ANALYSIS OF LINKAGE GROUP V (LEFT) IN *CAENORHABDITIS ELEGANS*: CORRELATION OF THE GENETIC AND PHYSICAL MAPS AND STUDY OF *UNC-60* AND *LIN-40*

by

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Analysis of Linkage Group V (left) in *Caenorhabditis elegans*: Correlation of the Genetic and Physical Maps and Study of *unc-60* and *lin-40*

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ABSTRACT

Correlation of the genetic and physical maps in the Linkage Group V (left) (LGV) region of *Caenorhabditis elegans* has been done. This was accomplished by the following three strategies: (1) Polymerase chain reactions (PCR) of deficiency homozygous nematodes using primers derived from cloned genomic DNA; (2) microinjection of cloned genomic DNA into the germline to rescue Unc-60; (3) Creation of duplications containing cloned genomic DNA from LGV(left) by germline transformation. The results have shown that the gene cluster region of LGV(left) has more DNA per map unit than the gene sparse regions. Nine cosmid-derived primers were placed on the genetic map and helped to anchor the molecular map in the region. The *unc-60* gene was rescued by germline transformation using the cosmids F53E2 and C32E5. PCR was also used to confirm the presence of cosmid clones in transformed strains.

The genes *unc-60* and *lin-40* were examined in more detail in this study. A new *unc-60* allele was isolated, *s1983*. Another putative *unc-60* allele, *s1986* was isolated and is likely a small deficiency. Alleles of *lin-40* were studied for their effect on the number of self-fertilization progeny produced by XX hermaphrodites. The results have shown that some of the *lin-40* alleles have a dominant effect on the mean number of progeny produced.

DEDICATION

For Noshir Kapadia, my chemistry teacher; the first man to show me the beauty of science and also to my brothers Warren and David, who continued to nurture my interest in science.

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There are many individuals who I would like to thank for helping me during my years at Simon Fraser University. Dr. David L. Baillie my senior supervisor was a continual source of genetic wisdom throughout my work. Raja Rosenbluth generously taught me worm genetics and helped with the writing of this thesis. Dr. Robert C. Johnsen performed the interallelic complementation, determination of the terminal phenotypes, T-tests and also assisted with the egg counts of the *lin-40* genes. Moreover, he patiently assisted me with my worm work and gave me many helpful discussions on my work and this thesis. All PCR experiments would not have been possible without Andrea Scouras, who performed the molecular parts of the experiments. Dr. Kim McKim and Cammy Matheson provided me with DNA for the *unc-60* injections. My committee members, Dr. Barry M. Honda, Dr. Ann Rose and Dr. Mike Smith were very supportive and helpful, especially during the writing of this thesis. Many thanks to the members of the Baillie lab, past and present: Denise Clark, Shiv Prasad, Mark Heschl, Yousef Amaar, Jacquie Schein, Brad Barbazuk, Dinar Suleman, Ann Marie Howell, Helen Stewart and Margaret Rogalski. A special thank you to Marco Marra for comments on parts of this thesis. Special thanks to Karen Beckenbach for the lessons in molecular biology. Other people who were helpful to me were John Boom, Gary Saunders, Ian Tan, Charlene Mayes and members of the Rose lab. Thanks to Dr. Paul Sternberg and his lab for teaching me the new injection methods. Most of all I would like to say how grateful I am to God and my support network consisting of my friends, my family and most especially Steven, who has stood by me and endured my struggle to complete this work.

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GENERAL INTRODUCTION

This thesis describes the correlation of the genetic map distance and physical map distance in a defined region of linkage group V left [LGV (left)] in the nematode *Caenorhabditis elegans*. Three approaches were used for this study. The polymerase chain reaction was applied to deficiency homozygous nematodes in order to map cloned genomic DNA sequences relative to the deficiency map of LGV. Microinjections were used to create transgenic nematode strains containing duplications of LGV(left) chromosomal regions. In addition, microinjection of genomic DNA fragments from the *C. elegans* physical map was used to identify a genetically characterized gene, *unc-60*.

This thesis also describes two genes from the LGV(left) region, *unc-60* and *lin-40*. These genes were examined in more detail because of their interesting genetic characteristics.

C. elegans was a good model organism for this study. The nematode has 5 autosomes and a sex chromosome (X) and can exist as a self-fertilizing hermaphrodite or as a male. Hermaphrodites have two X chromosomes and males have a single X. The *C. elegans* physical map of overlapping cosmid (Collins and Hohn 1978) and YAC (yeast artificial chromosome) (Burke *et al.* 1987) genomic clones is nearly complete (Coulson *et al.* 1986; 1991; Coulson and Waterston 1988) and the sequencing of the entire genome is being done (Sulston *et al.* 1992; Waterston *et al.* 1992). The map is 90% complete and is assembled into contiguous regions (contigs). There are fewer than 40 gaps left to close in the physical map (Sulston *et al.* 1992). Although the sequence of the genome is very informative, we cannot elucidate precise functions of genes from the sequence information. The identification and characterization of genes through genetic means and reintroduction of the genes into the genome is essential to our complete understanding of the nematode. Our laboratory has been studying the genomic organization of LGV(left) (Johnsen and Baillie 1988; 1991; Rosenbluth *et al.*

1988; Clark *et al.* 1988; 1990; Stewart *et al.* 1991) and LGIV (*unc-22* region) (Rogalski *et al.* 1982; Rogalski and Baillie 1985).

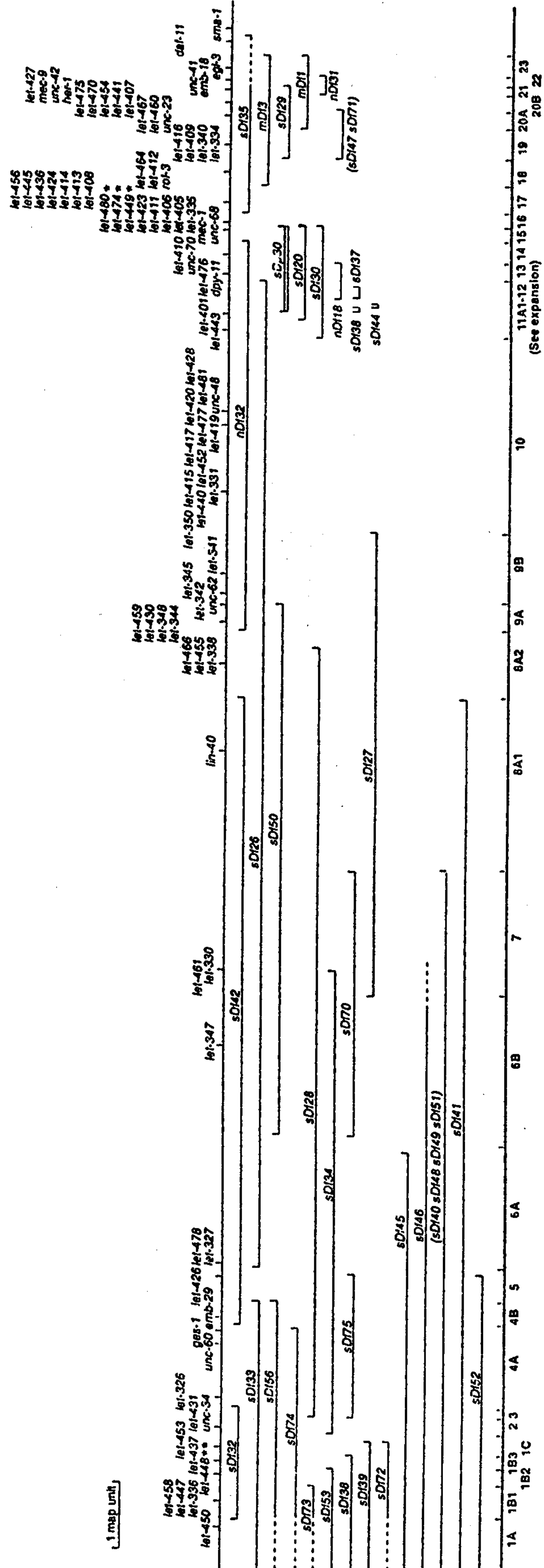
LGV(left) is recombinationally balanced by the reciprocal translocation *eT1(III;V)* (Rosenbluth and Baillie 1981). The region corresponds to approximately 7% of the total genetic map of *C. elegans* and comprises approximately 23 map units (m.u.). Figure 1 shows the LGV(left) genetic map (Johnsen and Baillie 1991). The LGV(left) region has been studied by saturation mutagenesis for essential genes such that 101 recessive lethal mutations have been mapped (Johnsen and Baillie 1988; 1991; Rosenbluth *et al.* 1988; Clark *et al.* 1990; Edgley and Riddle 1990; Stewart *et al.* 1991). There has also been 17 putative nonessential genes mapped in LGV(left). The region has been subdivided into zones by sets of deficiencies (Johnsen and Baillie 1988; 1991 Rosenbluth *et al.* 1988; Clark *et al.* 1988; 1990; Stewart *et al.* 1991).

Section I presents the data for the correlation of the genetic and physical maps on LGV(left) using PCR and germline transformations. I also describe the generation of stable small genomic duplications of LGV(left) by germline transformation.

Section II describes the isolation and characterization of a new *unc-60* allele and a putative *unc-60* deletion. I also show how the Unc-60 rescue by germline transformation helped to characterize the gene. In addition, I examine the effect of *lin-40* alleles on the number of self progeny produced by hermaphrodites.

Figure 1 Genetic map of LGV(left)

This map is from Johnsen and Baillie (1991). The map shows the genes and deficiencies characterized prior to this thesis. The essential genes are designated as *let* and non-essential genes are named according to Horvitz *et al.* (1979). Deficiencies are named from the laboratory in which they were generated. *sDfs* were isolated in D. L. Baillie's laboratory, *mDfs* were in D. Riddle's laboratory and *nDfs* in Horvitz's laboratory. Below the map, the zones defined from deficiency breakpoints are shown.



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GENERAL MATERIALS AND METHODS

The genetic nomenclature in this thesis follows that of Horvitz *et al.* (1979). Nematodes were cultured in Petri plates on nematode growth medium streaked with *Escherichia coli* strain OP50 bacteria (Brenner 1974).

Mutations used in this study

The wild-type strain N2 (var. Bristol) and strains carrying the following mutations were used in this study. '*s*' mutations arose in D. L. Baillie's laboratory. Unless noted otherwise, strains carrying other mutations were obtained from the stock collection at the Medical Research Council, Cambridge, England or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia. LGIII: *dpy-18(e364), let-500(s2165);* LGV: *dpy-11(e244), emb-29(s166), unc-46(e177), unc-60(m35, r398, s1983, s1986, s1331), let-326(s1404), let-327(s1496), let-347(s1035), let-426(s826, s1527), lin-40(s1053, s1345, s1351, s1352, s1358, s1360, s1373, s1506, s1593, s1611, s1634, s1669, s1675, s1704, s1916).* *lin-40(e2173)* was isolated by S.W. Emmons and was a gift from J. Hodgkin (MRC, Cambridge, England). Deficiencies from LGV are as follows: *sDf20, sDf26, sDf27, sDf28, sDf30, sDf32, sDf33, sDf34, sDf35, sDf39, sDf42, sDf45, sDf50, sDf51, sDf70* and *sDf75*. *mDf3* and *mDf1* were from D. L. Riddle's laboratory (Columbia, MO) (Brown 1984). *nDf18* and *nDf32* were from R. H. Horvitz's laboratory (M.I.T.) (Park and Horvitz 1986).

Characteristics of *eT1(III;V)*

The reciprocal translocation *eT1(III;V)* is comprised of *eT1(III)* segregating from LGIII and *eT1(V)* segregating from LGV (Rosenbluth and Baillie 1981). The translocation acts as a balancer for the right half of LGIII and the left half of LGV. Genes from the left halves of LGIII and V (including *unc-60*) are carried on *eT1(III)* whereas genes from the right halves are carried on *eT1(V)*. Aneuploid progeny from

heterozygotes do not survive, but *eT1(III;V)* homozygotes do and have a Unc-36 phenotype. Among the progeny of *eT1* heterozygotes, markers on LGIII(right) appear pseudolinked to markers on LGV(left). Thus, for example, the only viable progeny from *dpy-18/eT1(III);unc-60/eT1(V)* hermaphrodites are phenotypically wild-types, Unc-36s and Dpy Unc-60s, but no Non-Dpy Unc-60s or Non-Unc Dpys.

Origin of cosmid clones

All cosmid clones used in this study were obtained from A. R. Coulson and J. E. Sulston at the MRC in Cambridge, England. They are located in the LGV left region: C15C5, C32E5, F12B4, F16B4, F46A7, F53E2, K06H5, T01C2, T03F8, T10D1, T28A3, W06E4 and W06G11. The cosmid clones C15C5 and C32E5 carry the ampicillin resistance gene whereas all other cosmids carry the kanamycin resistance gene, neomycin phosphotransferase.

SECTION I: CORRELATING THE GENETIC AND PHYSICAL MAPS

Introduction

There are three distinct mapping strategies for genes. Genetic linkage maps are based upon the coinheritance of allele combinations. Genetic map distances are defined by percent recombination between alleles. Cytogenetically based maps are generated by *in situ* hybridization and order loci with respect to relative chromosomal positions or visible banding patterns. Molecularly based maps are measured in kilobases (Kb) and can consist of overlapping clones, restriction endonuclease sites or sequence tagged sites. Both cytogenetic and molecular maps are considered physical maps. The conversion and comparison between linkage maps and physical maps is not a simple task.

The human genome mapping project currently is limited in its comparison of the genetic to physical maps. Only individual chromosomes or chromosomal regions can be converted from one form to the other (Stephens *et al.* 1990). In the fruit fly *Drosophila melanogaster,* the polytene banding map is co-linear with the genetic map (first demonstrated by Bridges (1935; 1938) and Painter (1933; 1934a; 1934b) using chromosomal rearrangements). The genome mapping and cloning project in *D. melanogaster* has progressed much faster than the human mapping project because of the smaller genome size and the ease of genetic analysis in *D. melanogaster*. The physical map of overlapping cosmids, YACs and pacmids (bacteriophage P1 based vector) (Sternberg 1990) in *D. melanogaster* is nearing completion (Merriam *et al.* 1991). The resolution of *in situ* hybridization of nucleic acid probes to the polytene chromosomes is to a polytene band of 20 Kb (Rykowski, *et al.* 1988). Thus the comparison of the physical and genetic maps in *D. melanogaster* is well underway. Moreover, vertebrate genes are now being used to screen for homologous genes in *D. melanogaster*. The homologous sequences are correlated to an existing mutation or new mutations are selected to demonstrate its function (i.e. kinesin like genes from

vertebrates are being correlated to *D. melanogaster* cell division mutants (Endow and Hatsumi 1991; McDonald and Goldstein 1990).

Many organisms show areas of gene clustering on the genetic map. Gene clusters could be a result of a lower than average frequency of recombination per length of DNA or of a nonrandom distribution of genes along the DNA. Regions around the centromere have reduced recombination compared to the average and thus do not show a direct relationship between genetic and physical distance (Weaver and Hedrick 1992). In addition, there can be sex-specific recombination differences [e.g. humans (Wu *et al.* 1990)].

