-m})/(1-e^{-m})$, where f is the fraction of genes with more than one allele and m is the average number of alleles]. Of the nineteen genes in *hDf6*, ten were represented by more than one allele, giving a value of 0.53 for f. By calculation from the above formula, $m = 1.35$. By examination of a table of cumulative terms for the Poisson distribution (Beyer, 1976) the proportion of genes with no alleles is 0.25. Thus, a maximum of 75% of the essential genes in *hDf6* have now been identified. From these calculations one might conclude that a minimum of six genes are yet to be identified, giving a total minimum estimate of 25 essential genes in *hDf6*. This estimate is likely an underestimate because, as Muller (1929) pointed out, the zero allele class will always be underestimated if the mutability is not constant.

The frequency of recovery of mutations should be about the same across the entire *sDp2* region, since the screen places no bias on the position of the lethal mutations recovered as long as a wild-type allele is present on the duplication. If complete complementation analysis were carried out for any other interval in the *sDp2* region, the degree of saturation attained should be the same as for the *hDf6* interval. The set of EMS-induced *sDp2*-recovered mutations should therefore represent 75% of all essential genes in the entire *sDp2* region. My results are similar

Figure 7. Distribution of lethal mutations in *hDf6*. The observed distribution of lethal mutations is compared to that predicted by the truncated Poisson formula.



to those obtained by Clark *et al.* (1988). They predicted they had identified 65% of the essential genes in the *sDf2* region after screening 20,000 mutagenized chromosomes (D. V. Clark, personal communication). Meneely and Herman (1981) estimated they had identified one half of the genes in a region of the X chromosome after screening 11,500 mutagenized chromosomes. Their recovery rate was higher because they used a higher dose of EMS. Examination of the EMS dose response curve prepared by Rosenbluth, Cuddeford and Baillie (1983) indicates that double hit events are much more likely to be recovered at higher doses. These complicate the analyses by underestimating the total number of genes.

To estimate what fraction of the genome is contained in *hDf6,* the size of *hDf6* must be determined (see Figure 5). The size of *hDf6* can be estimated from recombination mapping data of genes known to be in or out of it. The right boundary is between *him-1* (0.5 map units from *dpy-5,* Table 7) and *unc-57* (1.0 map units from *dpy-5,* Table 5). Duplication mapping confirmed that *let-368, let-369* and *let-372* are to the left of the left breakpoint of *hDf6.* The left boundary must therefore be somewhere between the position of these genes and *let-356* and *let-366,* which are in *hDf6.* According to the recombination mapping data in Table 7, this is approximately 2 map units from *dpy-5.* Thus, *hDf6* extends from 0.75 map units left of *dpy-5* to 2.0 map units left of *dpy-5.* It is therefore about 1.25 map units across. This is 1/240 of the size of the total genome (300 map units).

It is possible to derive an estimate of the number of genes in *hDf6* by molecular studies if an estimate of its physical size is made. Its position in the genome must be taken into account for this estimate because the genetic map of *C. elegans* exhibits clustering of genes on the autosomes, first recognized for nonessential genes by Brenner (1974). It has since been shown to be the case for essential genes also (Section III.C.2.a; Rose and Baillie, 1980; Sigurdson, Spanier and Herman, 1984; Clark *et al.,* 1988; Rosenbluth *et al.,* 1988). Greenwald *et al.* (1987), Prasad (1988) and Starr *et al.* (1989) have shown that this clustering is due to suppression of recombination in the apparently gene dense regions. Kim and Rose (1987) showed that succeptibility to gamma radiation induced map expansion changes across the chromosome I gene

cluster. It is believed that regions of the genome which exhibit gamma radiation induced map expansion have a suppressed frequency of recombination per base pair relative to the genomic average. The differential sensitivity of regions of the gene cluster to map expansion indicates that the amount of DNA per map unit changes across the gene cluster. Estimates of the DNA/map unit value based on cloning in the *dpy-5* - *unc-13* region (Starr *et al.*, 1989) correlated well with the data of Kim and Rose (1987). For example, in the *dpy-14* - *unc-13* interval, Starr *et al.* (1989) found the DNA/map unit value to be 4.5 times the genomic average; Kim and Rose (1987) found that recombination in the same region increased four-fold after radiation treatment. *hDf6* is on the left edge of the gene cluster on chromosome I. Kim and Rose (1987) showed that the frequency of recombination in the *unc-11* - *dpy-5* interval increased 1.5 fold after radiation treatment. If the correlation between the amount of DNA/map unit and the increase in recombination frequency after radiation treatment extends to the left of *dpy-5,* one would predict that the *hDf6* region would contain 1.5 times the genomic average amount of DNA/map unit. Since the size of the genome has recently been re-estimated to be 100 Mb (J. Sulston, personal communication) the average size of a map unit would be 333 Kb. This would make *hDf6* approximately 625 Kb. Both Prasad (1988) and Starr *et al.* (1989) estimated there is one coding region for every 15 Kb in *C. elegans*. Heine and Blumenthal (1986) estimated 20 kb per gene. This would mean there would be room for 30 to 40 genes in *hDf6*. Five non-essential genes and nineteen essential genes have been mapped to the *hDf6* region. Including the six essential genes predicted to be present by the Poisson analysis, this would give a total of 30 genes in *hDf6*. Thus, the estimates of gene number in *hDf6* based on genetic or molecular analyses are in reasonable agreement.

The largest fraction of genes in the *hDf6* region is most likely the essential genes. Of the identified genes in *hDf6,* approximately four-fifths are essential. There may be, however, several genes which would not be identified in screens for lethal mutations. Genes with subtle null phenotypes or redundant members of gene families would not be identified. The true fraction of

essential genes could be determined when the number of coding regions identified by molecular analysis can be compared to the number of genes identified by mutational analysis.

Several different methods have been used to estimate the total gene number in *C. elegans*. Brenner's (1974) original estimate of 2,000 essential genes was based on the induction of X-linked recessive lethal mutations. A similar method was used by Baillie (D. L. Baillie, personal communication as cited in Moerman and Baillie, 1979) to arrive at an estimate of 4,000 essential genes. Rogalski (1983) obtained an estimate of 5,700 essential genes based on a saturation analysis of the small deficiency of chromosome IV, *sDf2*. Most recently, Clark *et al.* (1988) arrived at a minimum estimate of 3,500 essential genes by comparison of induction rates in a well characterized small region to a large region. However, they caution that their estimate may be an overestimate. If the essential gene density in the *hDf6* region were representative of the entire genome, an estimate of approximately 6,000 (25 x 240) total essential genes would be obtained. However, the *hDf6* region is probably not representative of the genome. *hDf6* is on the left edge of the gene cluster on chromosome I, so the gene/map unit density is expected to be higher than the genomic average (see Fig. 4). If the density is 1.5 times the genome average, this would give an estimate of approximately 4,000 essential genes. This is in good agreement with the estimates of Baillie (Moerman and Baillie, 1979) and Clark *et al.* (1988).

C. Forward Mutation Rates in *C. elegans*

C. elegans researchers often cite Brenner (1974) for his estimate of the average forward mutation rate per gene, that is, the rate of inducing a mutation in any given gene causing a mutant phenotype. Brenner had recovered 26 visible mutations from a screen of 736 mutagenized chromosomes. He estimated his target size as 77 genes, which was the total number of visible genes identified in screens of unmeasured sample sizes. Using these values, Brenner arrived at an estimate of 5×10^{-4} mutations per gene using 0.05 M ethyl methane sulfonate (EMS). The sample size of mutagenized chromosomes on which this estimate was based was quite small. The estimate of the target size was also inaccurate; many more than 77 genes in

C. elegans are now known to be mutable to a visible phenotype (Edgley and Riddle, 1987). The identification of visible mutations can be subjective because some mutant phenotypes are quite subtle. The majority of genes in *C. elegans* are essential genes, so an estimate based on mutability of visible genes may not represent the average gene. For these reasons, there is room for considerable error in Brenner's estimate.

The data presented in this thesis can also be used to derive an estimate of the average forward mutation rate per gene in *C. elegans*. The data for *let-354* is omitted from this calculation because its mutation rate is at least ten times higher than that for an average gene. From approximately 31,000 mutagenized chromosomes, 37 lethal mutations were recovered in *hDf6*. The target was the 24 essential genes predicted to be present in this region. Using these values, the average forward mutation rate per gene is 5×10^{-5}. This is ten-fold lower than Brenner's estimate. According to the dose response curve for EMS mutagenesis published by Rosenbluth, Cuddeford and Baillie (1983), the difference in EMS dose used would result in a two-fold lower induction frequency. This would lower Brenner's estimate to 2.5×10^{-4} mutations per gene, which is still five-fold higher than the estimate predicted by my data. The sample size of screened chromosomes on which this estimate is based is 40 times larger than Brenner's. The large sample allowed the recovery of mutations in less mutable targets. The identification of lethal mutations is also less subjective than identification of visible mutations.

The induction frequency for lethal mutations based on the *hDf6* analysis is in good agreement with previously published studies. If a two-fold correction is made for the EMS dose, the data on induction of X-linked lethal mutations from Meneely and Herman (1981) can be used to calculate an estimate of 5×10^{-5} mutations per gene. This estimate is identical to the estimate based on the results of this study. Using the data from Clark *et al.* (1988) based on an estimated 20,000 screened chromosomes (D. Clark, personal communication), the induction frequency is calculated to be 6×10^{-5} mutations per gene.

