Genetic Analysis of a Major Segment [LGV(left)] of the Genome of *Caenorhabditis elegans*

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ABSTRACT

From 10,900 F_1 progeny of ethyl methanesulfonate (EMS)-mutagenized *Caenorhabditis elegans* nematodes, we isolated 194 lethal mutations on the left arm of LGV, a region balanced by the reciprocal translocation of *eT1*. The analysis of 166 of those mutations resulted in the identification of one deficiency and alleles of 78 genes including 38 new genes, thus increasing the number of identified essential genes to 101. We estimate that there are a minimum of 120 essential genes in this region, which comprises approximately 7% of the recombinational distance, although only about 4.2% of the genes, in *C. elegans*. We calculate that there are a minimum of 2850 essential genes in the genome. The left arm of LGV has two recombinational gene clusters separated by a high-recombination and/or essential gene-sparse region. One gene in this region, *let-330*, is the largest EMS target on the left arm of LGV, with twice as many alleles (16) as the next most EMS-mutable genes, *let-332* and *rol-3*. Another gene in the sparse region, *lin-40*, and the region near *lin-40* are major targets for Tc1 mobilization-induced mutagenesis. The analysis of essential genes in large regions should help to define *C. elegans* in terms of all its genes and aid in the understanding of the relationship of genome structure to genome function.

WE have attempted to genetically identify and localize the majority of essential genes in a large region of the *Caenorhabditis elegans* genome: the 23 map units (m.u.) that are balanced by the reciprocal translocation *eT1(III;V)* (ROSENBLUTH and BAILLIE 1981) in the left arm of linkage group V [LGV(left)]. Recombinationally, it is the largest region in any higher eukaryote for which such an attempt has been made. The region represents about 7% of the *C. elegans* genetic map.

There are two motives for this work. The first is based on the ultimate goal of many investigators to define *C. elegans* in terms of all its genes. A genomic cosmid-Yac contig map of *C. elegans* is nearing completion (COULSON *et al.* 1986, 1988) and the systematic sequencing of the entire *C. elegans* genome has begun (A. COULSON, J. E. SULSTON and R. H. WATERSTON, personal communication). While the sequencing data may allow for the identification of all genes, it may not yield much information about their biological functions. The existence of genetic mutations would supply functional information. Essential genes constitute the largest class of genes for which mutant alleles causing discontinuous phenotypes can be detected by clear-cut Mendelian ratios in genetic crosses. Thus, a way to obtain mutations in a large number of genes is to screen the genome, region by region, for lethal mutations. By dividing the resulting mutations into complementation groups and characterizing their phenotypes, and then combining this information with the sequence information, functions for a large class of genes can be deduced. Regions other than LGV(left) that are being studied include: LGI(left) (ROSE and BAILLIE 1980; HOWELL and ROSE 1990; MCKIM and ROSE 1990); LGII(right) (SIGURDSON, SPANIER and HERMAN 1984); the *unc-22(IV)* region (ROGALSKI, MOERMAN and BAILLIE 1982; ROGALSKI and BAILLIE 1985; CLARK 1990); and the duplication *mnDp1* balanced region of LGX (MENEELY and HERMAN 1979, 1981).

The second motive for this study is our interest in the relationship of genome structure to genome function. In this regard, genetic analysis of most genes in a large chromosomal region would address several problems. Genes are not distributed evenly along the chromosomes of *C. elegans* (BRENNER 1974) (for the *C. elegans* genetic map see EDGLEY and RIDDLE 1990). Identification of most essential genes would more precisely delineate gene clusters from the gene sparse regions. Furthermore, results from a few small regions in which the coding elements have been identified and that are of known length, both physically and recombinationally, suggest that gene spacing along the chromosome and recombination distances do not have a strong correlation (GREENWALD *et al.* 1987; PRASAD and BAILLIE 1989; STARR *et al.* 1989). That

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is, recombination per unit length of DNA is not constant. Genetic mapping of most genes in a given region would aid in aligning the physical contig map with the genetic map and thus identify regions of relative low and high rates of recombination for further analysis. Finally, we have only a vague idea of the location of structural features governing chromosome behavior, such as meiotic pairing. Mapped genes would serve as landmarks for their localization.