On the *C. elegans* genetic map, there is an uneven distribution of genes along the autosomes and sex chromosome (Brenner 1974). What is the cause of this? Many studies have addressed this question in the nematode. Greenwald *et al.* (1987) used polymorphisms on LGVIII in proximity to *lin-12* to correlate the genetic and physical maps. They found that the amount of DNA per m.u. is approximately three fold greater around the cluster containing *lin-12.* Starr *et al.* (1989) isolated probes for DNA polymorphisms from the LGI gene cluster. They mapped the sites on the genetic and physical maps and found that there is more DNA per m.u. in the center of the gene cluster. Clark and Baillie (1992) demonstrated that the 0.2 m.u. *dpy-20-unc-22* region within the cluster region on LGIV contains approximately 200 Kb and thus is over three times the average in *C. elegans* of 333 Kb/m.u.(Edgley and Riddle 1990; J. E. Sulston, personal communication). The *dpy-20-unc-22* interval was also studied through a molecular analysis by Prasad and Baillie (1989). They found nine potential coding regions within 150 Kb in the *dpy-20-unc-22* region. Hodgkin (1987) demonstrated that the *tra-1* locus has a recombinational size of at least 0.15 m.u., about 4 times larger than the estimated lengths of the large muscle genes *unc-22* and *unc-54* (Moerman and Baillie 1979; Waterston *et al.* 1982).

Interestingly, genetic clustering is paralleled by physical clustering of select cDNA clones (from a sorted cDNA library) on the cosmid and YAC map (Waterston *et al.* 1992). However, the clustering of the cDNA clones is not nearly as extensive as the clustering of genes on the genetic map (due to recombination suppression).

On LGV, the majority of the genes reside near the center of the chromosome around *dpy-11*. Very few genes lie left of this area and extending all the way to the end of the chromosome. The physical map in this area is currently defined by at least two contigs, stP3 and eP74 (ACEDB, R. Durbin and J. Thierry-Mieg unpublished). They have not been linked together. LGV left is thus a good area to study the distribution of genes on the genetic and the physical maps.

Reintroduction of cloned genes back into organisms is a powerful method for the comparison of genetic to physical maps. Creation of transgenic organisms has been well documented in the past 10 years for organisms such as yeast, fruit flys, toads, nematodes, sea urchins, fish, mice and numerous other mammals. In *C. elegans*, germline transformation technology has developed rapidly and has become a major tool for the identification of genes and the elucidation of regulatory elements controlling them (Fire 1986; Stinchcomb *et al.* 1985; Mello *et al.* 1991). Microinjection of cosmids could molecularly define a gene's map position and also generate small genomic duplications. Duplications have been demonstrated to be useful in the mapping of essential genes LGI (Howell *et al.* 1987; Howell and Rose 1990; McDowell 1990; McKim 1990; A. M. Rose, personal communication).

In this section, I present the results from the PCR-based mapping of cosmid-derived primers to deficiencies on LGV(left). The results indicate that the gene sparse regions of LGV(left) are likely areas of lower than average DNA/m.u. while the area of the *dpy-11* cluster of LGV left appears to have a higher than average ratio of DNA/m.u. This section also describes the rescue of Unc-60 with two cosmid clones,

F53E2 and C32E5. Finally, I show how 8 small genomic duplications of LGV(left) were created using germline transformations.

Materials and Methods

Preparation of DNA to make oligonucleotide primers

Andrea Scouras designed primer pairs from genomic cosmid clones for polymerase chain reactions (PCR). Cosmid clones came from the contigs stP3 and eP74, as shown in Figure 3. Briefly, subclones of cosmids were generated and selected for single inserts of 0.5 to 1 Kb in size. The subclones were sequenced using two methods. The first utilized Terry Snutch's modified version of the dideoxy termination method (personal communication) of Sanger *et al.* (1977). The modification involves the addition of dimethyl sulfoxide to eliminate secondary structure of the DNA. This method was used on conjunction with the Sequenase version 2.0 kit (United States Biochemical Corporation, Cleveland Ohio). The second involved cycle sequencing using dye primers outlined in the Applied Biosystems Taq Dye Primer Cycle Sequencing Kit. The sequences obtained for each subclone were analyzed and deoxynucleotide primers were designed.

Oligonucleotide design

Oligonucleotides used for PCR mapping of deficiencies were designed using the computer program OLIGO (Rychlik and Rhoads 1989). The program determines duplex stability, specificity, self-complementarity of the oligonucleotide, the presence of palindromes and the ability of the oligonucleotide to form dimers. The primers used in this study are described in Table 1. They were synthesized by the IMBB Oligo service (Simon Fraser University).

Table 1 Oligonucleotide primers

This is a list of primers designed and used for the PCR reactions described in this thesis. L and R refer to left and right, the direction of synthesis from the priming site.

Oligonucleotide Primers	Sequence 5' - 3'
F12B4 (L)	ACC ACA TCA TCT CCC ATA CC
F12B4 (R)	GAC TTG AAT GAG AGC GAA TG
F46A7 (L)	GGT AGA TGG GCT GAA GGT AG
F46A7 (R)	CTA CCT TCA GGC CAT CTA CC
T01C2 (L)	AAG GAC ATC GGT TCA GGT TC
T01C2 (R)	AAT GAT AGG GTG TGT TGC CG
T03F8 (L)	GGA GAA CAC TTT ATC AGC CG
T03F8 (R)	AGT GAA TGA ACA GGG AAC CG
T10D1 (L)	TAG CCA GAA AAG CGA AAA TG
T10D1 (R)	AGA GAA AGA TAA AAG GAC CG
W03A10 (L)	TAA CAC CTG AGC AAA GTC GG
W03A10 (R)	CAG CGG AGC AAA GGA GAG TC
W06E4 (L)	AGA TAA CGG TGA CAT TGC TC
W06E4 (R)	GCT TAT CCA GGG TAT GTA TG
W06G11 (L)	AGC ATT TTT CAC GGG ATC TG
W06G11 (R)	TCG TTG AGG GAA TTG ATA AG
Y7E8 (L)	GAT GGC TAT GTT GTG CC
Y7E8 (R)	GTT TTC CAT TTT TTT CAA G
Y53A1 (L)	GAA AAA TTT TCG AAA AAT CG
Y53A1 (R)	AAA ACT TGT GGG ATT ATG G
Neomycin-3 (L)	CTG AAG CGG GAA GGG ACT GG
Neomycin-4 (R)	AGC ACG AGG AAG CGG TCA GC

PCR mapping of deficiency homozygotes

PCR was used as a tool to align the physical and genetic maps in the LGV(left) region. The PCR protocol was derived from Barstead and Waterston (1991). Genetic manipulation was necessary to obtain homozygous deficiency nematodes. The strains containing the deficiencies are balanced over the crossover suppressor *eT1*. The aneuploids generated from the self fertilization of the *eT1* heterozygotes are egg or early larval lethals and therefore are impossible to distinguish from the deficiency homozygotes (which also arrest as egg or early larval lethals). Therefore, the strains need to be outcrossed to remove the balancer chromosomes. The first method used deficiency heterozygous nematodes *dpy-18/eT1(III);sDfx/eT1* mated to *unc-46(e177)/+* males. This was done because some of the deficiency chromosomes carry the Unc-46 marker. The mating allowed for the selection of the chromosome V deficiency and the *unc-46* marked chromosome by selected Unc-46 nematodes. In this manner, Uncs were picked in the F_1 and allowed to lay eggs. The Uncs were brooded over a period of a couple of days then removed from the plates. The F_2 were allowed to mature and the plate was screened for unhatched eggs or in the case of *sDf70*, arrested L1 larvae. The unhatched eggs or arrested larvae are the individuals homozygous for the deficiency chromosomes. Not all deficiency strains contained the Unc-46 marker, and thus the Dpy-18 marker was used in some instances. As stated in General Materials and Methods, *dpy-18* is pseudolinked to LGV(left) genes and therefore can be used to select for the deficiency chromosome V. The Dpy-18 selection method was similar to that of the Unc-46 selection except that deficiency heterozygotes were mated to *dpy-18/+* males and Dpy nematodes were picked from the F_1. These were allowed to lay eggs and were brooded over a couple of days. Two to five eggs were picked per reaction using a drawn out glass capillary pipette. The eggs were picked up using a small volume of chitinase solution (20 mg/ml chitinase, 50 mM NaCl, 70 mM KCl,

2.5 mM $MgCl_2$ and 2.5 mM CaCl) and placed in a 500 ul eppendorf tube cap containing 2.5 ul of nematode lysis buffer (50 mM KCl, 10 mM Tris-HCl pH 8.2, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin and 5 ul of proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, IN) solution at 10 mg/ml. In the case of wild-type N2 or arrested L1 nematodes, whole nematodes were used in the PCR reactions and were picked directly into the cap of the eppendorf tube containing nematode lysis buffer. The eggs or nematodes were briefly spun down to the bottom of the tube and frozen for 10 minutes at -70^{0}C. Mineral oil was placed on top of the frozen mixture and the tubes were placed at 65^{0}C for one hour to allow digestion of the eggs and/or nematodes. The tubes were then heated to 95^{0}C for 15 minutes to inactivate the enzyme. The remaining ingredients were then added to the reaction: 25-35 pM of each primer, 2.5 ul 10X PCR reaction buffer (100 mM Tris pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% [w/v] gelatin), 4 ul dNTP mix (1.25 mM for each dNTP), 0.0125 ul Taq polymerase (5 U/ul) and double distilled sterile H_2O to bring the volume to 25 ul.

The PCR reaction conditions were as follows: one cycle of denaturing for 5 min at 94^{0}C, anneal for 1 min at 58^{0}C, and extension for 2 min at 72^{0}C followed by 25 cycles of denature 1 min at 94^{0}C, anneal 1 min at 58^{0}C, extension 2 min at 72^{0}C and finally one cycle of denature 1 min at 94^{0}C, anneal 1 min at 58^{0}C and extension 10 min at 72^{0}C. PCR Reactions were performed on the ABI model #TCX15A thermal cycler. PCR products were electrophoresed on 1.2, 1.5 and 2.0 % agarose gels containing EtBr and photographed under UV transillumination.

All PCR experiments were performed with positive and negative control reactions. The cosmid from which a primer pair was designed was used as a positive control template to see if the primers were working under the reaction conditions. Wild-type genomic DNA (from N2 nematodes) was used as a positive control to determine the size of the PCR product from genomic DNA and to see if there was a

single product. Negative controls consisted of reaction tubes containing all of the components except the template DNA (data not shown for all reactions). Other control primer pairs from either LGIV or LGV were used in all PCR reactions involved in the deficiency mapping. The control primers were chosen so that the control primer product was a different size than the experimental product. In addition, the control primers were not from an area thought to be deleted by the deficiencies being tested. The control primers established that the nematodes were lysed and their DNA was available for the PCR reaction.

Preparation of DNA for germline transformation

The cosmid and plasmid DNAs used for germline transformation were prepared by one of three methods. Method one was a protocol from John Sulston (personal communication). It was a modified alkaline lysis procedure from Maniatis *et al.* (1982). 10 ml liquid cultures were grown for 16-24 hours in large 50 ml Falcon tubes for maximum aeration. The phenol/chloroform extractions were omitted and the DNA was not subject to RNase treatment. The DNA was dissolved in 50 ul of TE buffer and 50 ul of a 4.4 M LiCl solution was added. This mixture was kept at 4^0C for 4 hours, spun down in a microcentrifuge for 5 minutes and the supernatant was retained. Two volumes of 95% EtOH were added to the supernatant and the mixture reprecipitated at -20^0C for 1 or more hours. After a 2 minute spin, the pellet was washed with 70% EtOH and dried briefly. The pellet was dissolved slowly in 100 ul of 0.1 M potassium acetate (pH 7.4). The DNA was reprecipitated at -20^0C with 2 volumes of 95% EtOH. The final pellet was washed with 70% EtOH, dried and resuspended in 10 mM KPO_4 (pH 7.5) and stored at -20^0C.

The second method was similar to the above, except the DNA was not treated with LiCl nor subjected to the numerous precipitations. Instead, the 50 ul of DNA recovered from the alkaline lysis preparation was placed on a Miniprep Spun Column

(Pharmacia) and spun for 2 minutes at 400 x g. The DNA was often difficult to elute from the columns, so an additional 50 ul of column buffer (TE pH 8.0) was placed on top of the column and it was spun a second time for 2 minutes. The DNA was used directly for germline transformations.

Method three was the large scale alkaline lysis as described in Maniatis *et al.* (1982). This was necessary to generate enough cosmid or plasmid DNA for many experiments. The DNA, prepared from 250 or 500 ml liquid cultures, was purified on CsCl density gradients (Maniatis *et al.* 1982). The gradients were spun for approximately 20 hours at 55,000 rpm in the Beckman model L8-80 ultracentrifuge. Two bands were collected from the tubes and the EtBr was extracted using water saturated butanol. The DNA was diluted and precipitated using 95% EtOH.

Quantification of the DNA generated from the CsCl purification was performed by spectrophotometry at A_{260} using the Ultrospec III UV/Visible spectrophotometer (Pharmacia). DNA purified from columns was quantified by electrophoresing a sample on a 0.7% agarose gel and comparing the intensity of EtBr staining to a known quantity of 1 Kb DNA Ladder (Bethesda Research Laboratories, Life Technologies, Inc.). DNA prepared by the Sulston method was quantified by spotting onto 1% agarose against known standards as described by Maniatis *et al.* (1982). It was then confirmed by gel electrophoresis.

DNA for germ line transformation was originally mixed with Fire's (Fire 1986) injection solution at 200-400 ng/ul. However, most injections later were performed using DNA at 100 ng/ul in TE buffer or double distilled sterile H_2O.

DNA prepared from each of the three methods was equally suited for germline transformations.

Germline transformation techniques

Two different methods were used to perform germline transformations. All experiments were performed using an inverted Zeiss microscope with Nomarski optics. Needles were pulled from World Precision Instruments Kwik-fil glass capillaries using a micro-pipette puller (Industrial Science Associates, Inc., Ridgewood, N.Y.) or using the Flaming/Brown Micropipette puller Model P-87 (Sutter Instrument Co.) at the settings heat=377, pull=140, velocity=140, time=140. The needle tips were either broken open on the cuticle of the worm or etched open using 50% HF (as described by Mello *et al.* 1991)

The initial injections were performed as described by Fire (1986). Worms were dehydrated and thus immobilized onto agarose pads. The method required individually injecting mature oocytes in the two gonad arms of the worm, usually 4 injections per arm. The worms were slowly rehydrated from the process over a period of 2-6 hours. Mello *et al.* (1991) devised a much more efficient method of injections. Gravid adult hermaphrodites 1/3 of their way through the egg laying cycle were picked and placed on agarose pads, but they were not allowed to dry out. Single injections were performed into the large syncytium of each gonad arm. The worms required no recovery time after injection and were immediately placed on seeded Petri plates.

Most DNA preparations were mixed with the plasmid pRF4, containing the dominant Roller gene, *rol-6(su1006)* (Kramer *et al.* 1990). The Roller gene acted as a marker to assess the transformation efficiency and act as a selection system for maintaining transformed strains. The pRF4 plasmid was used at 30-100 ng/ul. The final concentration of marker plus experimental DNA was usually 100 ng/ul. Roller F_1s were picked as transformants. All transformed strains discussed in this thesis are likely to contain the injected DNA as extrachromosomal arrays (*sExs*). Integration of

foreign DNA into the chromosomes is rare using the injection method of Mello *et al.* (1991).