The forward mutation rate can be used to estimate the number of chromosomes which need to be screened when attempting to identify all of the genes in a region, or all of the genes

of a given type in the genome. In order to have 95% confidence that any gene has been identified, the number of chromosomes screened must be three times that required to recover one mutant allele. For the average gene in *C. elegans,* this would require the screening of approximately 60,000 chromosomes. If Brenner's estimate had been correct, only 12,000 mutagenized chromosomes (0.015 M EMS) would need to be screened to identify an average gene in the genome. If this were true, the three large studies (Meneely and Herman, 1981; Clark *et al.,* 1988; this thesis) would have identified all of the genes in the regions studied.

Kemphues, Kusch and Wolf (1988) relied on Brenner's estimate of average forward mutation frequency to formulate the important conclusions of their study on maternal-effect lethal mutations in *C. elegans*. They identified 17 strict maternal-effect genes on chromosome II, 13 of which had one or two alleles and four of which had four or more alleles. They argued that if the data was subjected to a Poisson analysis, a minimum estimate of 37 strict maternal-effect genes would be obtained. They then calculated that this would give an average forward mutation rate of 8×10^{-5} mutations per gene. Since this was so much lower than Brenner's value, they did not believe that it could be correct. They then used only the genes with four or more alleles (with an induction frequency of 5×10^{-4}) to make their conclusions. They assumed that since all of these genes had more than three alleles, they had identified all of them. This led to an extremely low estimate of the predicted number of strict maternal-effect genes in the *C. elegans* genome. If they had known that the average forward mutation rate is actually only 5×10^{-5} per gene, they would have predicted the existence of many more strict maternal-effect genes.

D. Stage of Developmental Arrest

The stage of lethal arrest was determined for the nineteen essential genes represented by 54 EMS-induced *sDp2*-rescued mutations in *hDf6* (Table 15). The stage of lethal arrest was determined in strains which also carried mutations in *dpy-5* and *unc-13*. The presence of these mutations may affect the phenotypes of the lethal mutations. The *dpy-5* and *unc-13* mutations

may alter the viability or the stage of lethal arrest of the lethal mutations. For example, viable mutant alleles of *unc-11* are synthetically lethal in combination with *unc-13* mutations (Section III.C.2.b). Some of the other apparent lethal mutations may be synthetically lethal with the marker mutations, but this was not tested.

Most alleles of a gene, for which more than one allele has been identified, arrest development at approximately the same developmental stage. This finding has also been described by others (Shannon *et al.*, 1972; Rose and Baillie, 1980; Meneely and Herman, 1981; Rogalski, Moerman and Baillie, 1982; Rosenbluth *et al.*, 1988). Shannon *et al.* (1972) proposed that most variability between alleles is due to leakiness of later arresting mutations. Clark *et al.* (1988) and Leicht and Bonner (1988) report more variability between alleles than the other studies mentioned above. In some cases Clark *et al.* (1988) showed that the later arresting mutations blocked development at an earlier stage when heterozygous with a deficiency, indicating that they are indeed hypomorphs (Muller, 1932). Leicht and Bonner (1988) proposed that some of the apparent variability between alleles of the essential genes they analysed was due to linked second site mutations. Since the lethal mutations described in this thesis were generated using a low dose of EMS, variability between alleles is not likely due to linked second site mutations. The most variability in stage of arrest was found for alleles of *let-502*, which ranged from mid-larval to sterile adult. The dissimilarities in phenotype could represent varying severities of mutant alleles, or differing mutated functions which are required at different times in development.

There is considerable variability in the stage of arrest between genes in *hDf6*. If the earliest blocking allele is taken for genes with more than one allele, three genes are required for development past the first larval stage, seven are required for development past the mid-larval stage, six are required to mature to adulthood from the late larval stage, and three are required for fertility of adult hermaphrodites. No mutants which blocked development during embryogenesis were found. This is not surprising since *C. elegans* embryonic development is strongly maternally influenced. Maternal-effect lethal mutations were not studied in this thesis research, although it is possible to recover them using the *sDp2* lethal screening system.

Figure 8 shows the distribution of arrest stages for the essential genes in *hDf6*. They have been divided into three regions; a) is the region left of *hDf7*, b) is the *hDf7* region, and c) is the region right of *hDf7*. The results show that within a small region many of the lethals arrest at the same stage, and that this stage differs from region to region. For example, the three genes which are required for development past the first larval stage are clustered in the leftmost region of *hDf6*. Individuals with mutations in five of the six essential genes in *hDf7* arrest development as late larvae or sterile adults. Individuals with mutations in four of the six essential genes in the rightmost region of *hDf6* arrest development at a mid-larval stage. However, because of the small number of genes analysed, these differences are not statistically significant. The clustering of genes which are required at the same developmental stages may represent some type of clustering of genes with related functions.

Analyses of stages of arrest for lethal mutations have been carried out in studies of different regions of the *C. elegans* genome. Figure 9 shows the distribution of arrest stages for three small regions; a) the *hDf6* region, b) the *unc-15* region (Rose and Baillie, 1980), and c) the *unc-22* region (Clark *et al.*, 1988). The frequencies of arrest stages vary dramatically depending on the region of the *C. elegans* genome under analysis. Only three of the nineteen genes in *hDf6* appear to be required for development past the first larval stage. Rose and Baillie (1980) reported that worms carrying mutations in 13 of the 16 essential genes in the region around *unc-15 (I)* arrested as early larva. Mutations in one third of the essential genes listed in Clark *et al.* (1988) from the *unc-22 (IV)* region arrest as early larva. The *hDf6* region has the smallest proportion (0.16) of early larval lethals. The proportion of genes with mid-larval arrest phenotypes is approximately one third for both the *hDf6* and *unc-22* regions. The proportion of genes with late larval arrest phenotypes is much larger in the *hDf6* region [0.32, (6/19)] than in the *unc-22* region [0.17, (5/30)]. The proportion of genes with sterile adult mutations is somewhat larger in the *hDf6* region [0.16, (3/19)] than the *unc-22* region [0.10, (3/30)] (Clark *et al.*, 1988). The *hDf6* region is similar to some other genomic regions for the proportions of mid-larval and adult sterile mutations recovered, but differs in the scarcity of early larval mutants recovered. Meneely and Herman

Figure 8. Distribution of lethal arrest stages for genes in the *hDf6* region.

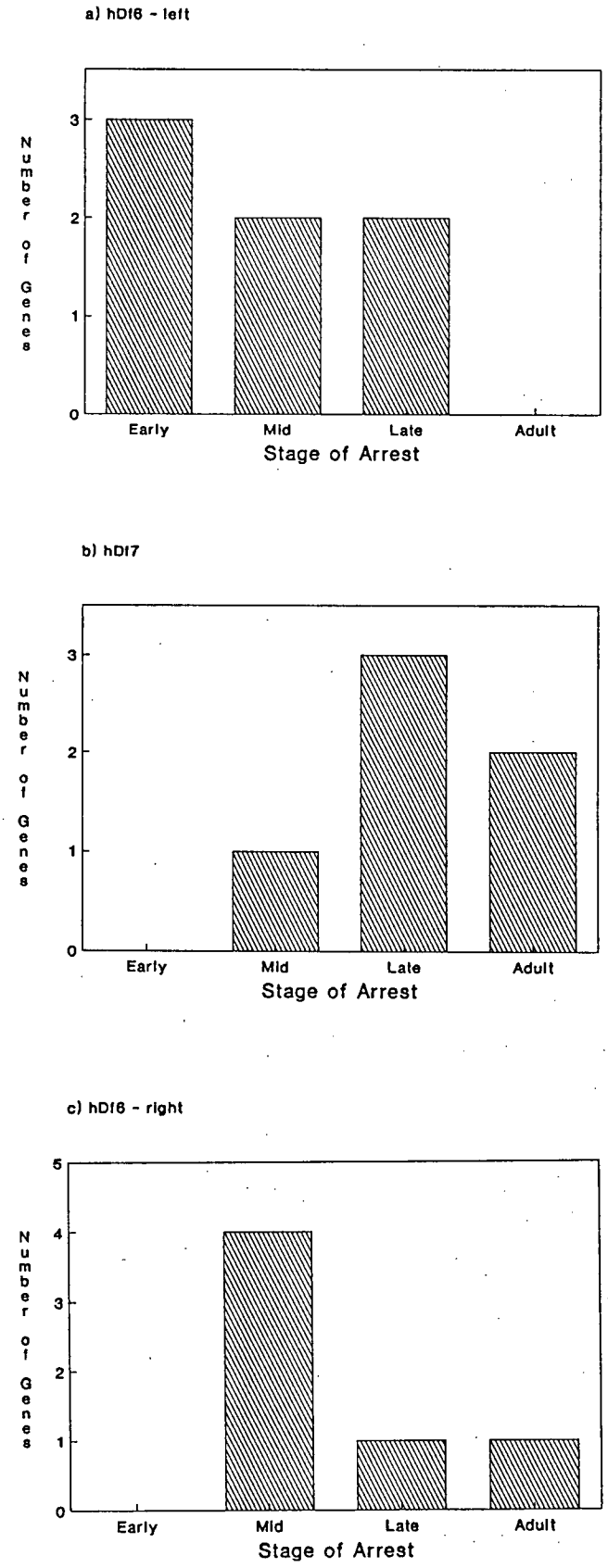
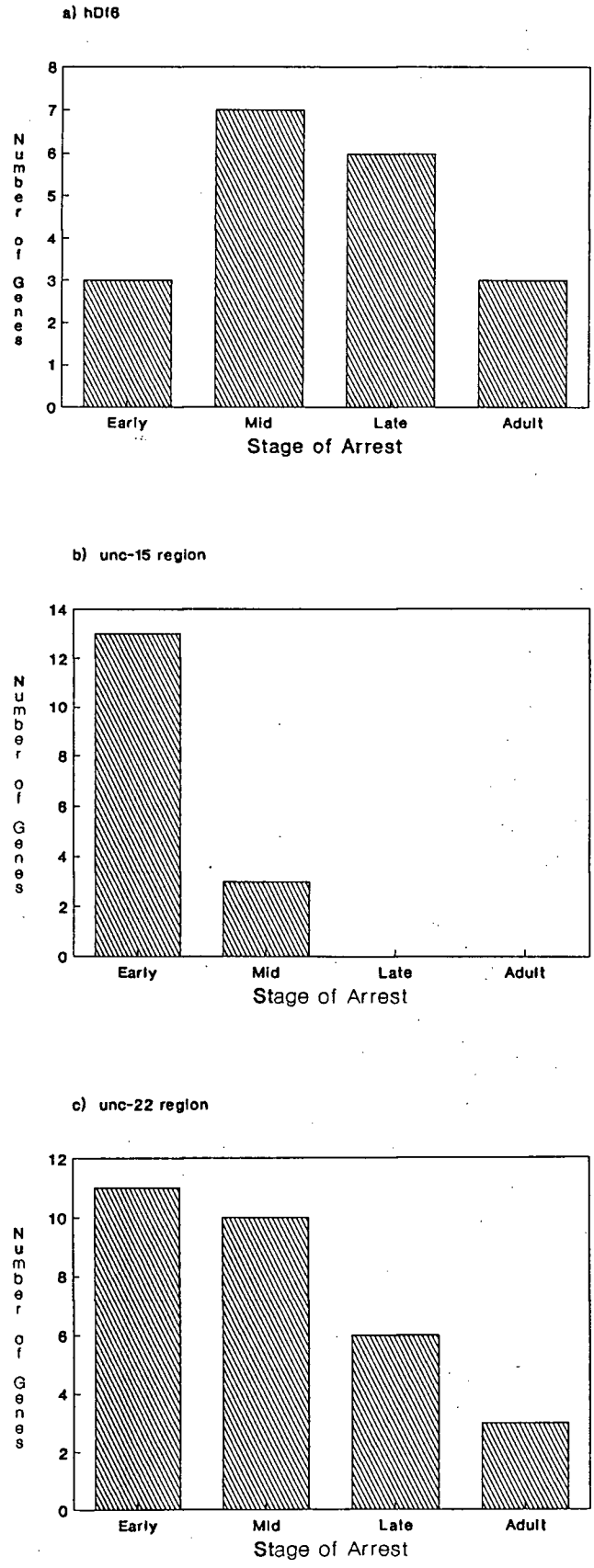