Recombination of LGV(left) is efficiently balanced by *eT1(III;V)*, which also balances the right arm of linkage group III [LGIII(right)] (ROSENBLUTH and BAILLIE 1981). The region has been subdivided into zones by sets of overlapping rearrangements (mostly deficiencies) (JOHNSEN and BAILLIE 1988; ROSENBLUTH *et al.* 1988; CLARK *et al.* 1990; STEWART, ROSENBLUTH and BAILLIE 1991). Prior to this paper, 17 putative nonessential genes (under laboratory conditions) and 63 essential genes had been identified on LGV(left) (JOHNSEN and BAILLIE 1988; ROSENBLUTH *et al.* 1988; CLARK *et al.* 1990; EDGLEY and RIDDLE 1990; STEWART, ROSENBLUTH and BAILLIE 1991).

Mutations in these genes had been isolated by a variety of screening procedures. The largest class of mutations were those recovered by screening over the entire *eT1* balanced region after treatment with ethyl methanesulfonate (EMS). Our intent in this study was to increase the number of lethal mutations using EMS mutagenesis so that the majority (at least two-thirds) of all essential genes on LGV(left) would be identified. Based on calculations in ROSENBLUTH *et al.* (1988), we estimated that screening 10,000 mutagenized chromosomes would identify over two-thirds of the essential genes.

We have screened 10,900 *eT1* balanced chromosomes treated with EMS and isolated 751 recessive lethal [including sterile adult and some *mel* (maternal effect lethal)] mutations in or close to the balanced region. We compared the number of lethal mutations that mapped to the two *eT1* balanced chromosomes. We used complementation tests to map the lethal mutations on LGV(left) against a set of deficiencies that divide LGV(left) into zones. Once the lethal mutations were mapped to zones, we used complementation tests to discern if the lethal mutations identified new genes or were alleles of known genes. We noted the approximate developmental blocking stages for most lethals at 20° and tested to see if any of those mutations were temperature sensitive (*ts*) with permissive temperatures of 15° or 25°. We also tested the sterile adults and *mel*s for putative sperm or production-of-sperm defects by mating them with wild-type males.

MATERIALS AND METHODS

General: The nomenclature follows the uniform system adopted for *C. elegans* (HORVITZ *et al.* 1979). The nematodes were cultured in Petri dishes on a simple agar nematode growth medium streaked with *Escherichia coli* (OP50). For details of this as well as procedures for observing and handling the worms see BRENNER (1974).

Mutations: The wild-type *C. elegans* (var. Bristol) N2 strain and strains carrying the following mutations were obtained from the MRC, Cambridge, England, from the Caenorhabditis Genetics Center at the University of Missouri, Columbia, Missouri, or as cited: *dpy-11(e224)V, dpy-18(e364)III, emb-29(g52)V, lin-40(e2173)V* [isolated by S. W. EMMONS, was supplied by J. HODGKIN (MRC Cambridge)], *rol-3(e754)V, unc-23(e25)V, unc-34(e566)V, unc-42(e270)V, unc-46(e177)V, unc-60(e677* and *m35)V, unc-62(e644)V, unc-68(e540)V, unc-70(e524)V, unc-76(e911)V, eT1(III;V)*. The deficiencies *mDf1* and *mDf3* (BROWN 1984) were from D. L. RIDDLE's laboratory (Columbia, Missouri). *nDf32* (PARK and HORVITZ 1986b), *nDf18* and *nDf31* originated in R. H. HORVITZ's laboratory (MIT). All mutations denoted with the *s* prefix arose in this laboratory.