Results

Correlation of the physical and genetic maps using PCR

The polymerase chain reaction was used as a tool to correlate the genetic and physical maps in the LGV left region. Primer pairs designed from cosmids and YACs containing genomic DNA (see Figure 3 for cosmids/YACs used) were used to amplify DNA from deficiency homozygous nematodes. Primer design and a table of primer pairs used is presented in Materials and Methods (Table 1). The presence of a PCR product indicates that the deficiency does not delete the DNA corresponding to the primer pair. However, this protocol would not necessarily allow for the mapping of primer sites relative to very small deficiencies (e.g. deficiencies containing less nucleotides than the number of nucleotides between primer pair sites). If a deficiency removed DNA between primer sites, the PCR product would be smaller than the predicted size and may not be resolved from the expected product on gels. The absence of a PCR product indicates that the deficiency deletes one or both primer pair sites. The results are summarized in a complementation table, Table 2. Table 3 describes the zone where each primer pair falls. Figure 2 presents a partial genetic map of LGV(left) showing genes and all of the deficiencies used in the PCR analysis. Figure 2 also shows the two contigs, stP3 and eP74. The cosmid and YAC sites were placed on the contigs according to the data in Table 2. Figure 3 shows the same PCR data in relation to the genetic map of Johnsen and Baillie (1991). This Figure depicts the relationship between the physical map distance (cosmid distances) to the genetic map distance (recombinational map distance).

As a sample of the criteria used to score the PCR experiments, Figure 4 demonstrates the results achieved for the primers from the cosmid W06G11. The presence of the experimental band at 490 bp indicates a '+' score on the

Table 2 Complementation Table of Cosmid/YAC Sites Against Deficiencies

Complementation table generated from PCR amplification of deficiency homozygotes. The horizontal axis lists the cosmid/YAC derived primers used in PCR experiments. The vertical axis lists the deficiencies used in the PCR experiments. ( ) indicates anomalous result; + indicates PCR product formed; - indicates no PCR product formed. Blank squares indicate experiments not performed.

	T03F8	W06E4	F12B4	W03A10	F46A7	Y7E8	W06G11	T01C2	Y53A1
sDf51		-							
sDf45	-	+	+						
sDf34	-	-	-						
sDf42	-	-	-	+	+	+			
sDf75	+	+							
sDf33	(-)	+							
sDf70		+	+						
sDf50		+	+	(+)	-	+	+	+	
sDf27			+	-		+	+		
sDf28	-		-	+	+				
nDf32				+		-	-	-	+
sDf30				+		+		-	+
sDf35							+	+	+
mDf3							+	+	+
mDf1							+	+	
sDf26					-	-	-	+	
nDf18								+	+
sDf20								(+)	+

Table 3 Zone Location of Mapped Cosmid/YAC Sites

Cosmid / YAC Primer Sites	Zone Mapped To
T03F8	5-6A
W06E4	6A
F12B4	6A
W03A10	8A2
F46A7	8A2-9A
Y7E8	10
W06G11	10
T01C2	14
Y53A1	16
ZK230 *	7
Y45G12 *	6A-6B
T18A6 *	6B

* Eric Lambie personal communication

Figure 2 Genetic and Physical Map of LGV(left)

The physical map of the two contigs called stP3 and eP74 are shown above the genetic map. The cosmid/YAC sites were placed along the contigs according to the PCR results from Table 3. The genetic map is a partial map derived from Johnsen and Baillie (1991). Only the deficiencies used in the PCR analysis are shown below the map.

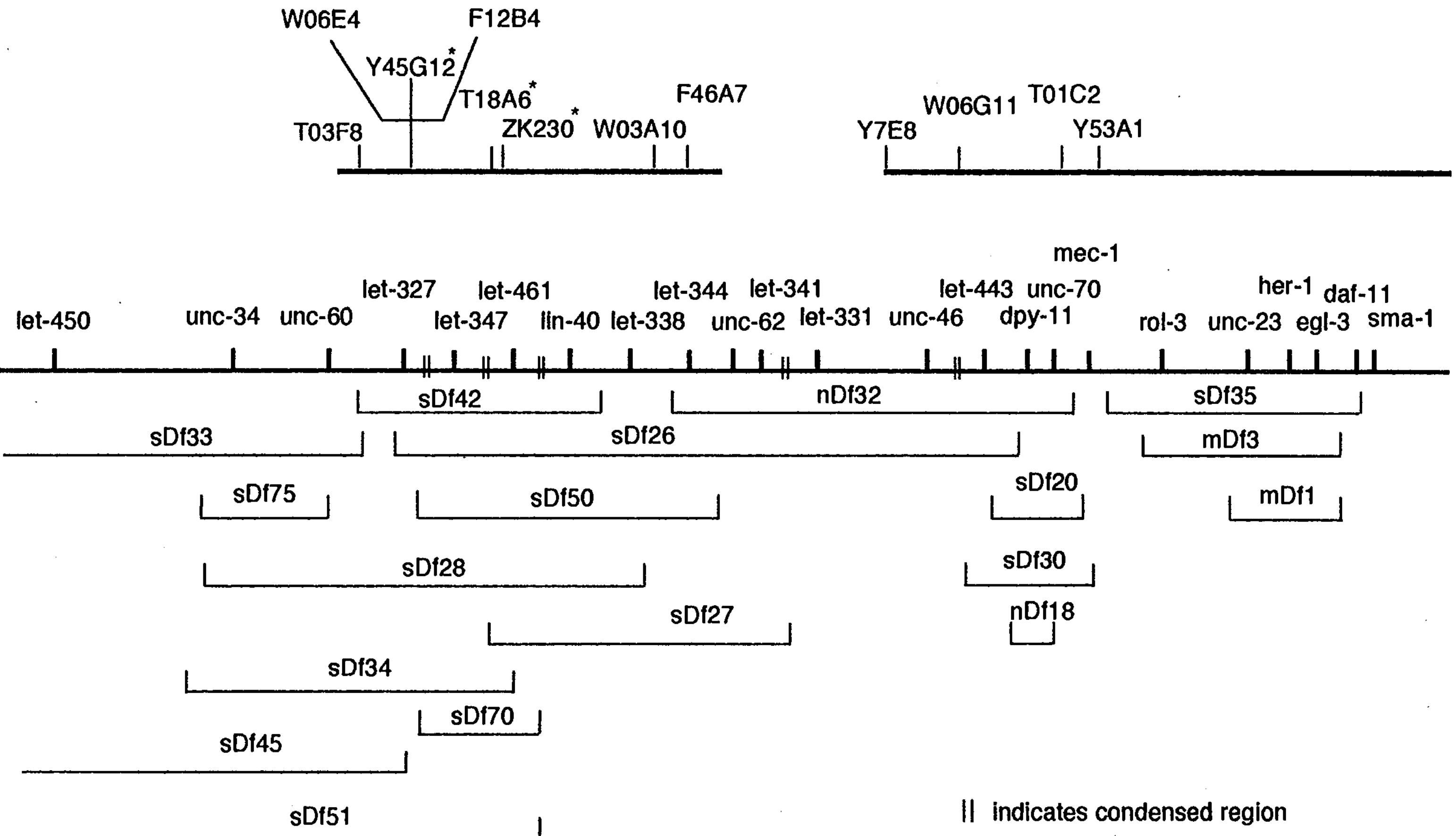

stP3
W06E4
F12B4
Y45G12*
T18A6*
ZK230*
W03A10
F46A7
T03F8
eP74
Y7E8
W06G11
T01C2
Y53A1
let-450
unc-34
unc-60
let-327
let-347
let-461
lin-40
let-338
let-344
unc-62
let-341
let-331
unc-46
let-443
dpy-11
unc-70
mec-1
rol-3
unc-23
her-1
egl-3
daf-11
sma-1
sDf42
nDf32
sDf35
sDf33
sDf26
mDf3
sDf75
sDf50
sDf20
mDf1
sDf28
sDf30
sDf27
nDf18
sDf34
sDf70
sDf45
sDf51
|| indicates condensed region
1 map unit
* Eric Lamble personal communication

Figure 3 Interpretive Map of the Correlation of the Recombination and Physical Distance in LGV(left)

The top line represents the contigs stP3 and eP74 and shows the mapped cosmid/YAC sites from the PCR results in Table 3. The contigs are drawn to show the estimated number of cosmids lying end to end from mapped sites (based upon the physical map from ACEDB [R. Durbin and J. Thierry-Mieg unpublished)]. Each mapped cosmid/YAC site has a line drawn from its physical map position to its genetic map position. The genetic map is from Johnsen and Baillie (1991).

(—) approximately 4 cosmid distances (1 cosmid = 40Kb)

T03F8 W06E4 Y45G12 F12B4/T18A6 ZK230 WO3A10 stP3 F46A7 Y7E8 WO6G11 eP74 T01C2 Y53A1

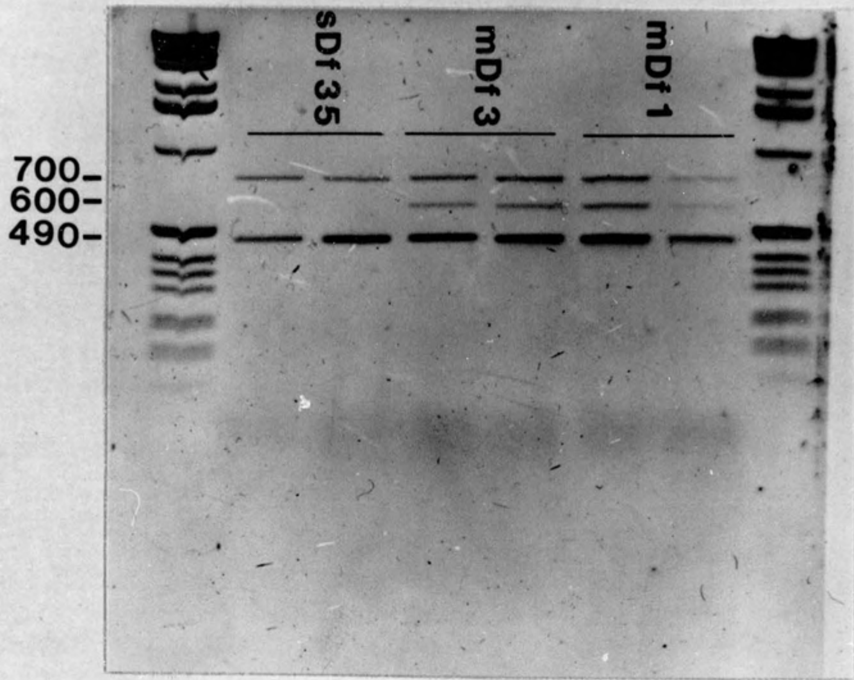
1 map unit

1A 1B1 1B2 1B3 1C 2 3 4A 4B 5 6A 6B 7 8A1 8A2 9A 9B 10 11A1-12 13 14 15 16 17 18 19 20A 20B 21 22 23

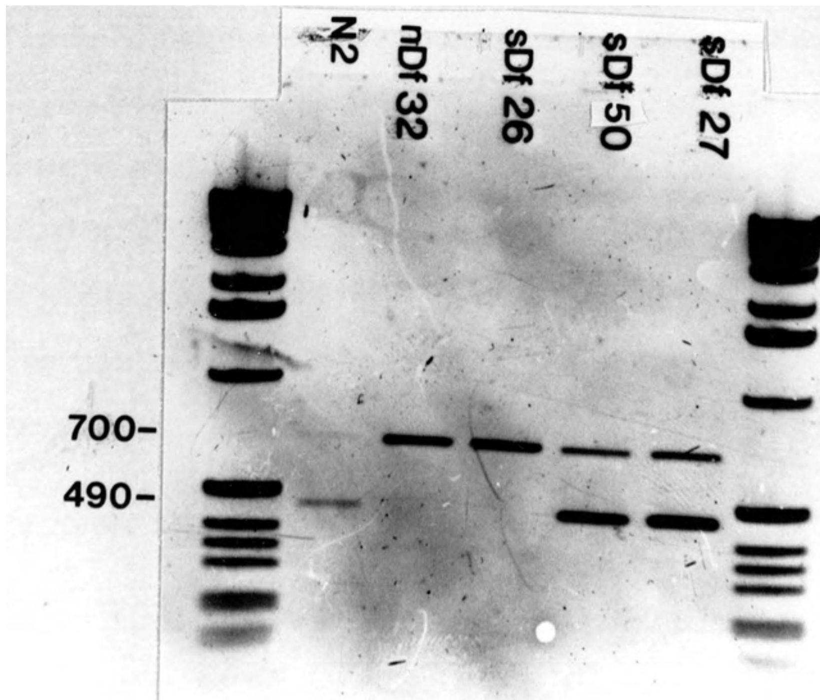
Figure 4 PCR Products from W06G11 Derived Primers

Panels A and B show ethidium stained gels of PCR products using primers designed from the cosmid W06G11. A '+' was scored on the presence of the experimental band (490 bp). A '-' was scored on the absence of the experimental band but the presence of the control band(s) at 600 and 700 bp. A 1 Kb DNA Ladder was used as a DNA size marker. Panel A shows two lanes for each of the deficiencies *sDf35, mDf3* and *mDf1* each lane representing individual PCR experiments. The experimental band is present in all of the lanes. Panel B shows single PCR experiments for the deficiencies *nDf32, sDf26, sDf50* and *sDf27.* The experimental band is absent in the *nDf32* and *sDf26* lanes.

A



B



complementation table (Table 3). *sDf35, mDf3* and *mDf1* all contain the experimental band (Figure 4 panel A). However, *nDf32* and *sDf26* (Figure 4 panel B) do not amplify the 490 bp product and thus were scored as a '-' (Table 3). Using these results, W06G11 was placed between the *sDf27* and *sDf30* breakpoints on the genetic map (see Discussion for explanation with *sDf30*). Control primers from LGIV (a gift from Marco Marra) were used in the reactions with W06G11 and can be seen at 600 and 700 bp in most of the lanes. These control primers sometimes gave two bands presumably because of two priming sites available in the nematode DNA.

Figure 5 illustrates the results obtained using the primers designed from the left end of the YAC, Y7E8 (the primers were a gift from A. R. Coulson and J. E. Sulston). The experimental band is 200 bp and the control band is 700 bp. Both *nDf32* and *sDf26* delete the site corresponding to the left end of Y7E8 (Figure 5 panels A and B). Since the experimental band can be seen in the *sDf27* and *sDf30* lanes, the Y7E8 site is believed to be between the break points of *sDf27* and *sDf30*. Figure 6 shows some of the PCR results for the primers designed from the cosmid W06E4. The experimental band is 350 bp and can be seen in the *sDf75, sDf70* and *sDf50* lanes but is absent in the *sDf34* and *sDf42* lanes. W06E4 derived primers also amplify a 350 bp product in *sDf45* and *sDf33* homozygotes (data not shown). This allowed for the placement of W06E4 primer sites between the breakpoints of *sDf45* and *sDf50*. Data obtained for the primers designed from T03F8, F12B4, W03A10, F46A7, T01C2 and Y53A1 is summarized in Table 3 (data not shown).