Figure 9. Distribution of lethal arrest stages for genes in three regions of the *C. elegans* genome. The data for b) was from Rose and Baillie, 1980, and for c) was from Clark *et al.*, 1988.



(1981), Sigurdson, Spanier and Herman (1984) and Rosenbluth *et al.* (1988), studied regions of the genome which were much larger. If the data from their large regions is divided into small intervals, regional differences in stages of lethal arrest become evident.

None of the sterile adult mutations in *hDf6* appear to be due to defective sperm production. L'Hernault, Shakes and Ward (1988) carried out an extensive screen for sperm-defective mutations on chromosome I. They identified eleven genes, none of which were in *hDf6*. From a Poisson analysis of the number of alleles recovered, they estimated there may be as few as three more *spe-* (sperm-defective) genes on chromosome I. There could be more than that since they did not screen enough chromosomes to saturate the region.

Alleles causing temperature sensitive dominant or normal recessive lethal phenotypes have been recovered for one of the genes in *hDf6, let-354*. Paul Mains isolated mutations in a screen for temperature sensitive dominant maternal-effect embryonic lethal mutations (P. Mains, personal communication). Three of these failed to complement *hDf6*. They also had a non-conditional recessive mid-larval lethal phenotype. All three failed to complement each other for the recessive phenotype. One of these, *ct42*, is an allele of *let-354*. *let-354* is a very large mutational target for both the recessive and dominant phenotypes. One third of all recessive lethal mutations in *hDf6* described in this thesis are alleles of *let-354*. Three of eight dominant temperature-sensitive maternal-effect embryonic lethal mutations recovered in a screen of the whole genome were alleles of *let-354* (P. Mains, personal communication). *hDf6* represents the null phenotype for *let-354* since the entire gene must be within *hDf6* because genes on both sides of it are included in *hDf6*. *hDf6* does not have a dominant temperature-sensitive maternal-effect lethal phenotype, so this could not be the null phenotype of any gene *hDf6* deletes. Furthermore, most temperature-sensitive mutations are the result of alterations in protein structure, not loss of function. The recessive mid-larval lethal phenotype is the most likely the null phenotype of *let-354*. One wild-type allele on a duplication can rescue two null alleles (*hDf6*), but can not rescue *ct42*. Thus, *ct42* is an antimorphic mutation because it interferes with the function of the wild-type gene product. The mutant *ct42* gene product does not retain the function necessary for

larval development at any temperature, but at the restrictive temperature somehow causes dominant maternal-effect embryonic lethality. One of the *sDp2*-recovered EMS-induced alleles of *let-354*, *h482*, showed a dominant temperature-sensitive maternal-effect embryonic lethal phenotype. This phenotype was not as extreme as *ct42*; some of the eggs hatched at 25 ^{o}C but none of the larvae grew after that. With *ct42*, most eggs do not hatch at 20 ^{o}C and none hatch at 25 ^{o}C (P. Mains, personal communication). *h482* was rescuable by the duplication and the strain was quite fertile at 20 ^{o}C. The mutation is similar to *ct42* but its antimorphic behaviour is not as strong. Perrimon *et al.* (1986) showed that in *Drosophila melanogaster* many maternal-effect lethal mutations are rare hypomorphic or antimorphic alleles of ordinary zygotic essential genes. Recently, the same has been shown to be true in *C. elegans* (Kemphues, Kusch and Wolf, 1988). The alleles of *let-354* which show maternal effects are probably of this type; the *let-354* gene product may not normally play a role in embryogenesis.

E. Comparison of Balancing Systems

This thesis describes the effectiveness of the free duplication, *sDp2*, as a lethal rescue system. Lethals are induced in a strain designed to allow detection of new mutations in the F2 generation. The screening strain can be easily maintained owing to the tight linkage between the markers used, *unc-15* and *unc-13* (Waterston, Fishpool and Brenner, 1977; Rose and Baillie, 1980) and between the markers and the *sDp2* breakpoint (Rose, Baillie and Curran, 1984). The progeny of over 31,000 individuals have now been screened using this strain with no indication of alteration in its genetic composition. Lethals could be isolated on the chromosome marked with *unc-13* or *unc-15*. In this thesis lethals on the *unc-13* chromosome were analysed because Unc-13s grow and mate better than Unc-15s. It is extremely important for a large scale lethal analysis to have all of the mutations linked to common markers for complementation analysis.

The lethal mutations are maintained in a strain with an Unc-13 phenotype that segregates only Unc-13 progeny, all of which have an identical genotype. Thus these strains are self-

maintaining over any number of generations and transfer of the lethal-bearing individuals does not require prior familiarity with the phenotype of the strain. Furthermore, *C. elegans* strains can be frozen, making the maintenance of thousands of lethal-bearing strains feasible.

Only lethal strains with the wild-type allele supplied by *sDp2* are fertile since the lethal strains are maintained with two normal homologues carrying the same lethal mutation. This strictly defines the region from which lethal mutations will be recovered to that duplicated by *sDp2*.

It is a straightforward procedure to produce males for complementation analysis when using this system since all the males resulting from an outcross have the lethal-bearing chromosome. Those that carry the duplication do not interfere with the analysis (Rose, Baillie and Curran, 1984). This simplifies and speeds up complementation testing by eliminating the need to maintain male strains that are heterozygous for the lethal or to identify the lethal-bearing males.

A result of this research was the discovery of a lethal mutation on *sDp2* in the *hDf6* region. It could have occurred spontaneously during stock maintenance. Rosenbluth, Cuddeford and Baillie (1983) measured the spontaneous lethal mutation rate over a 40 map unit region of the *C. elegans* genome and determined that 0.06% of hermaphrodites per generation would carry a new spontaneous lethal mutation somewhere in this region. Since *sDp2* is approximately one half of that size, 0.03% of hermaphrodites per generation woud be expected to carry a lethal mutation on the duplication. *sDp2* was generated at a dose of 7000 R of gamma radiation (Rose, Baillie and Curran, 1984). This high dose often causes multiple hits (Rosenbluth, Cuddeford and Baillie, 1983) so the lethal event was likely generated at the same time as the duplication.

Only one deficiency, *hDf7*, was recovered in a screen for gamma-induced lethal mutations. Since only mutations which are rescued by wild-type alleles on *sDp2* would be recovered, any deletions with one lethal breakpoint outside *sDp2* would be eliminated. *sDp2* carries a lethal mutation in the *hDf6* region, so deficiencies which span that site would not be recovered. For these reasons, *sDp2* is not a good balancer to use for recovery of gamma-induced deficiencies.