Characteristics of *eT1(III;V)*: *eT1(III;V)* is a reciprocal translocation that recombinationally balances the right half of LGIII and the left half of LGV. A total of about 43 m.u. (14% of the genome) is balanced (ROSENBLUTH and BAILLIE 1981). The balanced regions of each chromosome are approximately the same size recombinationally. On LGV, recombination in eT1 heterozygotes appears to be completely suppressed from the left end to a region between *dpy-11* (zone 13) (see Figure 1 and Figure 2 for locations of zones) and *unc-42* (zone 21) near the center of the chromosome. Ten sixteenths of the progeny of *+/eT1(III);+/eT1(V)* hermaphrodites stop maturing or are blocked early in development, this is considered to be the result of those animals having aneuploid genomes. Because of the aneuploidy and no crossing-over between markers and eT1 breakpoints, markers on LGIII(right) and LGV(left) are pseudolinked. The breakpoint of *eT1* on LGIII had been mapped close to and may be within *unc-36*.

BC2200, the strain used for EMS mutagenesis: Hermaphrodites from a homozygous *dpy-18;unc-46* strain were mated to *dpy-18/eT1;unc-46/eT1* males. Wild-type male offspring were mated to homozygous *eT1* hermaphrodites. One individual wild-type hermaphrodite from the progeny of the latter cross was used to establish the strain BC2200. The genotype is *dpy-18(e364)/eT1(III);unc-46(e177)/eT1(V)*. Due to the early death of the aneuploids and due to the fact that *dpy-18* and *unc-46* are in the balanced regions, hermaphrodites with this genotype give rise to progeny with a phenotypic ratio of one Dpy Unc:four wild-type:one Unc-36.

Definition of lethal, sterile and *mel* (maternal effect lethal) mutations: A recessive lethal mutation is one that when homozygous causes the worm not to mature (egg lethal, hatches, early-, mid- or late-larval lethal); or to mature but not produce fertilized eggs (sterile); or to produce fertilized eggs that do not mature (*mel* mutations). Second generation *mel*s are also considered to be lethal mutations.

EMS mutagenesis: Mutagenesis of BC2200 was done according to BRENNER (1974) except that the dose was decreased to 0.012 M EMS on the basis of the dose-response curve of ROSENBLUTH, CUDDEFORD and BAILLIE (1983). Mutagenesis was done at room temperature (approximately 21–23°) for 4 hr.

Screening for mutations: After mutagenesis, egg bearing young adult P_os were allowed to recover for two hours and then wild-type hermaphrodites were individually plated and left to lay eggs for 21 hr ("A" brood) at room temperature, then 45 hr at 15° on fresh plates ("B" Brood), after which the P_os were discarded. Wild-type F_1s were picked individually (for every P_o used, all F_1s were picked). The F_1's

progeny were screened for the absence of healthy egg-bearing Dpy Uncs (lethal, sterile and some *mel* mutations were picked up). The absence of fertile Dpy Unc F_2s indicated the presence of a lethal mutation in the *eT1* balanced regions of LGIII or LGV (or outside but close to the balanced region).

Mapping mutations to LGIII or LGV: Replacing the balancer (*eT1*) with wild-type chromosomes allows one to map the lethal to a chromosome and to calculate its distance from the appropriate marker [*dpy-18(III) or unc-46(V)*]. The lethal bearing strains were crossed to N2 males and several wild-type L4 F_1 hermaphrodites were picked. Any F_1s that gave Unc-36 progeny were discarded. If the progeny of lethal bearing *dpy-18/+;unc-46/+* were in an approximate three wild-type:one Dpy-18 ratio then the lethal mutation was on LGV(left) and the strain was retained. Otherwise the strain was discarded (*i.e.*, strains with mutations on only LGIII or on both LGIII and LGV were discarded).