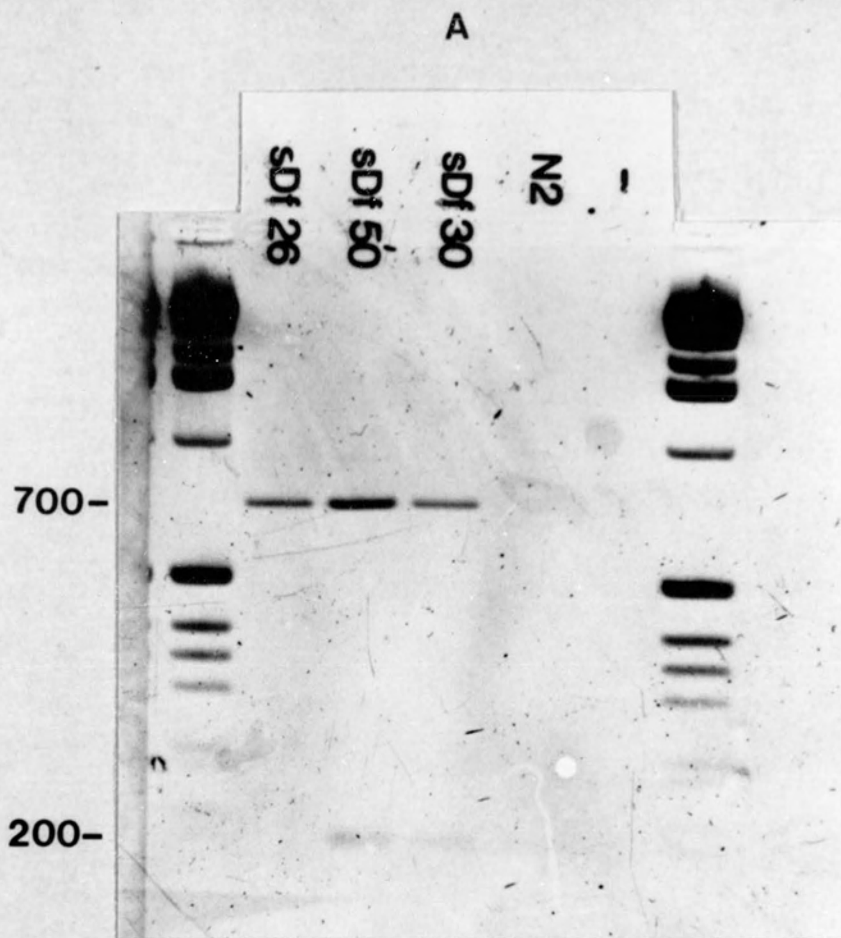
Germline transformation to rescue Unc-60

In order to provide an anchor on the LGV(left) physical map, transformation rescue of Unc-60 was attempted. *unc-60(m35)* (DR35) homozygous nematodes were chosen for injection because although they are paralyzed, they produce more progeny

Figure 5 PCR Products from Y7E8 Derived Primers

Panels A and B show ethidium stained gels of PCR products using primers designed from the left end of the YAC, Y7E8. A '+' was scored on the presence of the experimental band (200 bp). A '-' was scored on the absence of the experimental band but the presence of the control band at 700 bp. A 1 Kb DNA Ladder was used a DNA size marker. In panel A, the experimental band is weak and the band is absent in the *sDf26* lane. Panel B shows the experimental band absent in the *nDf32* lane.

A



B

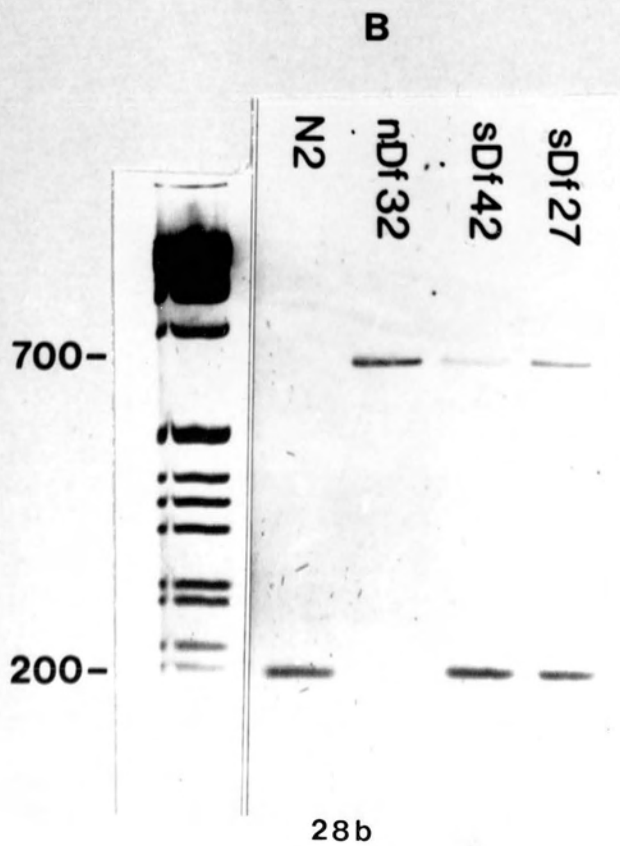
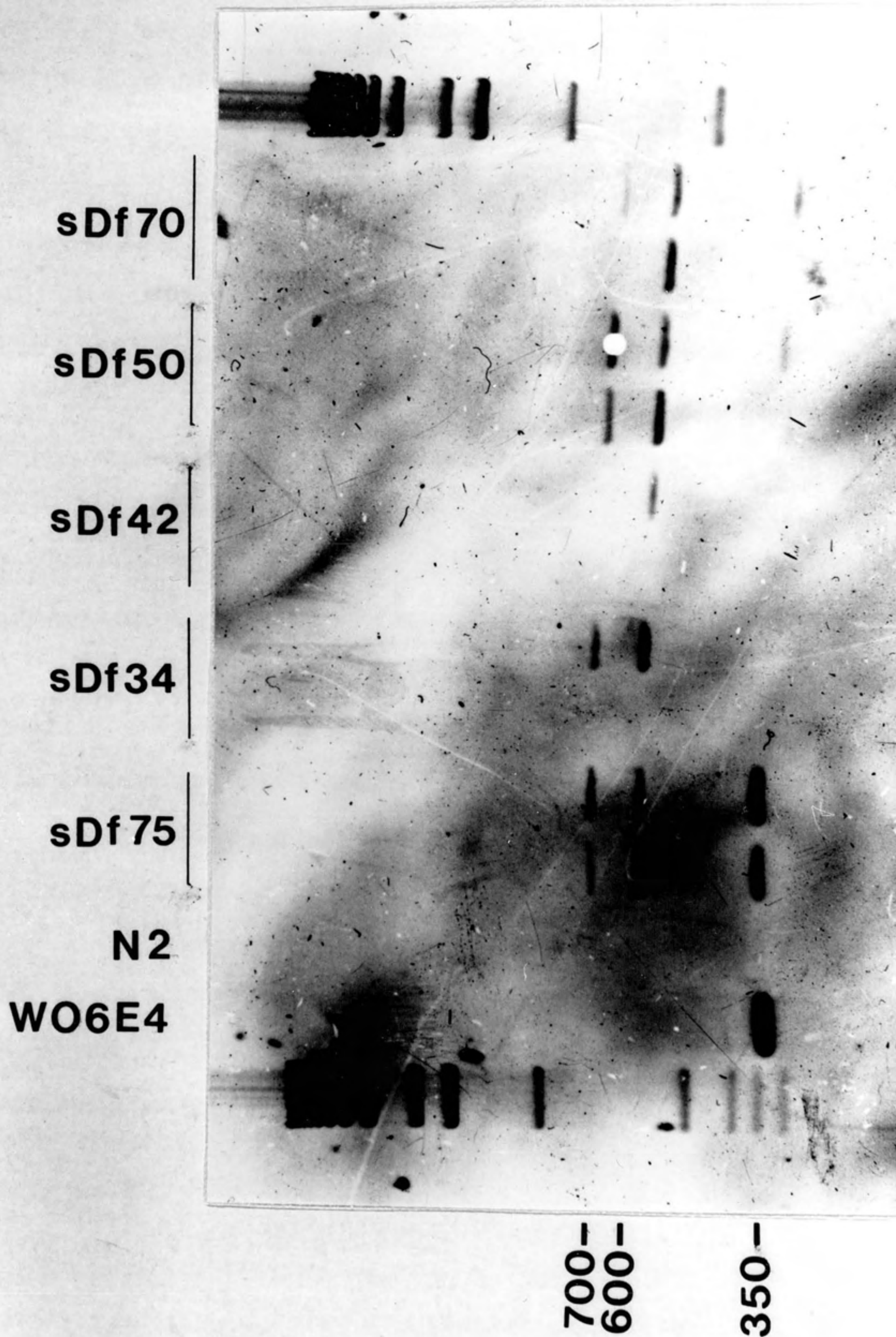


Figure 6 PCR Products from W06E4 Derived Primers

An ethidium stained gel of PCR products using primers designed from the cosmid W06E4. The experimental band is seen at 350 bp. There were duplicate PCR experiments performed for each deficiency tested, shown here with each reaction in a single lane. A '+' was scored on the presence of the experimental band (350 bp). A '-' was scored on the absence of the experimental band but the presence of the control band(s) at 600 and 700 bp. The experimental band is absent in the *sDf34* and *sDf42* lanes. A 1 Kb DNA Ladder was used a DNA size marker.


sDf70
sDf50
sDf42
sDf34
sDf75
N2
WO6E4
700-
600-
350-

than most *other unc-60* alleles. A rescue of the Unc-60 phenotype was scored by the absence of the paralysis (i.e. wild-type phenotype).

One of *unc-60*'s closest neighbours genetically, *ges-1*, had been cloned and placed on the map of overlapping cosmid and YAC genomic clones (see Figure 7). The cosmids from the *ges-1* cosmid contig, F53E2, K06H5 and T28A3 (T28A3 is equivalent to C38C3) were pooled together and injected into individual mature oocytes at 600 ng/ul to rescue Unc-60. Following successful rescue, individual cosmids were injected to see if a single cosmid could rescue the phenotype. The cosmid F53E2 was injected into individual mature oocytes at 200 ng/ul. The injections produced 8 individuals which exhibited a wild-type phenotype in the F_1 generation, but were again paralyzed in the subsequent generations. A different injection method developed by Mello (1991) was used to try to obtain a stable rescue; that is, a strain which continues to segregate rescued progeny from generation to generation. One stable rescued strain that probably carried an extrachromosomal duplication (Way and Chalfie 1988) of the cosmid F53E2 (*sEx6*) was generated by this method. The strain was named BC4026 with the genotype *unc-60(m35);sEx6* (F53E2). It segregated 10-40 % wild-type progeny. To confirm the rescue of the Unc-60 phenotype with the cosmid F53E2, the cosmid was injected into *unc-60(s1331) eT1(III);eT1(V)* (BC3581) nematodes. The cosmid was able to rescue the *s1331* allele also. Subclones of F53E2 were thereafter injected in order to identify a smaller DNA fragment that would rescue Unc-60. The subclones were provided by K. McKim and C. Matheson. None of the subclones injected rescued the gene (data not shown).

Complementation tests with transformed strains

The identification of *ges-1* and *unc-60* on the physical map provided an anchor point on the physical map for genes in the LGV(left) region. The anchor indicated potential physical map positions for the genes neighbouring *ges-1* and *unc-60* on the

Figure 7 *ges-1* Contig

This figure is a direct print out from the *C. elegans* genome database, ACEDB (R. Durbin and J. Thierry-Mieg unpublished). The 'cm' prefix indicates cDNA clones mapped to this region. YACs are identified by 'Y' and are represented by the long lines at the top of the figure. The cosmid clones are represented by the short lines in the middle of the figure. The cosmid T28A3 (as mentioned in Results) is equivalent to C38C3 on this map. Likewise, T10D1 is equivalent to ZK1101 on this map. Any name with a '#' indicates a phage clone placed on the contig. Below the stippled line are the two genetically mapped genes, *unc-60* and *ges-1*. Under the contig are notes about the joining of clones and individuals who have mapped sites in the area. At the lower right hand corner, the contig is shown with the identified markers. The darkly stippled area on this line indicates the area expanded on the top of the figure. This entire contig may be in the opposite orientation such that *ges-1* is to the right of *unc-60* (K. McKim personal communication).

cm5f10
cm5b1
cm17h12
cm5a3
cm14a7
cm7d7
cm10g6
(Y82G11)
(Y76G11)
(Y60H6)
(Y75B7)
(Y74C2)
(Y66C3)
(Y46H3)
(Y54C6)
M154 *
HK#71
HK#22
HK#4
C18B7 *
F52F10
C15C5 *
D2011 *
F16B4 *
Y3F1
K04F1
VT#YL56
VT#YL41
R12A1
K06A11
C51E2 *
T08A6
JM#L0001
C29B10 *
F53E2
C32E5 *
C38C3 *
K06H5
ZK1101
BC#S1001
unc-60
ges-1
K08D8etc was here(Y3F1 PROBE)
RcD1
<POSS JOIN TO eP74R:see Y40B10YW
sl dub;Y41G1cyB,Y44H1cyB,Y54C6cyB+
rsc M.Wakarchuk,D.Baillie
repel
J.McGhee
Cerep3
rep.(Waterston)
RcC9;RcD1;Rc123?
B.Johnsen,D.Baillie
unc-60
ges-1
TCUNC60C
rsn-7
MLCl3

genetic map. Therefore germline transformation was chosen as another tool to correlate the genetic and physical maps in the LGV(left) region. Most injections were performed into *eT1* heterozygous strains containing a lethal allele of interest (see Table 4 for list of strains injected). Test DNA was injected with the marker Rol-6 and F_1 Rollers were selected that segregated Rollers in subsequent generations. Injected test DNA is coassembled with pRF4 into stable extrachromosomal arrays at a low frequency if the DNAs are non-homologous (Mello *et al.* 1991) When possible, cosmid or plasmid test DNA with homology to pRF4 was chosen for injections. To ensure a Rol-6 strain contained the injected test DNA, two independently generated transformed strains were maintained. Later, PCR was used to confirm the presence of cosmids in the arrays (data presented under the heading "PCR amplification of transformed nematode DNA"). The extrachromosomal arrays generated by transformation could be used as a duplication of small regions of LGV(left). In this manner, matings of the duplication strain to heterozygous strains containing other lethal alleles were performed. Figure 8 outlines the method used for the complementation tests. Rol Non-Unc-36 F_1 progeny were picked from the matings and allowed to self fertilize. The F_2 progeny were screened for mature, fertile Dpy Uncs indicating that the duplication rescues the lethal phenotype. This method also allowed for the confirmation of the presence of injected test DNA within a stable Rol-6 strain.