Translocations in *C. elegans* suppress crossing-over from their breakpoint to one end of the chromosome (Rosenbluth and Baillie, 1981; Herman, Kari and Hartman, 1982; Ferguson and Horvitz, 1985; Rosenbluth, Cuddeford and Baillie, 1985; McKim, Howell and Rose, 1988). They are useful as balancers of recessive lethal mutations in their cross-over suppressed regions (Rosenbluth, Cuddeford and Baillie, 1983, 1985; Clark *et al.*, 1988; McKim, Howell and Rose, 1988; Rosenbluth *et al.*, 1988). Three translocations of chromosome I that have been analysed (*szT1, hT1* and *hT2*) suppress crossing over from the left end of the chromosome up to the breakpoint (McKim, Howell and Rose, 1988). Two of these translocations were used as balancers in this study; *szT1(I;X)* (Fodor and Deak, 1985) and *hT1(I;V)*. The boundary of suppression of crossing over is between *unc-13* and *unc-29* for both of these translocations, so the region they balance on chromosome I extends approximately one map unit farther to the right than the region balanced by *sDp2*. *szT1* was used in a screen for gamma-induced lethals and *hT1* was used in a screen for EMS-induced lethals.

One of the problems with using a translocation as a balancer in screens is that mutations are recovered which are linked to, but outside of, the boundaries of cross-over suppression. This is less of a problem with *szT1* screens because of the increased rate of recombination adjacent to the breakpoint on chromosome I. Lethal mutations outside of the *szT1* breakpoint will be lost by recombination three times faster than with a translocation like *hT1* which does not have this increase in recombination frequency. Another difficulty with using the translocations as balancers is that the males can not be used directly for complementation analysis.

The efficiency of recovering lethals that map under *hDp13* (Figure 1 b) is 1.4% using *sDp2* and 3% using *hT1*. The apparent two-fold difference in induction frequency may be due to three factors. Firstly, it may be accounted for in part by the lethal mutation on *sDp2*. This mutation could be a small deficiency such that mutations in several genes may not be recovered. Secondly, some mutations may not be recovered in a 2:1 ratio to wild type. This was proposed by Howell *et al.* (1987) for *sDp2* and shown to be true by Rosenbluth *et al.* (1988) for another region. Some lethal mutations may have a dominant influence on viability which would be enhanced in the

duplication system because the mutant allele is present in two doses. Since *hDf6* can be rescued by a duplication which does not carry a lethal mutation in that region (e.g. *hDp31*), null alleles of all genes in *hDf6* should be rescuable in a 2:1 ratio. Lastly, since recombination is not reduced in the presence of *sDp2* (A. M. Howell, unpublished results; K. McKim, unpublished results), some of the lethals would be lost due to recombination in *sDp2* screens. When the Unc-13s are set up to create the lethal-bearing strain, the probability that the lethal mutation would have crossed off one of the *dpy-5 unc-13* chromosomes is directly proportional to the distance from the lethal mutation to *dpy-5*. Since often more than one Unc-13 is transferred onto one plate, the probability of finding fertile Dpy-5 Unc-13s in the next generation is even higher. This is not a serious problem for lethals in the *hDf6* region because they are tightly linked to *dpy-5*. Approximately 2% of *hDf6* lethals would be lost by recombination (i. e., only one or two lethals in *hDf6* would have been lost). McKim, Howell and Rose (1988) showed that no recombination occurs in *hT1* heterozygotes from the left end of chromosome I to the breakpoint. No lethal mutations would be missed in *hT1* screens due to recombination.

The gamma-induced mutations recovered by *szT1* included the deficiency *hDf6*. This deficiency would not have been recovered by *sDp2* because of the lethal mutation carried by *sDp2*. This was the only deficiency found among 24 analysed lethal mutations on chromosome I recovered from the *szT1* screen (K. McKim, unpublished results). This frequency is much lower than that obtained by Rosenbluth, Cuddeford and Baillie (1985) who found that six of 35 analyzed gamma-induced lethal mutations recovered using *eT1* were deficiencies of chromosome V. It seems to be difficult to isolate deficiencies of chromosome I, but there is no obvious explanation for this difference in rate in this part of the genome. The existence of *sDf4* proves that there are no haplo-insufficient regions within the two map units left of *dpy-5*.

In summary, the induction frequency of lethal mutations may be higher with the translocation screens than with the duplication screen. However, lethal mutations are recovered in two genomic locations in translocation screens, whereas with a free duplication all mutations recovered are in a strictly defined region. Complementation analysis is much simpler with lethals

from the duplication screen. For a large scale project, the duplication system is preferable because the resultant lethal-bearing strains are much easier to work with.

F. Comparison of Mapping Systems

The lethal mutations recovered in the duplication and translocation screens spanned the left third of chromosome I. This region includes at least 17 map units. It was important to develop ways of positioning large numbers of mutations efficiently, especially to divide the large region into smaller regions for complementation analysis. Three different methods were used to map mutations in this study; recombination mapping, deficiency mapping and duplication mapping.

1. Recombination mapping

Recombination mapping using the *sDp2* system is relatively simple. If the Unc-13s from the *sDp2*-rescued lethal strains are mated to wild-type males, all of the outcross wild-type hermaphrodites carry the lethal-bearing chromosome. Many of them also carry *sDp2* and are not of use in recombination mapping. These can be selected against because they develop more slowly, are thinner and paler in appearance, and have a mildly uncoordinated phenotype. The lethal-bearing chromosomes are marked with two visible mutations. This allows for the right-left positioning of lethal mutations with respect to *dpy-5* to be determined. The two marker phenotypes (Dpy-5 and Unc-13) are easily visible. This increases the accuracy of recombination mapping since even rare recombinants are not likely missed. However, Unc-13s are less viable than wild types, so their frequency may sometimes be under-estimated. This would result in an underestimation of the distance from *unc-13* to *dpy-5* or the lethal mutation.

It is not as straightforward a procedure to obtain the correct heterozygous hermaphrodites for mapping experiments with the *szT1* system as with the *sDp2* system. The lethal-bearing strains are wild-type in phenotype and so progeny resulting from crossing can not be visibly distinguished

from self progeny. The outcross progeny will be of two types; *let-x dpy-5 unc-13/ + + +* and *+ + +/szT1*. The types can only be distinguished in the next generation by observing the progeny (the desired heterozygotes segregate no Lon-2 males).

Many mutations were positioned by recombination mapping to one of four regions; right of *dpy-5*, close to the left of *dpy-5*, far to the left of *dpy-5*, or inseparable from *dpy-5*. Recombination mapping is not an efficient method for dealing with large numbers of mutations because brooding the heterozygotes and scoring the progeny are labor intensive tasks. If the lethal mutation is close to *dpy-5*, large numbers of progeny may need to be scored in order to find an informative recombinant. Recombination mapping gives a right-left position with respect to a marker, but the distance from the marker is not precisely determined unless a large number of recombinants are scored.

2. Deficiency mapping

Deficiency mapping has been used very effectively in *C. elegans* to position large numbers of lethal mutations (Sigurdson, Spanier and Herman, 1984; Rosenbluth *et al.*, 1988). A series of overlapping deficiencies for chromosome I was not available. Deficiency mapping is easier than recombination mapping because it is not necessary to score large numbers of progeny to obtain results. The lethal mutation is positioned with respect to the two endpoints of the deficiency. For some deficiencies (e.g. *hDf6*) the heterozygous males will mate successfully and so many lethal stocks can be tested at one time, as opposed to recombination or duplication mapping where males must be obtained from each lethal strain. However, some deficiency strains are quite sick and difficult to work with. This may depend on the genotype of the strain. For example, the BC700 strain (*sDf4 + +/ + bli-4 dpy-14*) is very difficult to maintain, whereas the KR1069 strain (*sDf4/hT1*) grows well. The reduced viability of deficiency heterozygotes must be taken into account when determining the criteria for interpreting deficiency mapping results. Even in the *hT1* strain, the number of *sDf4* heterozygotes is reduced from what would be expected after crossing.

sDf4 is the largest deficiency of the *sDp2* region presently available. If a lethal mutation fails to complement *sDf4*, its position is well established. If it complements *sDf4*, however, it could be either to the left or the right of *sDf4*.

3. Duplication mapping

The duplications of chromosome I isolated by K. McKim (McKim and Rose, 1988) have proven to be very useful for positioning lethal mutations. Since the duplications form a linear array with one fixed end, they give left-right positioning of lethal and visible mutations with respect to each other. One minor problem with duplication mapping is that some of the duplications are extremely unstable in the oocyte germ line. Duplications of chromosome I seem to be easier to recover than deficiencies, and are probably more useful for mapping genes.

Meneely and Nordstrom (1988) discussed a possible problem associated with duplication mapping. They found that X-chromosome duplications increase gene expression of genes not under the duplication. If a mutation is a hypomorph, then increasing its expression may rescue the phenotype. It would then appear to map under the duplication even though it really did not. Their results may be specific for the sex chromosome and due to dosage compensation. If autosomal duplications behave in a similar manner, then special consideration should be given to mapping of leaky mutations. There is no evidence that the chromosome I duplications affect expression of genes they do not duplicate.

In summary, duplication mapping is an efficient way to map large numbers of lethal mutations. The positioning of the lethals can be much more precise than that determined by recombination mapping.

G. Summary of Findings/Contributions

The following is a list of the findings of this thesis.

1. *sDp2* was shown to be an effective balancer for the region of chromosome I which it duplicates.
2. In a collaborative laboratory effort, 31,606 chromosomes were screened for EMS-induced *sDp2*-rescued lethal mutations and 495 mutations were rescued.
3. *sDp2* was found to carry a lethal mutation in the *hDf6* region.
4. *szT1(I;X)* was used to recover gamma-induced lethal mutations. It was shown that *szT1* is an effective balancer for the left half of chromosome I, although it is not good for isolation of lethal mutations.
5. It was discovered that *szT1* heterozygotes have an increased frequency of recombination adjacent to the chromosome I breakpoint. This has not been found for any other translocation in *C. elegans*.
6. *hT1(I;V)* was shown to be useful for screening and balancing chromosome I lethal mutations, but was difficult to use for large scale complementation analysis.
7. *him-1* was shown to be an essential gene.
8. *unc-11* was shown to have a synthetic lethal interaction with *unc-13*.
9. *let-354* was shown to be a large mutational target. Approximately one third of all lethal mutations recovered in the *hDf6* region were alleles of *let-354*.
10. A new chromosome I deficiency, *hDf7*, was identified and characterized.
11. The average forward mutation rate per gene in *C. elegans* using 0.015 M EMS was estimated to be 5×10^{-5} mutations/gene. This value is five-fold lower than that estimated by Brenner (1974) even after a correction for EMS dosage is applied.
12. Nineteen of the estimated minimum of twenty-five essential genes uncovered by the deficiency, *hDf6*, were identified. This led to the prediction of the existence of 4,000 essential genes in the *C. elegans* genome.