Mapping to location on LGV(left): LGV(left) has been divided into zones by a set of rearrangement breakpoints, mostly deficiencies (ROSENBLUTH *et al.* 1988). A subset of these deficiencies (*sDf26*, *sDf30*, *sDf33*, *sDf34* and *sDf35*) uncover all of the region except for the zone between *sDf30* and *sDf35* (see Figure 1). All of the above deficiencies were balanced over *eT1* and were in strains that also carry *dpy-18*, and all but the strain containing *sDf35* carry *unc-46*. Males containing lethal mutations on LGV(left) [*dpy-18/eT1(III);let unc-46/eT1(V)*] were crossed to the above set of deficiencies. The absence of fertile Dpy Unc (Dpy-18 for *sDf-35*) progeny indicated failure to complement. Appropriate other deficiencies were then used, where necessary (see Figures 1 and 2), to identify the zone into which each mutation falls. Once a lethal had been mapped to a zone, it was complementation tested against representative alleles of all known genes in that zone (*ges-1* (zone 4A), *mec-1* (zone 15) and *unc-83* (zone 12) were not complementation tested against lethals) and if necessary to alleles of neighbouring zones; *e.g.*, a) if the mutation was in zone 15, zone 16 mutations were also tested; b) if the mutation was in zone 17 the gamma-irradiation-induced mutations (putative deficiencies) in zone 18 were tested. The results of the complementation tests allowed for the assignment of the mutations to appropriate genes.

Developmental blocking stage: All developmental blocking stages were noted at 20°. Prior to analysis, the worms were set at 20° for at least one generation to eliminate any possible temperature dependent maternal effects. Worms containing relatively early developmental blocking recessive lethal mutations were handled as follows: *dpy-18/eT1(III);let unc-46/eT1(V)* hermaphrodites were mated to *dpy-18/eT1(III);unc-46/eT1(V)* males. One-to-three Dpy Unc F_1s were set on plates for six hours and the number of eggs (F_2s) laid were counted. The next day the F_2s were scored for the presence of unhatched eggs (putative egg lethal mutations). On the third day, the F_2s were counted and the maturing nonlethal F_2s removed. On the fourth and seventh days the homozygous lethal bearing Dpy Uncs were observed and measured with an ocular micrometer. Worms containing late-larval to *mel* mutations were handled as follows: approximately 20 *dpy-18/eT1(III);let unc-46/eT1(V)* hermaphrodites were set on plates for 6 hr. The Dpy Unc progeny were transferred to new plates for observation and measurement on the fourth and seventh days.

The homozygous lethal containing Dpy Unc worms were classified according to their lengths. Dpy Unc-46s, carrying no lethal, reach a mature length of 0.8–0.9 mm. Worms shorter than 0.2 mm were considered to have not developed beyond hatching. Worms 0.2 and 0.3 mm were called early blockers, 0.3–0.5 mm mid blockers, 0.5–0.6 mm late blockers and greater than 0.6 mm with no internal fertilized eggs were called sterile adults. Worms with internal fertilized eggs or developmentally blocked progeny were considered to be homozygous for *mel* mutations. Worms that were fertile, but developed slowly, were noted and called "slow."

Test for temperature sensitive mutations: Worms were put at either 15° or 25° for at least one generation before being tested. *dpy-18/eT1(III);let unc-46/eT1(V)* hermaphrodites were brooded at either 15° or 25° and their progeny scored for the presence of Dpy Uncs. If Dpy Uncs were present, then these were brooded individually and scored for fertility.

Test for cross-fertilization with wild-type males: Sterile adult and *mel* mutations were tested for cross-fertilization with wild-type male sperm by attempting to mate three Dpy Unc hermaphrodites homozygous for the lethal mutation, with three N2 males. The presence of wild-type male and fertile wild-type hermaphrodite progeny indicated either defective production-of-sperm or defective sperm in the hermaphrodite.