A summary of the injection experiments and the results is presented in Table 4. The cosmid C32E5 was chosen for injection because it overlaps with the cosmid F53E2 for nearly its entire length, hence it might also rescue Unc-60 and possibly another gene. C32E5 was injected into *dpy-18(e364)/eT1(III);let-326(s1404) unc-46(e177)/eT1(V)* (BC2205) nematodes at 50 ng/ul plus 50 ng/ul of pRF4. *sEx9* did not demonstrate rescue of Let-326 but was used as a duplication in a complementation test with *unc-60(m35)*. *eT1(III;V);sEx9[pRF4{rol-6(su1006)} + C32E5]* (BC4031) was mated to

Table 4 Summary of Results From Injection Experiments

COSMIDS	STRAIN	ARRAY NAME	GENES TARGETED FOR RESCUE	RESCUE
C32E5	BC 2205 dpy-18(e364)/eT1(III);let-326(s1404) unc-46(e177)/eT1(V)	sEx9	let-326(s1404)	-
			unc-60(m35)*	+
F16B4, C15C5	BC 3551 dpy-18(e364)/eT1(III);let-426(s1527) unc-46(e177)/eT1(V)	sEx17, sEx18	let-426(s1527)	-
K06H5, T28A3, F53E2	BC 2205 dpy-18(e364)/eT1(III);let-326(s1404) unc-46(e177)/eT1(V)	sEx8, sEx12	let-326(s1404)	-
			let-426(s1527)*	-
T10D1	BC 3173 +/eT1(III);unc-60(m35) let-347(s1035) dpy-11(e224)/eT1(V)	sEx7	let-347(s1035)	-
			emb-29(s166)*	-
			let-327(s1496)*	-
			let-426(s826)*	-
			let-426(s1527)*	-
		sEx11	let-347(s1035)	-
			let-426(s1527)*	-
F53E2	DR 35 unc-60(m35)	sEx6	**unc-60(m35)**	+

* alleles tested by complementation tests

Figure 8 Strategy for Complementation Tests Using Duplications Created by Germline Transformation

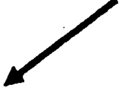
This figure shows the how extrachromosomal arrays were used as duplications in complementation tests. DNAs were injected in hermaphrodites and transformed individuals were selected. Transformed nematodes were mated to males containing an allele of interest. The F_1 individuals carrying the extrachromosomal array were allowed to self-fertilize and the F_2 were screened for rescued individuals.

pRF4[rol-6(su1006) + T10D1]

P-1 : + / eT1(III) ; unc-60(m35) let-347(s1035) dpy-11(e224) / eT1(V)

Pick unc-36 [eT1] Rol

P0 : eT1(III;V);sEx7[pRF4[rol-6(su1006)] + T10D1] X dpy-18(e364) / eT1(III) ; let-426(s1527) unc-46(e177) / eT1(V)



Pick L4 Rol

F1 : dpy-18(e364) / eT1(III) ; let-426(s1527) unc-46(e177) / eT1(V) ; sEx7

F2 : Screen for adult, fertile Dpy Unc-46

dpy-18(e364);let-426(s1527) unc-46(e177);sEx7

dpy-18(e364)/eT1(III);unc-60(m35)/let-500(s2165) eT1(V) (BC3480) males and 15 F_1 Rollers were set up on individual Petri plates. The F_2 generation was screened for Dpys. All F_1 rollers segregated fertile Non-Unc Dpys indicating that the cosmid C32E5 rescued the Unc-60 phenotype (in the individuals who received the cosmid duplication). However, the F_2 Dpys did not roll. To confirm the presence of the *rol-6* DNA in the rescued Dpys, these Dpys were then mated to wild-type (N2) males to resegregate the Roller phenotype. Since there were Rol-6 progeny, the homozygous *dpy-18(e364)* allele appears to suppress the expression of the *rol-6(su1006)* allele in these nematodes. It has been noted that *dpy-5(e61)* also suppresses the expression of rolling in transformed strains (J. McDowell, personal communication).

As discussed previously, the three cosmids K06H5, T28A3 and F53E5 were coinjected and found to rescue Unc-60. The rescue of Unc-60 was observed only in the F_1 generation. It was desirable to create a transformed strain that contained the three cosmids as a stable extrachromosomal array. The duplication of the cosmids could then be used to attempt rescue of *let-326(s1404)* or *let-426(s1527)*. BC2205 nematodes (see Table 4 or above) were injected with 150 ng/ul of the cosmid mixture together with 100 ng/ul of pRF4. No rescue of Let-326 was observed. A stable Rol-6 line *eT1(III;V);sEx12[pRF4{rol-6(su1006)} + K06H5, T28A3, F53E2]* (BC4033) was mated to a strain containing *let-426(s1527)*. The *sEx12* duplication was unable to rescue the *let-426(s1527)* allele.

A second experiment was performed using the triple cosmid mixture K06H5, T28A3 and F53E2 at 50 ng/ul plus 100 ng/ul of pRF4 injected into BC2205 nematodes. There was no rescue of Let-326. The resulting stable line *eT1(III;V);sEx8[pRF4{rol-6(su1006)} + K06H5, T28A3, F53E2]* (BC4030) was complementation tested with *unc-60(m35)* and *let-426(s1527)* alleles. The results showed that the *sEx8* array did not contain the information necessary to rescue the Unc-60 nor the Let-426 phenotypes.

The *ges-1* cosmid contig extends to the right with the cosmid T10D1 (Figure 7 where ZK1101 is equivalent to T10D1). T10D1 was a good candidate to attempt rescue of the surrounding essential genes. T10D1 was injected at 50 ng/ul plus 100 ng/ul of pRF4 into *+/eT1(III);unc-60(m35)let-347(s1035) dpy-11(e224)/eT1(V)* (BC3173) nematodes. Two stable Rol-6 lines, *eT1(III;V);sEx7[pRF4{rol-6(su1006)} + T10D1]* (BC4028) and *eT1(III;V);sEx11[pRF4{rol-6(su1006)} + T10D1]* (BC4029) were retained. Complementation tests were performed with the *sEx7* array to strains containing the alleles *let-327(s1496), let-426(s826)*, *let-426(s1527)* or *emb-29(s166)*. No rescue of the gene phenotypes was observed from these crosses. In addition, *sEx11* array was complementation tested with a strain containing *let-426(s1527)* and it failed to rescue the allele.

The cosmids F16B4 and C15C5 were chosen for injection into a strain containing *let-426* because of the results of PCR mapping of deficiency breakpoints in the *unc-60* region (K. McKim personal communication). McKim predicted that *let-426* could be contained in the cosmid F16B4. F16B4 is a cosmid which had no obvious sequence homology with pRF4, but F16B4 shares overlapping sequence with C15C5. Therefore, the strategy involved using the C15C5 ampicillin resistance gene as a homolog to the ampicillin resistance gene in the plasmid pRF4. The recombination between the homologous sequences would also involve the overlap between the two cosmids. The product of the recombination should be a molecule with C15C5, F16B4 and pRF4 together. C15C5 and F16B4 were coinjected into BC3551 nematodes at 35 ng/ul of each cosmid plus 30 ng/ul of pRF4. The resulting extrachromosomal array *sEx17* was not segregated at high frequency, so another injection experiment was performed. The array *sEx18* showed a higher frequency of transmission. Neither *sEx17* nor *sEx18* was able to rescue the *let-426(s1527)* allele.

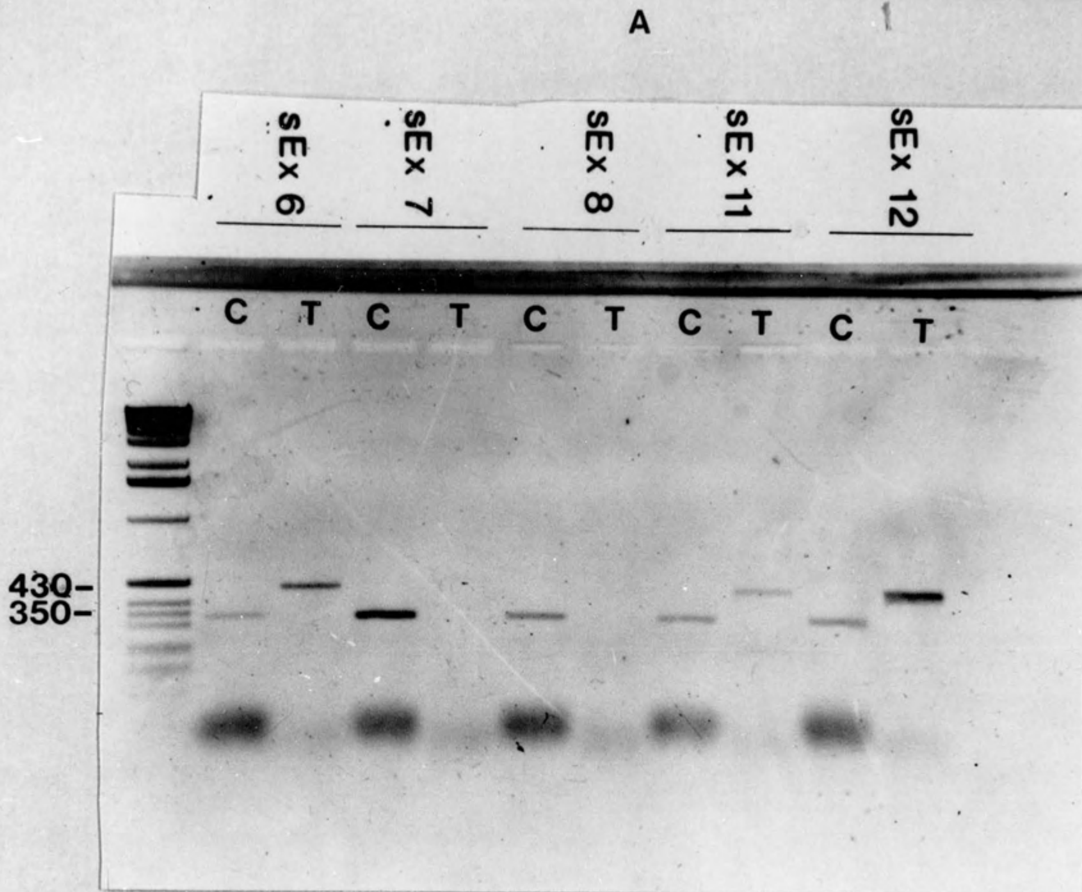
PCR amplification of transformed nematode DNA

PCR amplifications of DNA from nematodes transformed with various DNAs were performed. The DNA sequence of the neomycin phosphotransferase gene was obtained from the EMBL Data Bank and was used to design a set of primers (see Materials and Methods). The primers were used on transformed strains to determine if the injected cosmids (containing the neomycin phosphotransferase gene) were present in the strain. The results are shown in Figure 9. Two lanes are present for each DNA template; one lane contains the neomycin primers whereas the other lane contains the control primers, W06E4. The strains containing the arrays *sEx6, sEx11, sEx12,* and *sEx18* all contain the sequence for neomycin phosphotransferase (*sEx17* in all other PCR reactions with the neomycin primers showed the presence of the PCR product). As mentioned previously in regard to PCR experiments of deficiency homozygotes, both positive and negative control PCR experiments were done.

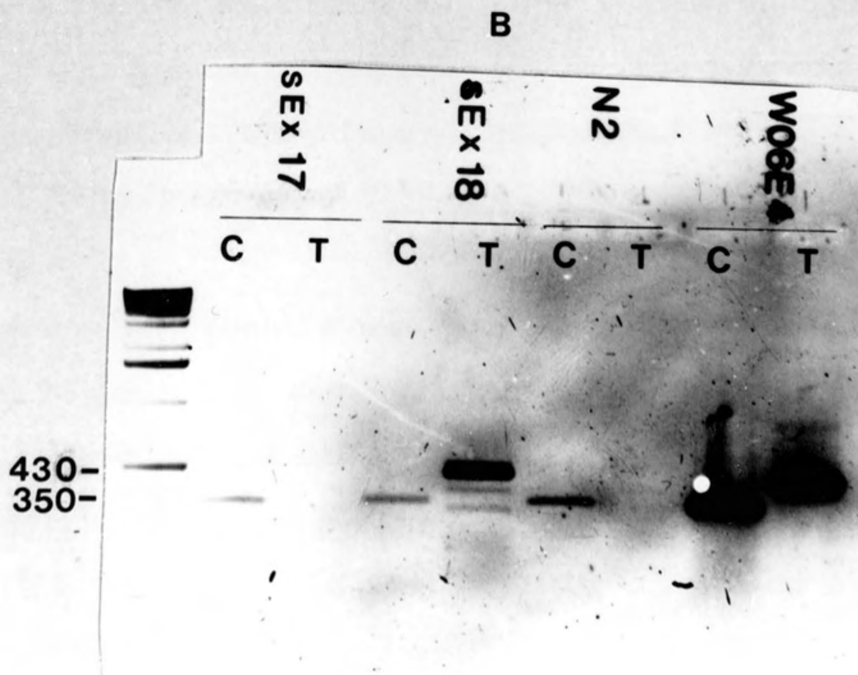
Figure 9 PCR Products From Transformed Strains Using Neomycin Primers

Panels A and B show ethidium stained gels of PCR products of transformed strains using primers designed from the neomycin phosphotransferase gene. The T lanes are test reactions performed with the primers designed from the neomycin phosphotransferase gene. The experimental band can be seen at 430 bp. The C lanes are control reactions using primers designed from the cosmid W06E4 with the product seen at 350 bp. The 1 Kb DNA Ladder was used as a DNA size marker. Panel A shows the experimental band in the T lanes of *sEx6, sEx11* and *sEx12*. Panel B shows the experimental band in the T lanes of *sEx18* and the control W06E4.

A



B



Discussion

PCR mapping of nine primer sites

PCR amplification of deficiencies proved to be a very informative and accurate method for demonstrating the alignment of the genetic and physical maps of LGV(left). Nine cosmid or YAC sequences were placed from the physical map to the genetic map (Figures 2 and 3). The PCR products were not sequenced to verify the amplification product. Although some of data from the PCR experiments show conflicting map positions, genetic map positions were often assigned based upon the work of others from the *C. elegans* community. Specifically, Eric Lambie mapped three primer sites in the LGV(left) region (personal communication). The contig configuration from ACEDB, the *C. elegans* genome database (R. Durbin and J. Thierry-Mieg unpublished) also allowed for the positioning of some of our sites which showed incongruous results.

One of the conflicts was the anomalous PCR result from the T03F8 cosmid-derived primers. There was a PCR product for *sDf75* but no product for *sDf33* which is conflicting with the other data presented in Table 3. The data was most easily explained if one assumed that the *sDf33* data was incorrect; therefore, T03F8 was placed between the right breakpoints of *sDf75* and *sDf45* in zone 5-6A on the genetic map (Figure 3).

Eric Lambie (personal communication) placed Y45G12, T18A6 and ZK230 primer sites on the genetic map. This helped to align the position of the F12B4 cosmid primer sites. The cosmid F12B4 overlaps with T18A6 for about 5 Kb and it is assumed that *let-347* lies between T18A6 and F12B4. The cosmids F12B4 and T18A6 cover the zone 6B. The left breakpoints of *sDf27, sDf50* and *sDf70* must be contained in one or both of these cosmids.

W06E4, Y45G12 and F12B4 all lie in zones 6A, an area where very few genes have been identified genetically. The actual physical distance between the right breakpoint of *sDf45* and the left breakpoint of *sDf50* is not known, so the amount of DNA in this area is difficult to estimate.

The map positions of W03A10 and F46A7 are tentative. The PCR results suggest that the primer sites from F46A7 should lie to the left of W03A10 because of the *sDf50* result. However, the physical map shows F46A7 to the right of W03A10 separated by approximately 18 cosmids. Therefore the *sDf50* result was considered incorrect and the W03A10 primer sites appear to lie between the right breakpoint of *sDf28* and the left breakpoint of *nDf32* corresponding to zones 8A2-9A. This is a region between the genes *let-338* and *let-344* on the genetic map.

The position of W06G11 on the map was based upon the PCR experiments performed with the W06G11 derived primers and also another set of primers to the left of W06G11 (a gift from Dr. R. Waterston, Washington University School of Medicine, St. Louis). The Waterston primer data (not shown) indicated that the primer sites fell just outside of the left breakpoint of *sDf30*. Therefore, even though W06G11 was not tested with deficiency homozygotes from *sDf30*, it was placed to the left of the *sDf30* breakpoint in zone 10.