13. The genes in *hDf6* were positioned into five regions based on the breakpoints of *hDf6, hDf7, hDp3* and *hDp25*.

14. The stages of lethal arrest were determined for the mutations in *hDf6*. They appear to show some clustering of genes with similar stages of arrest. There were fewer early arresting lethal mutations and more adult sterile mutations in *hDf6* than in other studied genomic regions.

H. Proposals for future experiments

The following is a list of experiments which could be done to further this work.

1. Lethal mutations could be tested with the amber suppressor tRNA mutants *sup-5* (Waterston and Brenner, 1978) or *sup-7* (Waterston, 1981). If suppressible alleles were found they would indicate that the mutation was in a protein coding region, and that the mutant probably represented the null phenotype. These alleles could then be tested with the tissue-specific amber suppressors (Hodgkin, 1985; Kondo, Hodgkin and Waterston, 1988) to assay for tissue-specific expression of the *let-* gene.

2. The tissues in which the essential genes must be expressed could be determined by genetic mosaic analysis using a modification of the method described by Herman (1984).

3. The phenotypes of the arrested Dpy-5 Unc-13s (or the arrested Unc-13s which result from complementation testing with a duplication which does not cover the *let-* gene) could be visually inspected by Nomarski optics to check for gross anatomical differences. Their muscle structure could also be examined by polarized light microscopy or antibody staining techniques.

4. All possible combinations of *let-354* alleles could be tested by *inter se* complementation analysis in order to look for intragenic complementation.

5. A fine structure analysis of *let-354* could be carried out. An approach similar to that described by Bullerjahn and Riddle (1988) for fine structure mapping alleles of an essential gene in the presence of a free duplication of the region could be employed.
6. The saturation analysis of *hDf6* could be continued. More lethal mutations could be recovered either in *hT1* screens, or in screens similar to *sDp2* screens but which use a duplication known to be able to rescue *hDf6*. New lethal mutations in *hDf6* could be used to identify the site of the lethal lesion on *sDp2*.
7. The genes in *hDf6* could be positioned with respect to the breakpoints of more duplications. Right-left positioning of lethals with respect to visible markers in the same zone could be undertaken.
8. A screen for transposon-induced mutations in visible or essential genes in *hDf6* could be carried out. These mutations could be used to clone specific genes and obtain overlapping cosmids clones around them. They could also be used to align the position of a gene with respect to available cosmid clones.
9. A set of overlapping cosmids clones is known to include the *unc-38* gene. If the cloned DNA extends into the *hDf6* region, cosmids to the right of the breakpoint could be used in transformation experiments to assign genes to cloned coding regions.

I. General Discussion

One current approach to studying eukaryotic genes and genome organization is to obtain the complete DNA sequence of a genome. It has been proposed that sequencing the human genome would be a worthwhile project. If this project is undertaken, it will result in the accummulation of incredible amounts of sequence data. The assignment of functions to the DNA sequence will be an extremely challenging task, however, since little genetic information is available for aligning the human physical and genetic maps. Coding regions can be identified

using the DNA sequence, but unless there is some identity with known sequences in the computer data bases, it will be difficult to determine the functions of the predicted proteins.

The complete sequencing of the genome of a model organism with a good system for genetic analysis may be a more reasonable project. Genomic descriptions will be most valuable if they are complete at both the genetic and molecular levels. Individual genes and genomic organization are being studied in many metazoan systems; especially in nematodes, Drosophila, and mice. The short generation time for *C. elegans* makes it an especially favourable organism for genetic analysis, and the small genome size makes it amenable to molecular analysis.

The work presented in this thesis provides another region of the *C. elegans* genome which is well characterized genetically. Twenty-four genes have been mapped to five zones defined by breakpoints of chromosomal rearrangements. The complete alignment of the physical and genetic maps of this region could be accomplished using cosmid clones and genetic transformation techniques. All of the genes in *hDf6* should be contained in a set of 20 cosmid clones with an average insert size of 30 kilobases. The rearrangement breakpoints would divide the cosmids into five small sets which could be used to microinject and rescue appropriate lethal mutations. All of the coding regions could be sequenced and compared to known sequences to look for important functional regions.

The major benefit of this model system is that genetic analysis can be used to analyze the functions of the coding regions. For example, it is possible to determine in which tissue type or developmental stage the gene product is required. The tissue type requirement can be determined genetically using tissue-specific amber suppressor tRNA mutations (Hodgkin, 1985; Kondo, Hodgkin and Waterston, 1988) or genetic mosaic analysis (Herman, 1984). The tissue type requirement can be determined biochemically by *in situ* hybridization of labelled antibodies to proteins or labelled probes for mRNA. Temperature shift experiments using temperature-sensitive mutations can determine the developmental time during which the gene product is required. Northern blots containing stage-specific RNA preparations could also be used to determine at which developmental stage a gene is expressed. Genetic analysis can also be used

to determine how gene products interact physically or in developmental pathways. Analyses of epistatic interactions between different mutations have been used to predict models for several developmental programs in *C. elegans;* including dauer larvae formation (Riddle, Swanson and Albert, 1981), sex determination (Hodgkin, Doniach and Shen, 1986), specification of the vulval cell lineages (Ferguson, Sternberg and Horvitz, 1987), and development of the hermaphrodite-specific neurons (Desai *et al.,* 1988).

C. elegans is presently one of the best metazoan organisms available for analysis of genomic organization. The molecular map of the *C. elegans* genome is almost complete and the genetic map is quite extensive. A fraction of genes are known to be evolutionarily conserved between nematodes and mammals (one example is the *unc-86* gene product which shares identity with the human *oct-1* and *oct-2* transcription factors (Finney, Ruvkun and Horvitz, 1988)), so information gained by studying genes and genome organization in *C. elegans* will provide information which is applicable to other systems (including humans).

BIBLIOGRAPHY

ANDERSON, P. and S. BRENNER, 1984 A selection for myosin heavy chain mutants in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **81**: 4470-4474.

BACHMANN, B. J., 1983 Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**: 180-230.

BAILLIE, D. L., K. A. BECKENBACH, and A. M. ROSE, 1985 Cloning within the *unc-43 unc-31 IV* interval of the *Caenorhabditis elegans* genome. Can. J. Genet. Cytol. **27**: 457-466.

BANERJI, J., L. OLSON and W. SCHAFFNER, 1983 A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell **33**: 729-740.

BELL, G. I., L. J. DeGENNARO, D. H. GELFAND, R. J. BISHOP, P. VALENZUELA and W. J. RUTTER, 1977 Ribosomal RNA genes of *Saccharomyces cerevisiae*: Physical map of the repeating unit and location of the region coding for the 5S, 5.8S, 18S and 25S ribosomal RNAs. J. Biol. Chem. **252**: 8118-8125.

BERGET, S. M., A. J. BERK, T. HARRISON, and P. SHARP, 1977 Spliced segments at the 5′ termini of Adenovirus-2 late mRNA: A role for heterogeneous nuclear RNA in mammalian cells. Cold Spring Harbor Symp. Quant. Biol. **42**: 523-529.

BEYER, W. H. (Editor), 1976 *CRC Handbook of Tables for Probability and Statistics*. CRC Press, Cleveland, Ohio.

BINGHAM, P. M., T. B. CHOU, I. MIMS and Z. ZACHAR, 1988 On/off regulation at the level of splicing. Trends Genet. **4**: 134-138.

BREITBART, R. E., A. ANDREADIS and B. NADAL-GINARD, 1987 Alternative splicing: A ubiquitous mechanism for the generation of multiple protein isoforms from single genes. Annu. Rev. Biochem. **56**: 467-495.

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

BULLERJAHN, A. M. E. and D. L. RIDDLE, 1988 Fine-structure genetics of *ama-1*, an essential gene encoding the amanitin-binding subunit of RNA polymerase II in *Caenorhabditis elegans*. Genetics **120**: 423-434.

CHALFIE, M. and M. AU, 1989 Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. Science **243**: 1027-1033.

CHOVNICK, A., W. GELBART, M. McCARRON, B. OSMOND, P. CANDIDO and D. BAILLIE, 1976 Organization of the rosy locus in *Drosophila melanogaster*. Evidence for a control element adjacent to the xanthine dehydrogenase structural element. Genetics **84**: 233-255.

CLARK, D. V., T. M. ROGALSKI, L. M. DONATI and D. L. BAILLIE, 1988 The *unc-22(IV)* region of *Caenorhabditis elegans*: Genetic analysis of lethal mutations. Genetics **129**: 345-353.

CORTESE, R., D. MELTON, T. TRANQUILLA and J. D. SMITH, 1978 Cloning of nematode tRNA genes and their expression in the frog oocyte. Nucleic Acids Res. **5**: 4593-4611.

COULSON, A., J. SULSTON, S. BRENNER, and J. KARN, 1986 Towards a physical map of the genome of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. **83**: 7821-7825.