RESULTS

We screened 10,900 EMS-treated chromosomes for the presence of lethal mutations on LGIII(right) or LGV(left). We isolated 751 mutations: 242 strains contained mutations only on LGV, 451 only on LGIII and 29 strains carried mutations on both LGV and LGIII. Thus 64% of the mutations mapped to LGIII and 36% mapped to LGV. These data support our previous findings regarding the distribution between LGIII and LGV for EMS-induced lethals balanced by *eT1* (JOHNSEN, ROSENBLUTH and BAILLIE 1986). Since the two regions are recombinationally approximately equal in size, the essential gene density on LGIII(right) is twice that of LGV(left). All strains but the ones carrying mutations only on LGV were discarded.

In the screen, we expected to recover lethal mutations that were close to but not in the *eT1* balanced regions. While analyzing the 242 strains containing mutations on LGV(left), 50 (27%) mutations were lost by segregation and therefore were outside the balanced region. Two other strains proved to have two lethal mutations each within the *eT1* balanced region on LGV(left). Therefore, a total of 194 mutations in the *eT1* balanced region of LGV were uncovered. This gave a 2% forward lethal mutation rate for 0.012 M EMS in the *eT1* balanced region of LGV [this calculation includes the 29 mutations that were discarded because they were in strains that also carried lethals on LGIII(right)].

The *eT1* breakpoints on LGIII and LGV are each in a gene cluster near the center of their respective chromosomes. Assuming that the same number of mutations (50) would have been outside the *eT1* balanced region on LGIII as were lost from LGV, we calculated a forward mutation rate for EMS of 5.8% over the region balanced by *eT1*. This is in agreement with ROSENBLUTH, CUDDEFORD and BAILLIE (1983),

where the screening of 1662 chromosomes yielded a forward mutation rate of 6.6%.

Of the 10,900 chromosomes screened for the presence of lethal mutation, 4750 (43.6%) came from the A brood and 6150 (56.4%) came from the B brood. Of the 194 mutations recovered on LGV(left), 84 (43.3%) came from the A brood and 110 (56.7%) came from the B brood. Therefore there is no significant difference in the mutation rates of the early and late broods. Furthermore, no gene had more than one allele originating from any given P_o, *i.e.*, there was no evidence for premeiotic events.

We mapped 166 of the 194 lethal mutations isolated. The other 28 were difficult to work with (*i.e.*, male strains could not be established or maintained) and, therefore, were not mapped. The analysis of the 166 recessive lethal mutations yielded one deficiency (*sDf56* which deletes zones 1–4B inclusive, see Figure 1), and alleles of 78 genes including 38 newly identified essential genes and the first lethal allele of *unc-60* (zone 4A). These 38 genes, together with previously detected genes, provide a total of 101 genes with recessive lethal mutations that have now been mapped on LGV(left). The 101 essential genes plus 17 putative nonessential genes total 118 genes mapped to zones on LGV(left). The number of genes per zone average 3.3, and range from one to 12. Therefore, the majority of genes on LGV(left) have been right/left positioned with respect to each other by deficiency mapping.

All the genes that have been mapped to zone on LGV(left) are listed in Table 1. The number of EMS-induced alleles from screens over the entire eT1 balanced region and the total number of alleles for each gene are shown in columns 3 and 4, respectively. The canonical allele is the first mutation listed for each gene (column 5). Some of the results are from ROSENBLUTH *et al.* (1988).

Figure 1 and Figure 2 are genetic maps of LGV(left). The genes are shown above the line representing the chromosome; and the rearrangements [deficiencies and one duplication (*sDp30*)] whose breakpoints divide the region into zones are below the line. The zone numbers are at the bottom of the figure. Our newly identified genes have helped to distinguish some of the breakpoints. In addition, three γ-ray irradiation-induced mutations that had been described as *let-402(s992)*, *let-336(s741)* and *let-336(s521)* (ROSENBLUTH *et al.* 1988), were now shown to be deficiencies *sDf37*, *38* and *39*, respectively.