T01C2 primers mapped between the right breakpoints of *nDf18* and *nDf32* in zone 14 where at least 5 genes lie.

The PCR results for the position of the YAC Y53A1 placed it either between the right breakpoint of *sDf30* and the left breakpoint of *sDf35* or to the right of the right breakpoint of *sDf35*. However, the physical map position of *her-1* made it impossible for Y53A1 to be to the right of *her-1*. Thus we placed it into the region between *sDf30* and *sDf35* defined by zones 16.

C. elegans does not have the same amount of DNA per map unit in all areas of the genome. The *C. elegans* genome is $1x10^8$ bp and has approximately 300 map units

(Edgley and Riddle 1990; J. E. Sulston, personal communication); thus, there is an average of 333 Kb/m.u. Greenwald *et al.* (1987), Starr *et al.* (1989), Prasad and Baillie (1989) and Clark and Baillie (1992) have shown that the DNA per map unit is more in clusters of genes than in gene sparse areas. The PCR results suggest a similar correlation. The region between the cosmids F12B4 and W03A10 is a gene sparse region of LGV(left). The minimum number of map units between F12B4 and W03A10 (around *let-347* to *let-338*) is approximately 5.6 m.u. (Rosenbluth *et al.* 1988). The physical map shows approximately 20 cosmids (lying end to end) between F12B4 and W03A10. If one estimates the average size of a cosmid to be 40 Kb, the physical distance is 800 Kb between F12B4 and W03A10. This gives an estimate of approximately 143 Kb per map unit, approximately 0.5 times less than the average of 333 Kb/m.u. This can be compared to the maximum map units between W03A10 and F46A7 and the maximum map units between T01C2 and Y53A1; two regions more gene dense than the interval between F12B4 and W03A10. There are 18 cosmids between W03A10 and F46A7. W03A10 is in the area close to *let-338* and F46A7 maps near to *let-344* in zone 9A. The two factor recombination data indicates that the distance from *let-338* to *let-344* is 1 m.u. (Rosenbluth *et al.* 1988). This area is approximately 720 Kb/m.u., 2 times the average. T01C2 falls in zone 14 and Y53A1 lies in zone 16, the physical map distance equaling an average of 50 cosmids. The two factor mapping data for a gene in zone 14 (i.e. *unc-70*) to a gene in zone 17 (i.e. *let-413*) is 1.5 m.u. (Rosenbluth *et al.* 1988). Therefore, there is 1,333 Kb/m.u. in this interval, four times the average amount for *C. elegans*. The PCR data clearly show that the regions near the *dpy-11* gene cluster on LGV(left) contain more DNA/m.u. compared to gene sparse areas.

Germline transformation generated duplications

Germline transformation was another method used for correlating the genetic and physical maps on LGV(left). The transgenic strains contained pieces of genomic DNA as extrachromosomal arrays (Mello *et al.* 1991). Cosmids were injected from the region surrounding *unc-60* in an attempt to create duplications of the *unc-60* region. These duplications in turn were used for the complementation tests with genetically defined genes.

The duplication *sEx9* (containing cosmid C32E5) was able to rescue the Unc-60 phenotype (see Results). No essential genes were rescued with the duplications *sEx7, sEx8, sEx11, sEx12, sEx17* or *sEx18*. This might have occurred for a number of reasons. It is most likely that the cosmid duplications did not contain the gene of interest in its entirety or that a point mutation had occurred in the gene. There may have also been sequences present which suppressed or altered the expression of the gene. In addition, the number of copies of the wild-type gene in the duplication may have been vastly inappropriate for expression. Germline transformation is being used by others for the correlation of the genetic and physical maps. J. McDowell (personal communication) is using a similar injection strategy to rescue essential genes from LGI.

PCR confirmation of transformed strains

Mello *et al.* (1991) reported that the extrachromosomal arrays formed during injection consisted of the marker Rol-6 and the DNA coinjected with it. A stably transmitted array has been shown to require at least a size of 600 kb. The process of array formation occurs by homologous recombination. If the coinjected sequences are not homologous to the *rol-6* plasmid pRF4, the test DNA may not be assembled into the concatamer. Most plasmid vectors contain the ampicillin resistance gene and therefore have an area of homology to pRF4.

All cosmids used in this study which have vectors containing the neomycin phosphotransferase gene, are non-homologous to pRF4. However, by increasing the amount of test DNA in relation to the amount of pRF4 injected, the coassembly of DNA of both types into arrays is favored (Mello *et al.* 1991). Moreover, injecting at least one cosmid with homology to pRF4 (i.e. cosmids with a vector containing ampicillin resistance) and then adding overlapping cosmids (which may or may not have homologous sequences to pRF4) should facilitate the recombination of all the injected sequences into an array. This could be tested by Southern analysis, PCR analysis and sequencing of the arrays.

It was necessary to confirm that the cosmid or plasmid DNAs injected were present in the strains selected as Rollers. In the absence of a rescue of a gene there was no method to select for the cosmid or plasmid DNA present as extrachromosomal arrays. Figure 9 shows the results of the PCR amplification of the neomycin phosphotransferase gene in the transformed stains. Primers were designed from the neomycin phosphotransferase gene because it is not present in wild-type nematodes but is characteristic of all cosmid vectors conferring kanamycin resistance (Cross and Little 1986; Gibson *et al.* 1987a; 1987b; Little and Cross 1985). We had difficulty obtaining a product from the neomycin primers when control primers were present, presumably due to either the interaction of the two primer pairs or competition of the primers for the reaction components. Therefore, each reaction contained either the experimental primers or the control primers from the cosmid W06E4 [from LGV(left)]. As presented in Results, most of the stable duplications contained the neomycin phosphotransferase sequence. These results merely indicate the presence of the neomycin phosphotransferase sequences, not necessarily the entire cosmid. Moreover, Mello *et al.* (1991) and Stinchcomb *et al.* (1985) suggest that there is a chance for sequence rearrangement and/or deletion of injected DNA. Thus, in the absence of a rescue of a gene or further molecular confirmation of the presence of the DNA

injected, one is assuming that the neomycin phosphotransferase derived primers are diagnostic for the presence of the entire cosmid.

Analysis of transformed strains

The injection of the cosmids K06H5, T28A3 and F53E2 at 150 ng/ul generated the extrachromosomal array *sEx12*. The PCR product from the strain containing *sEx12* (Figure 9 Panel A) can be interpreted in a couple of ways. All of the cosmids injected, K06H5, T28A3 and F53E2 contain the neomycin phosphotransferase gene. The presence of the PCR product from *sEx12* may indicate the presence of 1, 2 or all three of the cosmids. It is impossible to distinguish between the cosmids using these primers. Southern blots of DNA from *sEx12* could be probed with sequences unique to each cosmid in order to identify the cosmids present in the *sEx12* concatamer. Additionally, primers specific for each cosmid could be designed and used in PCR experiments to answer the above question. As described in Results, the *sEx12* concatamer could not rescue the essential gene *let-426(s1527)*. The failure to rescue may have resulted from any of the reasons discussed earlier (see section above).

sEx8 was generated using the same three cosmids K06H5, T28A3 and F53E2 injected at concentrations of 50 ng/ul along with 100 ng/ul of pRF4. It however did not give a product from the PCR experiment using the neomycin primers. Prior to the PCR experiments, a complementation test was performed with the *sEx8* strain. Because *sEx8* may have contained the cosmid F53E2 which was known to rescue *unc-60*, the strain was used in a complementation test to a strain carrying *unc-60(m35)*. No rescue of the Unc-60 phenotype was observed and this was easily explained with the negative PCR result. A complementation test using *sEx12* with *unc-60* was not performed.

sEx7 and *sEx11* were strains descended from a hermaphrodite injected with the cosmid T10D1 along with pRF4. However, *sEx11* contains the neomycin

phosphotransferase sequence whereas *sEx7* does not (see Figure 9). These two strains were created independently but with the same amount of T10D1 DNA injected (50 ng/ul). As reported in Results, *sEx7* was unable to rescue *let-327(s1496), let-347(s1035), let-426(s826), let-426(s1527)* nor *emb-29(s166). sEx11* was also unable to rescue the genes *let-347(s1035)* or *let-426(s1527).*

sEx6 was able to rescue the Unc-60 phenotype and was also shown to contain the neomycin phosphotransferase sequence from PCR experiments. This provides evidence for the effectiveness of using neomycin primers to confirm the presence of an entire cosmid whose vector contains the neomycin phosphotransferase gene. *sEx9* also demonstrated rescue of Unc-60 but was not tested for neomycin phosphotransferase sequences using PCR. Unc-60 was rescued by mating *sEx9* to a strain carrying the *unc-60(m35)* allele.

The arrays *sEx17* and *sEx18* both were shown to have the neomycin phosphotransferase sequences. They both failed to rescue the *let-426(s1527)* allele. The PCR mapping on LGV(left) suggests that the cosmids between T03F8 and W06E4 would be more likely to rescue Let-426.

Other methods for correlating the genetic and physical maps could have been used in this study. One method would have been to perform Southern analysis of cosmid subclones hybridized to genomic DNA from deficiency strains. This method relies on the determination of the number of copies of the sequence corresponding to the subclone probe (one or two). The intensity of the signal on the autoradiogram suggests the number of copies. If there was one copy, it would indicate that the deficiency deletes the probe sequence and two copies would indicate the normal diploid state. This method however can be less accurate because of the difficulty interpreting densitometry scans of the autoradiogram.

SECTION II: ANALYSIS OF *UNC-60* AND *LIN-40*

Introduction

unc-60 is a gene affecting the muscle of *C. elegans*. Waterston *et al.* (1988) observed the muscle of *unc-60* mutants and found that the thin filaments were disorganized and in pools at the ends of muscle cells. Homozygous *unc-60*s have varied degrees of paralysis (Waterston *et al.* 1980). Four alleles are dystrophic, becoming more paralyzed as the nematode matures: *e677, e723, e890* (isolated by S. Brenner) and *m35* (isolated by D. Riddle). Allele *r398* is a suppressor of *unc-105* (P. Anderson personal communication) and nematodes become more motile as they mature (antidystrophy). The alleles *s1307, s1308, s1309, s1310* and *s1331* were isolated by McKim *et al.* (1988). *s1310* and *s1331* were induced on the *eT1* chromosome. The allele *s1307* is antidystrophic like *r398*. The *s1308* and *s1309* alleles show classic paralysis. K. McKim studied *unc-60* genetically (McKim *et al.* 1988) and fine structure mapped the 8 alleles, *e677, e723, e890, m35, r398, s1307, s1308,* and *s1309.* R. C. Johnsen during a screen for recessive lethal alleles on LGV(left) (Johnsen and Baillie 1991) discovered another *unc-60* allele, *s1586*. There were 11 alleles of *unc-60* when this study of the LGV(left) region was initiated. My goal in continuing the characterization of *unc-60* was to isolate new *unc-60* alleles which would help in the molecular identification of the gene.

The *lin-40* locus was originally identified with the *e2173* allele. Nematodes homozygous for *e2173* appear small, uncoordinated in movement, have an abnormal vulva and gonad and are sterile (isolated by S. Emmons and J. Hodgkin). There are currently 16 alleles of *lin-40* all of which are lethal alleles (Johnsen 1990). Interallelic complementation tests with the 16 alleles of *lin-40* demonstrated that there are 5 complementation classes (Johnsen 1990). Class A is represented with one allele, *s1506*. The B class alleles are *s1593, s1669* and *s1675*. The C class has 4 alleles, *s1053, s1611, s1634* and *s1704*. The single D class allele is *e2173*. The largest class

is E, represented by *s1916, s1345, s1351, s1352, s1358, s1360* and *s1373.* The class A allele complements B, C and D group alleles but fails to complement the E class alleles. Class B alleles complement A and C but fails to complement D and E class alleles. Class C alleles complements A and B but fails to complement D and E class alleles. Class D alleles complement A class alleles but fail to complement all other classes. Class E alleles fail to complement all classes. This is diagrammed in Figure 10.

Johnsen (1990) also examined the developmental blocking stages of the *lin-40* alleles. The homozygous class A (*s1506*) and the class E (*s1351, s1345, s1352, s1358, s1360, s1373, s1916*) hermaphrodites are blocked at early-mid larval stage. Homozygous class B (*s1669, s1675, s1593*) hermaphrodites are maternal effect lethal alleles which have protruding vulvae. The homozygous class C (*s1053, s1611, s1634, s1704*) and class D (*e2173*) hermaphrodites are sterile adults also with protruding vulvae.

In this section, I describe the isolation of a recessive *unc-60* allele and a putative deficiency of *unc-60.* Allele *s1983* is a visible allele whereas *s1986* is a putative lethal allele. I describe the terminal phenotype of *s1986* and the evidence for *s1986* being a deficiency. In addition, I describe the dominant effect of *lin-40* mutations on the mean number of self progeny produced by hermaphrodites.

Figure 10 *lin-40* Complementation Map

This map is from Johnsen (1990). It was based upon the results of interallelic complementation testing of the 16 *lin-40* alleles.

s1506

A

s1669
s1675
s1593

B

s1611
s1634
s1053
s1704

C

e2173

D

s1345 s1351 s1352 s1358 s1360 s1373 s1916

E

Materials and Methods

Mutagenesis with formaldehyde

Formaldehyde mutagenesis was performed as described by Johnsen and Baillie (1988) and Moerman and Baillie (1981). A formaldehyde solution was prepared by warming 5 g of paraformaldehyde (Fisher #T353) in 50 ml of 65^0C distilled water and then adding NaOH to clear the solution. HCl was used to adjust the solution to pH 7.2. This solution was diluted to 500 ml by adding M9 buffer (Brenner 1974) to give a 1% [w/v] solution. This solution was further diluted with M9 buffer to give a 0.11% solution.

dpy-18(e364)/unc-60(s1331) eT1(III);+/let-500(s2165) eT1(V) (BC3352) nematodes were rinsed off of stock plates and collected by centrifugation. They were then resuspended in the 0.11% formaldehyde solution at room temperature (20-23^0C) for four hours. The worms were aliquoted with a Pasteur pipette onto a unseeded Petri plate where they remained for two hours. One to three young adult wild-type worms were collected and placed onto seeded Petri plates. After three days F_1 progeny were screened for Unc-60s. Any paralyzed or Unc nematode was considered a candidate for an Unc-60 and was picked for further observations.

Gamma radiation mutagenesis

dpy-18(e364)/unc-60(s1331) eT1(III);+/let-500(s2165) eT1(V) (BC3352) nematodes were mutated with 1500 R of gamma rays as recommended by Rosenbluth *et al.* (1985). Nematodes were rinsed off of stock plates into a 15 ml centrifuge tube and washed three times with M9 buffer. The nematodes were spotted onto Petri plates for irradiation. The radiation was performed using a ^{60}Co radiation unit (Gammacell 200, Atomic Energy of Canada Ltd.). Immediately after treatment, the nematodes were

transferred to a fresh Petri plate for 30 minutes and then they were transferred to fresh plates for subsequent screening of Unc-60s (as described above).