COULSON, A., R. WATERSTON, J. KIFF, J. SULSTON, and Y. KOHARA, 1988 Genome linking with yeast artifical chromosomes. Nature **335**: 184-186.

COX, G. N., S. CARR, J. M. KRAMER and D. HIRSH, 1985 Genetic mapping of *Caenorhabditis elegans* collagen genes using DNA polymorphisms as phenotypic markers. Genetics **109**: 513-528.

CROW, E. L. and R. S. GARDNER, 1959 Confidence intervals for the expectation of a poisson variable. Biometrika **46:** 441-453

DESAI, C., G. GARRIGA, S. L. McINTIRE and H. R. HORVITZ, 1988 A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. Nature **336**: 638-646.

EDGLEY, M. L. and D. L. RIDDLE, 1987 Caenorhabditis elegans. In *Genetic Maps 1987*, Vol. 4, Edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

EMMONS, S. W., L. YESNER, K. RUAN, and D. KATZENBERG, 1983 Evidence for a transposon in *C. elegans*. Cell **32**: 55-65.

FERGUSON, E. L. and H. R. HORVITZ, 1985 Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. Genetics **110:** 17-72.

FERGUSON, E. L., P. W. STERNBERG and H. R. HORVITZ, 1985 A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*. Nature **326**: 259-267.

FILES, J. G. and D. HIRSH, 1981 Ribosomal DNA of *Caenorhabditis elegans*. J. Mol. Biol. **149**: 223-240.

FINNEY, M., G. RUVKUN and H. R. HORVITZ, 1988 The *C. elegans* cell lineage and differentiation gene *unc-86* encodes a protein with a homeodomain and extended similarity to transcription factors. Cell **55**: 757-769.

FODOR, A. and P. DEAK, 1985 The isolation and genetic analysis of a *C. elegans* translocation (*szT1*) strain bearing an X-chromosome balancer. J. of Genet. **64:** 143-157.

FRIEDMAN, D. I., M. J. IMPERIALE and S. ADHYA, 1987 RNA 3' end formation in the control of gene expression. Annu. Rev. Genet. **21**: 453-488.

GILLIES, S. D., S. L. MORRISON, V. T. OI and S. TONEGAWA, 1983 A tissue-specific enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. Cell **33**: 717-728.

GREENWALD, I., A. COULSON, J. SULSTON and J. PRIESS, 1987 Correlation of the physical and genetic maps in the *lin-12* region of *Caenorhabditis elegans*. Nucleic Acids Res. **15:** 2295-2307.

GREENWALD, I. and H. R. HORVITZ, 1982 Dominant suppressors of a muscle mutant define an essential gene of *Caenorhabditis elegans*. Genetics **101**: 211-225.

HEDGECOCK, E., J. CULOTTI, D. HALL and B. STERN, 1987 Genetics of cell and axon migration in *Caenorhabditis elegans*. Development **100**: 365-385.

HEINE, U. and T. BLUMENTHAL, 1986 Characterization of regions of the *Caenorhabditis elegans* X chromosome containing vitellogenin genes. J. Mol. Biol. **188**: 301-312.

HENDRIX, R. W., J. W. ROBERTS, F. W. STAHL and R. A. WEISBERG, 1983 *Lambda II.* (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

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

HERMAN, R. K., 1984 Analysis of genetic mosaics of the nematode *Caenorhabditis elegans*. Genetics **108**: 165-180.

HERMAN, R. K., C. K. KARI and P. S. HARTMAN, 1982 Dominant X-chromosome non-disjunction mutants of *Caenorhabditis elegans*. Genetics **102:** 379-400.

HERSHEY, A. D. (Editor), 1971 *The Bacteriophage Lambda.* (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

HILLIKER, A. J., A. CHOVNICK and S. H. CLARK, 1981 The relative mutabilities of vital genes in *D. melanogaster*. Drosophila Inform. Serv. **56**: 64-65.

HILLIKER, A. J., S. H. CLARK, A. CHOVNICK and W. M. GELBART, 1980 Cytogenetic analysis of the chromosomal region immediately adjacent to the *rosy* locus in *Drosophila melanogaster*. Genetics **95**: 95-110.

HOCHMAN, B., 1971 Analysis of chromosome 4 in *Drosophila melanogaster*. II. Ethyl methanesulfonate induced lethals. Genetics **67**: 235-252.

HODGKIN, J. A., 1985 Novel nematode amber suppressors. Genetics **111**: 287-310.

HODGKIN, J. A., T. DONIACH and M. SHEN, 1986 The sex determination pathway in the nematode *Caenorhabditis elegans*: Variations on a theme. Cold Spring Harbor Symp. Quant. Biol. **50**: 585-593.

HODGKIN, J. A., H. R. HORVITZ and S. BRENNER, 1979 Nondisjunction mutants of the nematode *C. elegans*. Genetics **91:** 67-94.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129-133.

HOWELL, A. M., D. COOL, J. HEWITT, B. YDENBERG, M. J. SMITH and B. M. HONDA, 1987 Organization and unusual expression of histone genes in the sea star *Pisaster ochraceus*. Journal of Molecular Evolution **25:** 29-36.

HOWELL, A. M., S. G. GILMOUR, R. A. MANCEBO and A. M. ROSE, 1987. Genetic analysis of a large autosomal region in *Caenorhabditis elegans* by the use of a free duplication. Genet. Res. **49**: 207-213.

JACOB, F. and J. MONOD, 1961 Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3**: 318-356.

JEFFREYS, A. J. and R. A. FLAVELL, 1977 The rabbit beta-globin gene contains a large insert in the coding sequence. Cell **12**: 1097-1108.

JOHNSON, C. D., J. B. RAND, R. K. HERMAN, B. D. STERN and R. L. RUSSELL, 1988 The acetylcholinesterase genes of *C. elegans*: Identification of a third gene (*ace-3*) and mosaic analysis of a synthetic lethal phenotype. Neuron **1**: 165-173.

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.

KARLSSON, S., and NEINHUIS, A. 1985. Developmental regulation of human globin genes. Ann. Rev. Biochem. **54**: 1071-1108.

KEMPHUES, K. J., M. KUSCH and N. WOLF, 1988 Maternal-effect lethal mutations on linkage group II of *Caenorhabditis elegans*. Genetics **120**: 977-986.

KIM, J. S. and A. M. ROSE, 1987 The effect of gamma radiation on recombination frequency in *Caenorhabditis elegans*. Genome **29:** 457-462.

KOHARA, Y., K. AKIYAMA and K. ISONO, 1987 The physical map of the whole *E. coli* chromosome: Application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**: 495-508.

KONDO, K., J. HODGKIN and R. H. WATERSTON, 1988 Differential expression of five $tRNA_{UAG}$(trp) amber suppressors in *Caenorhabditis elegans*. Mol. Cell. Biol. **8**: 3627-3635.

LANDEL, C. P., M. KRAUSE, R. H. WATERSTON and D. HIRSH, 1984 DNA rearrangements of the actin gene cluster accompany reversion of three muscle mutants. J. Mol. Biol. **180**: 497-513.

LASKO, P. F. and M. L. PARDUE, 1988 Studies of the *vestigial* microregion of *Drosophila melanogaster*. Genetics **120**: 495-502.

LEFEVRE, G. and W. WATKINS, 1986 The question of the total gene number in *Drosophila melanogaster*. Genetics **113**: 869-895.

LEICHT, B. G. and J. J. BONNER, 1988 Genetic analysis of chromosomal region 67A-D of *Drosophila melanogaster*. Genetics **119**: 579-593.

LEWIS, J. A., WU, C. H., BERG, H. and LEVINE, J. H. 1980. The genetics of levamisole resistance in the nematode *C. elegans*. Genetics, **95**: 905-928.

L'HERNAULT, S. W., D. C. SHAKES and S. WARD, 1988 Developmental genetics of chromosome I spermatogenesis-defective mutants in the nematode *Caenorhabditis elegans*. Genetics **120**: 435-452.

MacLEOD, A. R., J. KARN and S. BRENNER, 1981 Molecular analysis of the *unc-54* myosin heavy-chain of *Caenorhabditis elegans*. Nature **291**: 386-390.

MARCHANT, G. E. and D. G. HOLM, 1988 Genetic analysis of the heterochromatin of chromosome 3 in *Drosophila melanogaster*. II. Vital loci identified through EMS mutagenesis. Genetics **120**: 519-532.

MATHEWS, C. K., E. M. KUTTER, G. MOSIG and P. B. BERGET (Editors), 1983 *Bacteriophage T4*. (American Society of Microbiology, Washington, D.C.).

MAXSON, R., R. COHN and L. KEDES, 1983 Expression and organization of histone genes. Ann. Rev. Genetics **17**: 239-277.

McCARRON, M., 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.

McKIM, K. S., A. M. HOWELL and A. M. ROSE, 1988 The effects of translocations on recombination frequency in *Caenorhabditis elegans*. Genetics **120**: 987-1001.

McKIM, K. S. and A. M. ROSE, 1988 Construction of an array of duplications to facilitate rapid mapping of LG I genes in *Caenorhabditis elegans*. Genome **30** (Suppl. 1): 298.

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.

MENEELY, P. M. and R. K. HERMAN, 1981 Suppression and function of X-linked lethal and sterile mutations in *Caenorhabditis elegans*. Genetics **97**: 65-84.

MENEELY, P. M. and K. D. NORDSTROM, 1988 X chromosome duplications affect a region of the chromosome they do not duplicate in *Caenorhabditis elegans*. Genetics **119**: 365-375.

MILLER, J. H. and W. S. REZNIKOFF (Editors), 1978 *The Operon*. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

MOERMAN, D. G. and D. L. BAILLIE, 1979 Genetic organization in *Caenorhabditis elegans*: Fine-structure analysis of the *unc-22* gene. Genetics **91**: 95-103.