Fifty-four of the mutations analyzed by ROSENBLUTH *et al.* (1988) were recovered from screens similar to ours, that is, from screens over the entire *eT1* balanced region for EMS-induced recessive lethal mutations. Combining their mutations with ours gave 220 mutations in 89 of the 101 identified essential genes. Mutations defining the other 12 genes came either from screens over selected regions of LGV(left) (ROSENBLUTH *et al.* 1988) or from screens using mutagens that can cause rearrangements [γ-ray irradiation (ROSENBLUTH, CUDDEFORD and BAILLIE 1983) and formaldehyde (JOHNSEN and BAILLIE 1988)] or from a mutagen that has preferred sites of action [mobilization of the transposon Tc1 (CLARK *et al.* 1990)].

In Table 1, the developmental blocking stages (based on the measured lengths of the worms) of the various alleles are listed. We have changed the canonical allele assignments for some of the essential genes previously identified in this laboratory. The canonical alleles were only changed to reflect an earlier blocking recessive lethal phenotype or an EMS-induced allele of a gene previously identified only by a γ-ray irradiation-induced mutation. The developmental blocking stages of the canonical alleles range from egg lethality to slow development. The number of genes identified in each category is shown in Figure 3. Because we screened for only recessive lethal mutations, and not *mel* mutations, the *mel* class may be underrepresented. Previously ROSENBLUTH *et al.* (1988) screened EMS mutagenized *eT1* balanced chromosomes, and retained all F_1s until the Dpy Uncs were tested for fertility, thus selecting for *mel* mutations. In our screen, *mel* mutations were isolated because they caused other phenotypic effects, such as slow growth or thinness due to few eggs, and subsequently were shown to be *mel*. Because a similar fraction of *mel* mutations were isolated in our screen (6 from 10,900 mutagenized chromosomes) as were isolated by ROSENBLUTH *et al.* (1988) (2 from 2738 mutagenized chromosomes), we concluded that most of the *mel* mutations that we generated were isolated. The isolation of these *mel* mutations implies that a large fraction of *mel* mutations have phenotypic effects other than inviable progeny.

We screened our mutations on LGV(left) for temperature sensitivity by testing if any had permissive temperatures of either 15° or 25°. Worms homozygous for four mutations [*let-422(s1578)* (zone 11B5), *let-476(s1621)* (zone 13), *let-470(s1629)* (zone 20B) or *let-441(s1414)* (zone 20B)] were fertile at 15°. At 20°, *let-441(s1414)* escapes, (an escaper is defined as an occasional individual, who carried a lethal factor in an effective dose, that overcomes the crisis and develops further [HADORN (1945) cited in HADORN (1961)]) and occasionally a worm is fertile. At 15°, six of nine homozygous *let-441(s1414)* worms tested were fertile; at 25°, the worms were not healthy and none were fertile. Worms homozygous for *let-461(s1486)* (zone 7), *let-418(s1617)* (zone 11B4) and *let-442(s1430)* (zone 15) blocked development at a later stage at 15° than at either 20° or 25°, these mutants were not tested

TABLE 1

Genes mapped to zone on LGV(left)