Complementation Tests of new *unc-60* alleles

The *unc-60* alleles *s1983* and *s1986* were mated to male stocks containing either the *unc-60(m35)* or the *unc-60(r398)* alleles as follows: *dpy-18(e364)/eT1(III);unc-60(x)/let-500(s2165)eT1(V)* hermaphrodites mated to *+/dpy-11(e224) unc-60(m35)(V)* and *+/unc-60(r398)(V)* males. The F_1 progeny were screened for Unc-60s. If Unc-60s were seen, it indicated that the new *unc-60* allele failed to complement the tested allele and was therefore a true allele of *unc-60.*

Other complementation tests were performed with the allele *s1986* because it was thought to be a deficiency (see Results). *dpy-18(e364)/eT1(III);unc-60(s1986)/let-500(s2165)eT1(V)* (BC3393) hermaphrodites were mated to male deficiency strains *dpy-18(e364)/eT1(III);unc-46(e177) sDfx/eT1(V).* The F_1 progeny were screened for Dpys. If *unc-60(s1986)* complements *sDfx* then Dpys will be present and thus *s1986* is not deleted by the deficiency. In a similar manner, BC3393 was mated to a strain containing *let-347(s1035)* and the F_1 progeny screened for Dpys. The presence of Dpys in this instance would confirmed that the *s1986* allele was not an allele of *let-347* or that *s1986* did not delete some or all of the *let-347* gene.

Terminal phenotype of *unc-60(s1986)*

Nematodes of the genotype *dpy-18(e364)/eT1(III);unc-60(s1986)/let-500(s2165) eT1(V)* (BC3393) were mated to *dpy-18(e364)/eT1(III);unc-46(e177)/let-500(s2165) eT1(V)* (BC3952) males. Five F_1 Dpys were picked and set on Petri plates to lay eggs for 6-12 hours. Thereafter, the Dpys were transferred to fresh Petri plates for a similar time period. As the progeny matured they were removed from the Petri plates. At 4

and 7 days, the plates were examined for the arrested Dpy Unc-60 larvae and measured using a micrometer.

Egg counts of *lin-40* and deficiency strains

dpy-18(e364)/eT1(III);lin-40(x)/eT1(V) heterozygous strains were mated to wild-type (N2) males and the wild-type F_1 hermaphrodites were placed individually on Petri plates. Thirty worms were set up so that there would be at least 10 outcross progeny in the sample of 30 picked. Each twelve hours, the worms were transferred to a fresh plate and their eggs scored immediately. This continued until there were no more eggs laid. Most of the strains were also scored for all late larval and mature F_1 progeny by R. C. Johnsen (1990).

Results

Precomplementation screens for *unc-60* alleles

Eleven alleles of *unc-60* existed at the beginning of this study (Brenner 1974; Park and Horvitz 1986; McKim *et al.* 1988 and Johnsen and Baillie 1991). To facilitate the cloning of the gene, an attempt was made to generate small intragenic deficiencies. The strain *dpy-18(e364)/unc-60(s1331) eT1(III);+/let-500(s2165) eT1(V)* (BC3352) was constructed for a precomplementation screen (McKim *et al.*, 1988) using the mutagens formaldehyde and gamma rays.

dpy-18/unc-60(s1331) eT1(III);+/let-500(s2165) eT1(V) (BC3352) nematodes were mutagenized with 0.11 % formaldehyde and the F_1 progeny were screened for Unc-60 individuals. Approximately 132,000 chromosomes were screened generating a novel allele, *unc-60(s1983)* on the normal LGV chromosome. This allele is recessive and homozygous viable. Subsequent complementation tests performed with *unc-60(m35)* confirmed the identity of *s1983* as an allele of *unc-60.*

A second precomplementation screen was performed using *dpy-18(e364)/unc-60(s1331) eT1(III);+/let-500(s2165) eT1(V)* (BC3352) nematodes mutagenized with 1500R gamma rays. Approximately 85,000 chromosomes were screened for *unc-60* mutations. The one putative *unc-60* allele generated from the screen, *s1986* is lethal in a homozygous state. *s1986* was complementation tested with *unc-60(m35)* and *unc-60(r398)* but no Unc-60s were seen in the F_1 generation (see Discussion). *s1986* complements *sDf50, sDf32, sDf42, sDf39* and *let-326(s1404)*. The terminal phenotype of *s1986* was examined. The Dpy18 Unc60 nematodes hatch and move about but arrest shortly thereafter.

Brood sizes of *lin-40* and deficiency strains

Some heterozygous strains bearing *lin-40* alleles had a smaller or larger brood size compared to wild-type (N2) and *+/dpy-18;+/unc-46* nematodes (Johnsen 1990). Because all of the *lin-40* alleles are lethal alleles, it is difficult to score developmentally arrested larvae. Therefore the number of eggs laid was used to determine the self-fertilization brood sizes of strains carrying *lin-40* alleles. Table 5 lists the deficiencies and *lin-40* alleles used in the analysis. The total number of eggs laid were scored from strains containing most of the *lin-40* alleles. Heterozygotes of the genotypes *dpy-18(e364)/eT1(III);lin-40(x) unc-46(e177)/eT1(V)* were outcrossed to N2 males and wild-types of the genotype *+/eT1(III);+/eT1(V)* or *+/dpy-18(e364);+/lin-40(x) unc-46(e177)* were picked in the F_1. The F_1 nematodes were allowed to lay eggs for 12 hours then transferred to a fresh Petri plate. The eggs were counted before hatching. As the progeny matured, plates containing *eT1* nematodes were discarded as these were not the outcross progeny. The strains bearing the *lin-40* alleles also contained the two mutations *dpy-18(e364)* and *unc-46(e177)*; therefore, the brood size of the strain *+/dpy-18;+/unc-46* was used as a control.

The results of the egg count for the *lin-40* alleles is shown in Table 5. To determine if a brood size was significantly different from the control strain, T-tests were performed. The effect of individual alleles and the effect of an entire class of alleles were used for comparison to the average mean brood size of *+/dpy-18;+/unc-46* nematodes.

The class C alleles show a significant decrease in brood size and the class D allele shows a significant increase in brood size compared to the control. Neither class A nor E showed any difference compared to *+/dpy-18;+/unc-46*. The B class was unusual because the individual alleles did not demonstrate a significant effect on brood size but the combined class B data did exhibit a larger brood size. Heterozygous

deficiency strains *sDf42, sDf50* and *sDf27* which all delete *lin-40* (see map) also were shown to have a dominant effect of increasing the mean number of self-fertilization progeny size.

Table 5 Brood Sizes of *lin-40* and Deficiency Strains

Strain	Class	No. of Broods	Average Size	Significantly* larger than *dpy-18;unc-46*	Significantly* smaller than *dpy-18;unc-46*
dpy-18;unc-46		18	343		
N2(wild type)		10	340	no	no
sDf27		19	416	yes	
sDf34		10	275		yes
sDf42		11	363	yes	
SDf50		8	377	yes	
sDf70		12	343		no
lin-40(s1506)	A	15	345	no	no
lin-40(s1669)	B	16	356	no	
lin-40(s1675)	B	21	359	no	
combined B class		37	358	yes	
lin-40(s1053)	C	12	313		yes
lin-40(s1634)	C	22	302		yes
lin-40(s1704)	C	4	308		yes
combined C class		38	306		yes
lin-40(e2173)	D	20	419	yes	
lin-40(s1351)	E	19	333	no	no
lin-40(s1358)	E	4	317	no	no
lin-40(s1360)	E	10	351	no	no
lin-40(s1373)	E	8	334	no	no
lin-40(s1916)	E	5	349	no	no
combined E class		46	337	no	no

* T-test (95% confidence)

Discussion

Molecular and Genetic Characteristics of *unc-60*

Formaldehyde and gamma rays were chosen as mutagens because of their demonstrated ability to induce rearrangements in *C. elegans* chromosomes (Johnsen and Baillie 1988; Rosenbluth *et al.* 1985). Small internal deficiencies in the unc-60 gene could facilitate the molecular isolation of the gene. The deletion might be detectable as a restriction fragment polymorphism and could then be used as a molecular tag to isolate the gene. The method of precomplementation was chosen to isolate new *unc-60* alleles (McKim *et al.* 1988). Other screening methods would not have allowed the easy separation and identity of new *unc-60* alleles. General screens for uncoordinated or paralyzed mutations would have turned up many different types of *unc* mutations. McKim *et al.* (1988) made an *eT1* chromosome that carried an *unc-60* mutation, *s1331* which was suited for the precomplementation screen. *dpy-18(e364)/unc-60(s1331) eT1(III);+/let-500(s2165) eT1(V)* hermaphrodites were used for the gamma and formaldehyde mutagenesis. When an Unc-60 was found during screening, the new *unc-60* allele could be isolated from the *s1331* allele by replacing the *eT1(III)* chromosome.

s1986 is a putative lethal allele of *unc-60* isolated during the screening of 85,000 hermaphrodites (and therefore 85,000 chromosomes). No Unc-60s were observed in the progeny of complementation tests performed with *s1986* mated to the *unc-60* alleles *m35* or *r398*. This was surprising but may have occurred because the nematodes with the genotype *unc-60(m35)/unc-60(s1986)* or *unc-60(r398)/unc-60(s1986)* may have been too small and sickly to be observed among the wild-type progeny on the plates (a deficiency could lead to very sick nematodes). To test if *s1986* was a deficiency, further complementation tests were performed. As

reported in Results, complementation tests were performed with genes and deficiencies around *unc-60* but no allele failed to complement *s1986*. However, the genes *emb-29* and *ges-1* were not tested for complementation with *s1986*. Deficiencies which delete *unc-60* should now be tested to confirm that *s1986* is indeed an *unc-60* allele.

Sequence analysis of *unc-60* by K. McKim and C. Matheson in Dr. D. L. Baillie's laboratory (unpublished results) showed that the lethal allele *s1586* was a 500 bp deletion. The terminal phenotype of the allele *s1586* (Johnsen and Baillie 1991) demonstrated early larval lethality. This terminal phenotype is very characteristic of deletions in the chromosome (Johnsen and Baillie 1988; Stewart *et al.* 1991). It seems likely that *s1986* is a deletion because it also has a terminal phenotype of early larval lethality. However, further molecular and genetic data are needed to confirm the nature of the *s1986* allele.

The other *unc-60* allele isolated was *s1983*. 132,000 chromosomes were screened in total after formaldehyde mutagenesis. *s1983* is a recessive allele which has classic paralysis. It should now be fine structure mapped with the other alleles.

To expedite the molecular analysis of *unc-60*, microinjection experiments were performed. The rescue of a gene by germline transformation is a good confirmation of the genes identity. *ges-1* had been positioned on the physical map of overlapping cosmid and YAC clones and it mapped within 1 m.u. of *unc-60* on the genetic map (Figure 7). Therefore, cosmids were obtained from the *ges-1* region from A. R. Coulson and J. E. Sulston (MRC, Cambridge, England). The cosmid F53E2 rescued the *m35* and *s1331* alleles. The subsequent rescue of *unc-60(m35)* with the cosmid C32E5 allowed for the isolation and molecular analysis of the *unc-60* gene. K. McKim and C. Matheson identified the coding regions of the gene and sequenced them (unpublished results). They have identified *unc-60* as a cofilin-destrin like protein. Subsequent injections of subclones (provided by K. McKim and C. Matheson) of the F53E2 cosmid were unable to rescue the Unc-60 phenotype (data not shown).

lin-40 dominantly affects brood sizes

The *lin-40* locus has been defined by five complementation classes A, B, C, D and E (Johnsen 1990). The complementation map (Figure 10, Introduction) is a graphic representation the of interallelic complementations. The *lin-40* alleles used in this study were generated by a variety of mutagens. 0.12 M EMS generated *s1506* (class A), *s1593* (class B), *s1053, s1611, s1634, s1704* (class C) and *e2173* (class D). EMS generated alleles in *C. elegans* are generally G:C to A:T transitions (Dibb *et al.* 1985). 0.11% formaldehyde was used to isolate *s1669* and *s1675* (class B). Johnsen and Baillie (1988) demonstrated that 0.1% formaldehyde induced mutations are putative point mutations, deletions, or other complex lesions. *s1916* (class E) was induced by 120 J/m^2 UV light (Stewart *et al.* 1991). UV light mutagenesis was demonstrated to induce primarily chromosomal rearrangements and some putative point mutations (Stewart *et al.* 1991). The remaining class E alleles *s1345, s1351, s1352, s1358, s1360* and *s1373* were generated by mobilization of the transposable element Tc1 (Clark *et al.* 1988). Tc1 mutagenesis can cause point mutations and chromosomal rearrangements (Clark *et al.* 1988).

Wild-type XX hermaphrodites produce approximately 327 sperm and then switch to oocyte production (Hodgkin and Barnes 1991). The number of progeny generated from self-fertilization is limited by the number of sperm produced. *tra-2* (Hodgkin 1988) and *tra-3* (Lewis *et al* 1980; Hodgkin 1985) have been shown to cause a dominant increase in the mean number of self-progeny from XX hermaphrodites. Some alleles of *fem-3* (Barton *et al.* 1987) cause a decrease in the brood size of XX hermaphrodites. Dominant effects of reducing brood sizes has been observed for deletions of more than one gene (Johnsen and Baillie 1988; Stewart *et al.* 1991). The *tra-2, tra-3* and *fem-3* mutations all affect the level of hermaphrodite spermatogenesis

without a measurable effect on somatic sexual development (Hodgkin and Barnes 1991).

R. E. Rosenbluth and R. C. Johnsen (personal communication) noticed that the strains containing *sDf27, sDf42* and *sDf50* had a larger self-fertilization brood size compared to other *eT1* balanced heterozygotes. This was surprising since *sDf27* deletes a large number of genes. *sDf27, sDf42* and *sDf50* delete the same essential genes *let-347, let-461, let-330* and *lin-40*. However, the deficiency strains *sDf70* and *sDf34* did not show an increased brood size. This implied that the genes *let-347, let-461* and *let-330* contained in the region of overlap of the 5 deficiencies (*sDf70, sDf34, sDf27, sDf42* and *sDf50*) could not be responsible for the increase in brood size. This left *lin-40* as the only identified candidate gene that could be responsible for this effect.

12 of the 16 *lin-40* recessive alleles were examined for their dominant effect on hermaphrodite self-fertilization brood size (see Table 5 and Results). The results suggest that *lin-40* has a dominant effect on the number of self-fertilization progeny produced in the nematode. *lin-40* is therefore likely to be involved in the switch from spermatogenesis to oogenesis. T-tests were applied to the brood size data to determine if the differences were statistically significant within a 95% confidence level. Only the B and D classes of alleles demonstrated a large brood size compared to the control strain *+/dpy-18(e364);+/unc-46(e177)* nematodes. The combined B class results are statistically significant even though the two B class alleles on their own are not significantly different from the control. It was expected that the class E alleles would have shown a dominant effect of increasing brood size because they are putative null alleles. The E class has the greatest number of alleles which is common for null alleles. Moreover, Tc1 insertional mutagenesis often causes disruption of a gene and the concomitant loss of gene function (Emmons *et al.* 1983). However, deficiencies which delete *lin-40* do cause an increase in self-fertilization brood size. This implies that the class E alleles may in fact not be null alleles. Possibly, all of the Tc1 alleles

were the result of the same mutagenesis event (i.e. a hot spot for Tc1 insertion). Thus the E class would be defined by 2 mutations, the Tc1 and the UV light alleles.

The self-fertilization brood size of *sDf70* heterozygous nematodes is similar to the control *+/dpy-18;+/unc-46*. *sDf70* does not delete *lin-40* or as many genes as *sDf27, sDf42* and *sDf50*. The deficiencies *sDf27, sDf42* and *sDf50* all have statistically significant large progeny counts and delete approximately the same number of genes including *lin-40*.