MOERMAN, D. G., G. M. BENIAN and R. H. WATERSTON, 1986 Molecular cloning of the muscle gene *unc-22* in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. **83**: 2579-2583.

MULLER, H. J., 1929 The gene as the basis of life. Proc. Fourth Int. Congr. Plant Sci. [Ithaca, 1926] **1**: 897-921.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations. pp 213-255. In: *Proceedings of the Sixth International Congress of Genetics*, Edited by D. JONES. Brooklyn Botanic Gardens, Menasha, Wisconsin.

NASH, D. and F. C. JANCA, 1983 Hypomorphic lethal mutations and their implications for the interpretation of lethal complementation studies in Drosophila. Genetics **105**: 957-968.

NELSON, D. W. and B. M. HONDA, 1985 Genes coding for 5S ribosomal RNA of the nematode *Caenorhabditis elegans*. Gene **38**: 245-251.

PARK, E.-C. and H. R. HORVITZ, 1986 *C. elegans unc-105* mutations affect muscle and are suppressed by other mutations that affect muscle. Genetics **113**: 853-867.

PERRIMON, N., D. MOHLER. L. ENGSTROM and A. P. MAHOWALD, 1986 X-linked female-sterile loci in *Drosophila melanogaster*. Genetics **113**: 695-712.

PETERS, K. and A. M. ROSE, 1988 Complementing alleles of the *bli-4* locus of *Caenorhabditis elegans*. Genome **30**: s294.

PRASAD, S., 1988 Molecular analysis within the *dpy-20* to *unc-22* region of chromosome IV in *Caenorhabditis elegans*. Ph.D thesis, Simon Fraser University, Burnaby, B.C.

RIDDLE, D. L., M. M. SWANSON and P. ALBERT, 1981 Interacting genes in dauer larva formation. Nature **290**: 668-671.

RITOSSA, F. M., K. C. ATWOOD and S. SPIEGELMAN, 1966 A molecular explanation of the bobbed mutants of Drosophila as partial deficiencies of "ribosomal" DNA. Genetics **54**: 819-834.

ROGALSKI, T. M., 1983 A genetic characterization of region around the *unc-22* gene in *Caenorhabditis elegans*. PRASAD, S., 1988 Molecular analysis within the *dpy-20* to *unc-22* region of chromosome IV in *Caenorhabditis elegans*. Ph.D thesis, Simon Fraser University, Burnaby, B.C.

ROGALSKI, T. M., D. G. MOERMAN and D. L. BAILLIE, 1982 Essential genes and deficiencies in the *unc-22* region of *Caenorhabditis elegans*. Genetics **102**: 725-736.

ROGALSKI, T. R. and D. L. BAILLIE, 1985 Genetic organization of the *unc-22* IV region of *Caenorhabditis elegans*. Mol. Gen. Gen. **201**: 409-414.

ROGALSKI, T. M., A. M. E. BULLERJAHN and D. L. RIDDLE, 1988 Lethal and amanitin-resistance mutations in the *Caenorhabditis elegans ama-1* and *ama-2* genes. Genetics **120**: 409-422.

ROGALSKI, T. M. and D. L. RIDDLE, 1988 A *Caenorhabditis elegans* RNA polymerase II gene, *ama-1 IV*, and nearby essential genes. Genetics **118:** 61-74.

ROSE, A. M., 1980 Genetic studies on the gene coding for paramyosin in *Caenorhabditis elegans*: *unc-15* and the adjacent region. Ph.D. thesis, Simon Fraser University, Burnaby, B.C., Canada.

ROSE, A. M. and D. L. BAILLIE, 1979 Effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics **92:** 409-418.

ROSE, A. M. and D. L. BAILLIE, 1980 Genetic organization of the region around unc-15 (I), a gene affecting paramyosin in *Caenorhabditis elegans*. Genetics **96**: 639-648.

ROSE, A. M., D. L. BAILLIE, E. P. M. CANDIDO, K. A. BECKENBACH and D. NELSON, 1982 The linkage mapping of cloned restriction fragment length differences in Caenorhabditis elegans. Mol. Gen. Genet. 188: 286-291.

ROSE, A. M., D. L. BAILLIE and J. CURRAN, 1984 Meiotic pairing behavior of two free duplications of linkage group I in *Caenorhabditis elegans*. Mol. Gen. Genet. **195:** 52-56.

ROSENBLUTH, R. E. and D. L. BAILLIE, 1981 Analysis of a reciprocal translocation, *eT1(III;V)*, in *Caenorhabditis elegans*. Genetics **99:** 415-428.

ROSENBLUTH, R. E., C. CUDDEFORD and D. L. BAILLIE, 1983 Mutagenesis in *Caenorhabditis elegans*. I. A rapid eukaryotic mutagen test system using the reciprocal translocation, *eT1(III;V)*. Mutation Research **110**: 39-48.

ROSENBLUTH, R. E., C. CUDDEFORD and D. L. BAILLIE, 1985 Mutagenesis in *Caenorhabditis elegans*. II. A spectrum of mutational events induced with 1500R of gamma-radiation. Genetics **109**: 493-511.

ROSENBLUTH, R. E., T. M. ROGALSKI, R. C. JOHNSEN, L. M. ADDISON and D. L. BAILLIE, 1988 Genomic organization in *Caenorhabditis elegans*: deficiency mapping on linkage group V(left). Genet. Res. **52**: 105-118.

SCHAFFNER, W., E. SERFLING and M. JASIN, 1985 Enhancers and eukaryotic gene expression. Trends Genet. 1: 224-230.

SCHIBLER, U. and F. SIERRA, 1987 Alternative promoters in developmental gene expression. Annu. Rev. Genet. 21: 237-258.

SHANNON, M. P., T. C. KAUFMAN, M. W. SHEN and B. H. JUDD, 1972 Lethality patterns and morphology of selected lethal and semi-lethal mutations in the zeste-white region of *Drosophila melanogaster*. Genetics **72**: 615-638.

SIGURDSON, D. C., G. J. SPANIER and R. K. HERMAN, 1984 *C. elegans* deficiency mapping. Genetics **108**: 331-345.

SMITH, C. L., J. C. ECONOME, A. SCHUTT, S. KLCO and C. R. CANTOR, 1987 A physical map of the Escherichia coli K12 genome. Science 236: 1448-1453.

SNYDER, M., and N. DAVIDSON, 1983 Two gene families clustered in a small region of the Drosophila genome. J. Mol. Biol. **166**: 101-118.

STARR, T., A. M. HOWELL, J. McDOWALL, K. PETERS and A. M. ROSE, 1989 Isolation and mapping of DNA probes within the linkage group I gene cluster of *Caenorhabditis elegans*. Genome, in press.

SULSTON, J. E. and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans*. Genetics **77**: 95-104.

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

SULSTON, J. E. and H. R. HORVITZ, 1981 Abnormal cell lineages in mutants of the nematode *Caenorhabditis elegans*. Dev. Biol. **82**: 41-55.

SULSTON, J. E., E. SCHEIRENBERG, J. G. WHITE and J. N. THOMSON, 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. **100**: 64-119.

von MENDE, N., D. McK. BIRD, P. S. ALBERT and D. L. RIDDLE, 1988 *dpy-13*: A nematode collagen gene that affects body shape. Cell **55**: 567-576.

WAHLI, J. 1988. Evolution and expression of vitellogenin genes. Trends Genet. **4**: 227-232.

WARD, S., D. J. BURKE, J. E. SULSTON, A. R. COULSON, D. G. ALBERTSON, D. AMMONS, M. KLASS and E. HOGAN, 1988 The genomic organization of transcribed major sperm protein genes and pseudogenes in the nematode *Caenorhabditis elegans*. J. Mol. Biol. **199**: 1-13.

WATERSTON, R. H., 1981 A second informational suppressor, *sup-7 (X)*, in *Caenorhabditis elegans*. Genetics **97**: 307-325.

WATERSTON, R. H. and S. BRENNER, 1978 A suppressor mutation in the nematode *Caenorhabditis elegans* acting on specific alleles of many genes. Nature **275**: 715-719.

WATERSTON, R. K., R. M. FISHPOOL and S. BRENNER, 1977 Mutants affecting paramyosin in *Caenorhabditis elegans*. J. Mol. Biol. **117**: 679-698.

WATERSTON, R. H., D. HIRSH and T. R. LANE, 1984 Dominant mutations affecting muscle structure in *Caenorhabditis elegans*. J. Mol. Biol. **180**: 473-496.

WATERSTON, R. H., J. N. THOMSON and S. BRENNER, 1980 Mutants with altered muscle structure in *Caenorhabditis elegans*. Dev. Biol. **77**: 271-302.

WRIGHT, T. R. F., 1987 The genetic and molecular organization of the dense cluster of functionally related, vital genes in the DOPA decarboxylase region of the *Drosophila melanogaster* genome. pp. 95-120. In: Structure and Function of Eukaryotic Chromosomes. Edited by W. Hennig. Springer-Verlag, Berlin.

APPENDIX I

Below are complementation tables summarizing the data used to assign gene names to the new essential genes described in this thesis. In each table, a "+" indicates complementation and a "-" indicates failure to complement. Heterozygous lethal-bearing males are listed across the top of the tables, and *sDp2*-rescued lethal-bearing hermaphrodites are indicated down the side. The tables are ordered with the regions of the chromosome going from the left end to *dpy-5*.

1) Region left of *sDf4*

		lin-6 *h92*	*lin-6* *h99*	*let-357* *h89*	*let-357* *h132*	*let-360* *h96*	*let-362* *h86*	*let-362* *h93*	*let-365* *h108*	*let-365* *h129*
lin-6	*h92*		-	+	+	+	+		+	+
lin-6	*h99*	-								
let-357	*h89*		+		-	+	+		+	+
let-357	*h132*									+
let-360	*h96*		+	+	+		+		+	+
let-362	*h86*	+	+		+	+		-	+	+
let-362	*h93*	+					-			
let-365	*h108*			+	+		+			-
let-365	*h129*		+		+					

2) Region in *sDf4* to the left of *hDf6*

		let-368 *h121*	*let-369* *h125*	*let-372* *h126*
let-368	*h121*		+	+
let-369	*h125*	+		+
let-372	*h126*	+		

3a) Region between the left breakpoints of *hDf6* and *hDf7*

		let-356 *h501*	*let-356* *h679*	*let-356* *h871*	*let-366* *h112*	*let-366* *h265*	*let-366* *h411*	*let-366* *h505*	*let-366* *h852*	*let-366* *h890*
let-356	*h83*		-		+	+	+			+
let-356	*h679*	-		-				+	+	
let-356	*h871*				+					
let-366	*h112*	+	+			-	-	-		-
let-366	*h265*				-					
let-366	*h422*				-					
let-366	*h852*			+	-					
let-373	*h234*		+	+		+	+	+	+	+
let-501	*h498*							+		
let-501	*h714*		+	+		+		+	+	+
let-509	*h521*			+	+				+	+
let-509	*h522*								+	
let-509	*h867*								+	

3b) Region between the left breakpoints of *hDf6* and *hDf7*

		let-373 *h234*	*let-373* *h573*	*let-501* *h498*	*let-501* *h714*	*let-509* *h521*	*let-509* *h522*	*let-509* *h867*	*let-510* *h740*	*let-511* *h755*
let-356	*h83*				+					
let-356	*h679*		+	+		+		+	+	+
let-366	*h112*	+	+	+	+			+	+	+
let-366	*h422*	+								
let-366	*h505*		+							
let-373	*h234*		-	+	+	+		+	+	+
let-501	*h498*		+							
let-501	*h714*		+	-				+	+	+
let-509	*h521*		+	+			-	-	+	+
let-509	*h522*		+	+				-	+	+
let-509	*h867*		+	+			-			
let-511	*h755*								+	

4) *hDf7* region

		let-353 *h46*	*let-503* *h313*	*let-503* *h418*	*let-504* *h844*	*let-504* *h888*	*let-505* *h426*	*let-506* *h300*	*let-507* *h439*
let-353	*h46*		+		+	+	+	+	+
let-503	*h418*	+	-		+	+	+	+	+
let-504	*h448*	+	+	+	-	-	+	+	+
let-505	*h426*					+		+	
let-507	*h439*					+	+	+	

5a) Region in *hDf6* and *hDp3*

		let-351 *h43*	*let-354* *h79*	*let-354* *h90*	*let-354* *h201*	*let-354* *h267*	*let-354* *h370*	*let-354* *h482*	*let-354* *h508*	*let-354* *h693*
let-351	*h43*			+	+	+	+		+	+
let-354	*h79*			-	-	-	-	-	-	-
let-354	*h390*	+	-			-				
let-354	*h441*		-			-				
let-354	*h504*		-							
let-354	*h803*		-							
let-354	*h809*		-							
let-354	*h841*		-							
let-354	*h863*		-							
let-374	*h251*				+	+	+		+	+
let-375	*h259*				+	+	+		+	+
let-375	*h391*	+	+			+				
let-502	*h392*	+	+			+			+	
let-502	*h732*		+							
let-502	*h783*		+							
let-508	*h452*		+			+			+	

5b) Region in *hDf6* and *hDp3*

		let-354 *h819*	*let-354* *h866*	*let-374* *h251*	*let-375* *h259*	*let-502* *h392*	*let-502* *h509*	*let-502* *h732*	*let-508* *h452*	*let-508* *h995*
let-351	*h43*	+		+	+		+	+	+	
let-354	*h79*	-	-	+	+					+
let-354	*h390*			+	+					
let-354	*h803*							+		
let-354	*h809*							+		
let-354	*h841*							+		
let-354	*h863*							+		
let-374	*h251*	+					+	+		+
let-375	*h259*			+			+	+		+
let-375	*h391*	+		+	-					
let-502	*h392*			+	+			-		
let-502	*h783*								-	
let-502	*h835*		+			-	-		+	+
let-508	*h452*	+		+	+	+	+	+		-

6a) Region between *dpy-5* and the right breakpoint of *hDf6*

		him-1 *h134*	*let-352* *h45*	*let-355* *h81*	*let-359* *h94*	*let-361* *h97*	*let-361* *h113*
him-1	*h134*		+				
let-352	*h45*						+
let-355	*h81*	+	+		+		+
let-359	*h94*	+	+	+			+
let-361	*h97*	+					-
let-361	*h113*				-		+
let-363	*h98*			+	+		
let-363	*h111*	+		+	+		
let-363	*h114*	+					
let-363	*h131*	+		+	+	+	
let-364	*h104*	+					
let-367	*h119*		+			+	
let-370	*h128*		+				
let-371	*h123*	+					+

6b) Region between *dpy-5* and the right breakpoint of *hDf6*

		let-363 h98	*let-363 h111*	*let-363 h114*	*let-363 h131*	*let-364 h104*	*let-367 h119*	*let-370 h128*	*let-371 h123*
him-1	*h134*						+	+	
let-352	*h45*	+				+	+	+	+
let-355	*h81*	+				+	+	+	+
let-359	*h94*			+		+	+	+	+
let-361	*h97*		+			+			+
let-361	*h113*	+					+	+	
let-363	*h98*			-	-			+	
let-363	*h111*	-		-	-	+			+
let-363	*h114*		-		-				
let-363	*h131*		-	-		+			+
let-364	*h104*	+	+	+			+	+	+
let-367	*h119*	+	+					+	
let-371	*h123*			+			+	+	

APPENDIX II

Construction of Strains for DNA Polymorphism Mapping

The availability of both a genetic and physical map of the chromosome I gene cluster will allow the detailed study of genes and genomic organization in this large autosomal segment of the *C. elegans* genome. Since the physical map of the genome is nearly completed (Coulson *et al.* 1986; Coulson *et al.* 1988), the availability of genetically positioned cloned DNA probes from this region would allow for the alignment of the physical and genetic maps. One method for the isolation of DNA probes within a region of interest is Tc1-linkage selection (Baillie *et al.* 1985). Tc1-linkage selection takes advantage of the fact that the BO strain contains ten times the number of Tc1 elements found in N2 worms (Emmons *et al.* 1983). Thus BO derived DNA can be distinguished from N2 DNA by determining which fragments contain new Tc1 elements. I have constructed an interstrain hybrid for Tc1-linkage selection. Two linked mutations (*dpy-5(h14)*, *unc-29(h2)*) were induced in the Bergerac (BO) strain by L. Harris and A. M. Rose. By replacing the *dpy-5 unc-29* segment of the Bristol (N2) genome with this marked BO DNA, new Tc1 elements derived from the BO strain were crossed into an N2 background. The interval between these loci covers most of the chromosome I gene cluster. A hybrid, KR408, which was essentially free of BO DNA except for the BO-derived *dpy-5 unc-29* interval was constructed. Tc1-hybridizing fragments in the DNA of this strain have provided DNA markers for the *dpy-5 unc-29* interval (Starr *et al.*, 1989).

Construction of the hybrid strain, KR408

The BO *dpy-5 unc-29* double mutant strain was crossed to wild-type N2 males. F1 hermaphrodites were picked and allowed to self fertilize. One of their Dpy-5 Unc-29 offspring was isolated to generate the first backcross strain. This procedure was repeated until the sixth backcross strain was generated. This strain, KR408, contained BO DNA between the *dpy-5 (h14)* and *unc-29 (h2)* markers and approximately 97% N2 DNA for the rest of the genome.

Construction of chromosome I mapping strains

Two strains which could be used to map polymorphic probes to chromosome I were constructed using methods modified from Rose *et al.,* (1982). Outcross progeny of the genotype *dpy-5 unc-29 (BO) / + + (N2); unc-22 (N2) / + (N2)* were allowed to self fertilize. Dpy-5 Unc-29 and Unc-22 progeny were isolated. DNA was isolated from the progeny of 50 Dpy-5 Unc-29 hermaphrodites (by T. Starr). This DNA preparation contained BO DNA in the *dpy-5 unc-29 (I)* region and N2 for the remainder of the genome. DNA was also isolated from the progeny of 50 Unc-22 hermaphrodites (by T. Starr). This DNA preparation contained N2 DNA from chromosome IV and a mixture of N2 and BO DNA for chromosome I. These DNA preparations were could then be used to verify that probes detecting strain polymorphisms isolated from KR408 mapped to chromosome I.

Construction of three-factor mapping strains

Construction of recombinant chromosome I's containing both N2 and BO DNA allowed for three-factor analysis of polymorphisms isolated from KR408. Hermaphrodites of the genotype *dpy-5 unc-29 (BO) / + + (N2)* were allowed to self. Recombinant Dpy-5 and Unc-29 individuals were isolated (*dpy-5 unc-29 (BO) / dpy-5 (BO) + (N2)* and *dpy-5 unc-29 (BO) / + (N2) unc-29 (BO)*). From their progeny, individuals which were homozygous for the recombinant chromosome were isolated to generate recombinant strains. Thirteen Dpy-5 strains and nine Unc-29 strains were thus made available for mapping polymorphisms with respect to these two markers.