All larger and smaller self-fertilization egg counts come from strains with the sterile or maternal effect lethal phenotypes of *lin-40*. The larval lethal alleles of *lin-40* demonstrate no significant effect on progeny number. The dominant effect of the *lin-40* alleles on brood size is not likely an effect of an antimorphic or neomorphic gene product because the deletion of the gene also causes a large progeny count. The early larval lethality is also not likely the result of antimorphic alleles because antimorphs are usually rare and half of the alleles we have isolated are early blockers of development.

The interallelic complementation results of *lin-40* could be interpreted to indicate three individual genes. If the class A, B and C alleles were individual genes, the Class D and E alleles would need to be deficiencies of these genes. D would be a deficiency of 2 genes and E would delete all three. However, D and E alleles are unlikely to be deficiencies because of the frequency with which they were isolated. In addition, the similarity of phenotypes for all classes of alleles argues against them being different genes.

The *lin-40* gene may be comprised of a multi-subunit protein. The interallelic complementation could be due to the effects of a mutation located in a domain whose function could be replaced or repaired by another domain. Other genes in *C. elegans* have been shown to have a complex interallelic complementation pattern: *unc-15* (Rose

and Baillie 1980); *cha-1-unc-17* (Rand 1989); *bli-4* (Peters *et al.* 1991) and *srl* (W. B. Barbazuk, personal communication).

The complementation pattern of *lin-40* shows similarities to the *maroon-like* locus in *Drosophila melanogaster* (Chovnick *et al.* 1969; Finnerty *et al.* 1970; Finnerty and Chovnick 1970). *maroon-like* had 5 complementation classes and was considered to be a single gene after fine structure analysis and enzyme level analysis.

lin-40 is a very complex locus and the observations discussed here do not lead to a simple conclusion. Clearly, more study of the *lin-40* locus is necessary. Suppressors of *lin-40* and non-lethal alleles could be isolated and studied. The PCR mapping of deficiencies on LGV left near *lin-40* suggest that the cosmids between ZK230 and W03A10 could rescue Lin-40. The gene could then be located within the cosmid and isolated for sequence analysis. cDNA isolation and sequence analysis could also be done to determine the exon and intron positions. Finally, mutations of *lin-40* could be sequenced and studied for their effect on the protein function.

CONCLUSION

This study used traditional genetics and recent advances in molecular biology to examine the relationship between physical distance and recombinational distance in *C. elegans*. A region of approximately 1/12 of the total *C. elegans* genome was studied, namely the left end of LGV. Because of the previous extensive work identifying essential genes and generating deficiencies in this region, a study of the correlation of the genetic map to the physical map was possible. Moreover, the assembly of the entire genome into contigs of cloned DNA and the concomitant sequencing of the genome provided the starting points for this and other studies.

Two methods were chosen for this analysis of LGV(left); PCR and germline transformation. The PCR mapping of nine primer sites along the two major contigs on LGV(left) will allow for many more genes to be analyzed at the molecular level. In addition, the physical map will help in the identification of genes not previously identified through genetic means.

Germline transformation was used to identify the physical position of a genetically defined gene, *unc-60*. Germline transformations were also used to create duplications of small genomic areas which can help to localize the search for a particular gene. There were eight small duplications created in this study. One of them, *sEx9*, rescued the gene Unc-60 and allowed for the cloning of the gene. The isolation and characterization of two putative *unc-60* alleles was also completed.

The LGV(left) region includes the gene *lin-40*. The *lin-40* locus was examined because of its complex intracomplementation pattern and its phenotypic properties. The dominant effect of the gene on brood sizes in hermaphrodites was analyzed. Molecular characterization of the gene could now be done because of the identification of cosmids in the vicinity of *lin-40*.

REFERENCES

Barstead, R. J. and R. H. Waterston. (1991) Vinculin is essential for muscle function in the nematode. J. Cell Biol. 114: 715-724.

Barton, M. K., T. B. Schedl and J. Kimble. (1987) Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. Genetics 115: 107-119.

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

Bridges, C. B. (1935) Salivary chromosome map with a key to the banding of the chromosomes of *Drosophila melanogaster*. J. Hered. 26: 60-64.

Bridges, C. B. (1938) A revised map of the salivary gland X chromosome of *Drosophila melanogaster*. J. Hered. 29: 11-13.

Brown, S. J. (1984) Genetic interactions affecting muscle organization in the nematode *C. elegans*. Ph. D. Thesis. University of Missouri, Columbia, USA.

Burke, D. T, G. F. Carle and M. V. Olson. (1987) Cloning large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236: 806-812.

Chovnick, A., V. Finnerty, A. Schalet and P. Duck. (1969) Studies on genetic organization in higher organisms. I. Analysis of a complex gene in *Drosophila melanogaster*. Genetics 62: 145-160.

Clark, D. V. and D. L. Baillie. (1992) Genetic analysis and complementation by germline transformation of lethal mutations in the *unc-22 IV* region of *Caenorhabditis elegans*. Mol. Gen. Genet. 232: 97-105.

Clark, D. V., R. C. Johnsen, K. S. McKim and D. L. Baillie. (1990) Analysis of lethal mutations induced in a mutator strain that activates transposable elements in *Caenorhabditis elegans*. Genome 33: 109-114.

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 119: 345-353.

Clark, S. G., M. J. Stern and H. R. Horvitz. (1992) *C. elegans* cell-signalling gene *sem-5* encodes a protein with SH2 and SH3 domains. Nature 356: 340-344.

Collins, J. and B. Hohn. (1978) Cosmids: A type of plasmid gene-cloning vector that is packageable *in vitro* in bacteriophage lambda heads. Proc. Natl. Acad. Sci. USA 75: 4242-4246.

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. USA 83: 7821-7825.

Coulson, A., R. H. Waterston, J. Kiff, J. Sulston and Y. Kohara. (1988) Genome linking with yeast artificial chromosomes. Nature 335: 185-186.

Cross, S. H. and P. F. R. Little. (1986) A cosmid vector for systematic chromosome walking. Gene 49: 9-22

Dibb, N. J., D. M. Brown, J. Karn, D. G. Moerman, S. L. Bolten and R. H. Waterston. (1985) Sequence analysis of mutations that affect the synthesis, assembly and enzymatic activity of the *unc-54* myosin heavy chain of *Caenorhabditis elegans*. J. Mol. Biol. 183: 543-551.

Edgley, M. L. and D. L. Riddle. (1990) The nematode *Caenorhabditis elegans*, pp. 3.111-3.133 in *Genetic Maps Locus Maps of Complex Genomes*, Ed. 5, 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 *Caenorhabditis elegans*. Cell 32: 55-65.

Endow, S. A. and M. Hatsumi. (1991) A multimember kinesin gene family in *Drosophila*. Proc. Natl. Acad. Sci. USA 88: 4424-4427.

Finnerty, V. and A. Chovnick. (1970) Studies on genetic organization in higher organisms. Genet. Res., Camb. 15: 351-355.

Finnerty, V. G., P. Duck and A. Chovnick. (1970) Studies on genetic organization in higher organisms, II. Complementation and fine structure of the maroon-like locus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 65: 939-946.

Fire, A. (1986) Integrative transformation of *Caenorhabditis elegans*. EMBO J. 5: 2673-2680.

Gibson, T. J., A. R. Coulson, J. E. Sulston and P. F. R. Little (1987) Lorist2, a cosmid with transcriptional terminators insulating vector genes from interference by promoters within the insert: effect on DNA yield and cloned insert frequency. Gene 53: 275-281.

Gibson, T. J., A. Rosenthal and R. H. Waterston. (1987) Lorist6, a cosmid vector with *Bam*HI, *Not*I, *Sca*I and *Hind*III cloning sites and altered neomycin phosphotransferase gene expression. Gene 53: 283-286.

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*. Nucl. Acids Res. 15: 2295-2307.

Hodgkin, J. (1985) Novel nematode amber suppressors. Genetics 111: 287-310.

Hodgkin, J. (1987) A genetic analysis of the sex-determining gene, *tra-1*, in the nematode *Caenorhabditis elegans*. Genes and Development 1: 731-745.

Hodgkin, J. (1988) The nematode *Caenorhabditis elegans*. Wood, W. B. *et al.* (eds) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 243-279.

Hodgkin, J. and T. M. Barnes. (1991) More is not better: brood size and population growth in a self-fertilizing nematode. Proc. R. Soc. Lond. B. 246: 19-24.

Horvitz, H. R., S. Brenner, J. Hodgkin and R. K. Herman. (1979) A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. 175: 129-133.

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.

Howell, A. M. and A. M. Rose. (1990) Essential genes in the *hDf6* region of chromosome I in *Caenorhabditis elegans*. Genetics 126: 583-592.

Johnsen, R. C. (1990) Genetic analysis of the left half of linkage group V in *Caenorhabditis elegans*. Ph. D Thesis, Simon Fraser University, Burnaby B. C., Canada.

Johnsen, R. C. and D. L. Baillie. (1988) Formaldehyde mutagenesis of the eT1 balanced region in *Caenorhabditis elegans*. Mut. Res. 201: 137-147.

Johnsen, R. C. and D. L. Baillie. (1991) Genetic analysis of a major segment [LGV(left)] of the genome of *Caenorhabditis elegans*. Genetics 129: 735-752.

Kramer, J. M., R. P. French, E. Park and J. J. Johnson. (1990) The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. Mol. Cell Biol. 10: 2081-2089.

Little, P. F. R. and S. H. Cross. (1985) A cosmid vector that facilitates restriction enzyme mapping. Proc. Natl. Acad. Sci. USA. 85: 3159-3163.

Lewis, J.A., C.-H. Wu, H. Berg and J. H. Levine. (1980) The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. Genetics 95: 905-928.

Maniatis, T., E. F. Fritsch and J. Sambrook. (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

McDonald, H. B. and L. S. B. Goldstein. (1990) Identification and characterization of a gene encoding a kinesin-like protein in *Drosophila*. Cell 61: 991-1000.

McDowell, J. S. (1990) Essential genes in the *hDp16/hDp19* region of chromosome I in *Caenorhabditis elegans*. M. Sc. Thesis, University of British Columbia, Vancouver, British Columbia, Canada.

McKim, K. S. (1990) Analysis of chromosome I rearrangements in *Caenorhabditis elegans*. Ph. D. Thesis. University of British Columbia, Vancouver, British Columbia, Canada.

McKim, K. S., M. F. P. Heschl, R. E. Rosenbluth and D. L. Baillie. (1988) Genetic organization of the *unc-60* region in *Caenorhabditis elegans*. Genetics 118: 49-59.

Mello, C. C., J. M. Kramer, D. Stinchcomb and V. Ambros. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J 10: 3959-3970.

Merriam, J., M. Ashburner, D. L. Hartl and F. C. Kafatos. (1991) Toward cloning and mapping the genome of *Drosophila*. Science 254: 221-225.

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. and D. L. Baillie. (1981) Formaldehyde mutagenesis in *Caenorhabditis elegans*. Mut. Res. 80: 273-279.

Painter, T. S. (1933) A new method for the study of chromosome rearrangements and the plotting of chromosome maps. Science 78: 585-586.

Painter, T. S. (1934a) A new method for the study of chromosome aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. Genetics 19: 175-178.

Painter, T. S. (1934b) Salivary chromosomes and the attack on the gene. J. Hered. 25: 465-476.

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.

Peters, K., J. McDowell and A. M. Rose (1991) Mutations in the *bli-4 (I)* locus of *Caenorhabditis elegans* disrupt both adult cuticle and early larval development. Genetics 129: 95-102

Prasad, S. S., D. L. Baillie. (1989) Evolutionarily conserved coding sequences in the *dpy-20-unc-22* region of *Caenorhabditis elegans*. Genomics 5: 185-198.

Rand, J. B. (1989) Genetic analysis of the *cha-1-unc-17* complex in *Caenorhabditis*. Genetics 122: 73-80.

Rogalski, T. M., and D. L. Baillie. (1985) Genetic organization of the *unc-22 IV* gene and the adjacent region in *Caenorhabditis elegans*. Mol. Gen. Genet. 201: 409-414.

Rogalski, T. M., D. G. Moerman and D. L. Baillie. (1982) Essential genes and deficiencies in the *unc-22 IV* region of *Caenorhabditis elegans*. Genetics 102: 725-736.

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.

Rosenbluth, R. E. and D. L. Baillie. (1981) The genetic 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)*. Mut. Res. 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 1500 R 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., Camb. 52: 105-118.

Rychlik, n. and R. E. Rhoads. (1989) A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. Nucleic acids Res. 17: 8542-8551.

Rykowski, M. C., S. J. Parmelee, D. A. Agard and J. W. Sedat. (1988) Precise determination of the molecular limits of a polytene chromosome band: Regulatory sequences for the *notch* gene are in the interband. Cell 54: 461-472.

Sanger, F., S. Nicklen and A. R. Coulson. (1977) DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA 74: 5463-5468.

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 32: 365-372.

Stephens, J. C., M. L. Cavanaugh, M. I. Gradie, M. L. Mador and K. K. Kidd. (1990) Mapping the human genome: current status. Science 250: 237-244.

Sternberg, N. (1990) Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs. Proc. Natl. Acad. Sci. USA 87: 103-107.

Stewart, H. I., R. E. Rosenbluth and D. L. Baillie. (1991) Most ultraviolet irradiation induced mutation in the nematode *Caenorhabditis elegans* are chromosomal rearrangements. Mut. Res. 249: 37-54.

Stinchcomb, D. T., J. E. Shaw, S. H. Carr and D. Hirsh. (1985) Extrachromosomal DNA transformation of *Caenorhabditis elegans*. Mol. Cell. Biol. 5: 3484-3496.

Sulston, J., Z. Due, K. Thomas, R. Wilson, L. Hillier, R. Staden, N. Halloran, P. Green, J. Thierry-Mieg, L. Qui, S. Dear, A. Coulson, M. Craxton, R. Durbin, M. Berks, M. Metzstein, T. Hawkins, R. Ainscough and R. Waterston. (1992) The *C. elegans* genome sequencing project: a beginning. Nature 356: 37-41.

Waterston, R., C. Martin, M. Craxton, C. Huynh, A. Coulson, L. Hillier, R. Durbin, P. Green, R. Shownkeen, N. Halloran, M. Metzstein, T. Hawkins, R. Wilson, M. Berks, Z. Du, K. Thomas, J. Theirry-Mieg and J. Sulston. (1992) A survey of expressed genes in *Caenorhabditis elegans*. Nature Genetics 1: 114-122.

Waterston, R. H., K. C. Smith and D. G. Moerman. (1982) Genetic fine structure analysis of the myosin heavy chain gene of *unc-54* in *Caenorhabditis elegans*. J. Mol. Biol. 185: 1-15.

Way, J. C. and M. Chalfie. (1988) *mec-3*, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in *C. elegans*. Cell 54: 5-16.

Weaver, R. F. and P. W. Hedrick. (1992) Genetics. Wm. C. Brown Publishers, Dubuque, IA.

Wu, J., N. L. Carson, S. Myers, A. J. Pakstis, J. R. Kidd, C. M. Castiglione, L. Anderson, L. S. Hoyle, M. Genel, M. Verdy, C. E. Jackson, N. E. Simpson and K. K. Kidd. (1990) The genetic defect in multiple endocrine neoplasia type 2A maps next to the centromere of chromosome 10. Am. J. Hum. Genet. 46:624.