Zone	Gene	No. of EMS-induced alleles[a]	Total No. of alleles	Mutation	Mutagen[b]	Phenotype	Comments
1A	*let-450*	0	1	*s2160*	For		Lost
1B1	*let-336*[c]	3	3	*s1413*	EMS	Early larval	
				s1420	EMS	Early larval	
				s1495	EMS	Early larval	
	let-447	1	2	*s1457*	EMS	Mel (egg lethal)	Multicell pre-lima bean period (more eggs over *Df*)
				s1654	For	Mel (egg lethal)	Multicell pre-lima bean period (more eggs over *Df*)
	let-458[c]	1	1	*s1443*	EMS	Early larval	
1B2	*let-448*	0	1	*s1363*	Tc1	Mid larval	
1B3	*let-437*[c]	1	1	*s1405*	EMS	Mid larval	Abnormal tail on hermaphrodites
1C	*let-453*[c]	1	1	*s2167*	EMS	ND	In same strain as *let-417(s1424)*
2	*let-431*	2	2	*s1044*	EMS	Sterile adult	
				s1049	EMS	Sterile adult	
	unc-34	0	3	*e566*	EMS	Unc	
4A	*ges-1*	0	6			Ges[d]	
	let-326	2	2	*s1404*	EMS	Early larval	
				s238	EMS	Mid larval	
	unc-60	1	10	*e677*	EMS	Unc	A visible *unc* allele
				m35	EMS	Unc	A visible *unc* allele
				s1586	EMS	Early larval	
4B	*emb-29*	1	5	*s819*	EMS	Egg lethal	
				g52	EMS	Egg lethal	Temperature sensitive
				s1613	EMS	Egg lethal	
				s1666	For	Egg lethal	
5	*let-426*	1	2	*s1527*	EMS	Early larval	
				s826	EMS	Mid larval	
6A	*let-327*	3	3	*s247*	EMS	Slow	Translucent, cold sensitive
				s1485	EMS	Slow	Translucent
				s1496	EMS	Slow	Morphological abnormalities, lethal at 25°
	let-478[c]	1	1	*s1620*	EMS	Early larval	Mel egg lethal over *sDf50*
6B	*let-347*	1	1	*s1035*	EMS	Late larval	
7	*let-330*	16	17	*s1425*	EMS	Early larval	
				s573	EMS	Mid	
				s1429	EMS	Early larval	Occasionally mid larval
				s1433	EMS	Mid larval	Slow developing as a heterozygote
				s1449	EMS	Mid larval	
				s1450	EMS	Early-mid larval	
				s1463	EMS	Early larval	
				s1468	EMS	Early larval	
				s1497	EMS	Early-mid larval	Sterile adult-Mel over *sDf34*
				s1515	EMS	Early larval	
				s1517	EMS	Early larval	
				s1518	EMS	Mid larval	
				s1531	EMS	Mid larval	
				s1543	EMS	Mid larval	Hypomorph? Some slow sickly progeny over *Df*
				s1583	EMS	Early-mid larval	
				s1638	EMS	Mid larval	Mel larval lethal over *let-330(s1433)*
				s1702	Spo	Mid larval	
	let-461[c]	1	3	*s1486*	EMS	Early larval	Mid larval at 15°, Sel[e] and Lag[f] alleles
8A1	*lin-40*	6	16	*e2173*	EMS	Sterile adult	Vulva protrudes
				s1053	EMS	Sterile adult	Vulva protrudes
				s1345	Tc1	Early-mid larval	
				s1351	Tc1	Early-mid larval	
				s1352	Tc1	Early-mid larval	
				s1358	Tc1	Early-mid larval	
				s1360	Tc1	Early-mid larval	
				s1373	Tc1	Early-mid larval	

TABLE 1—Continued

Zone	Gene	No. of EMS-induced alleles[a]	Total No. of alleles	Mutation	Mutagen[b]	Phenotype	Comments
				s1506	EMS	Mid larval	
				s1593	EMS	Late larval	Vulva protrudes
				s1611	EMS	Sterile adult	Vulva protrudes
				s1634	EMS	Sterile adult	Vulva protrudes
				s1669	For	Mel (egg lethal)	Vulva protrudes (no eggs laid)
				s1675	For	Mel (hatches)	Vulva protrudes (no eggs laid)
				s1704	EMS	Sterile leaky/*Df*	Vulva protrudes [in same strain as *let-403(s246)*]
				s1916	UV	Early-mid larval	
8A2	*let-338*	1	2	*s1020*	EMS	Mid larval	
				s503	γ-ray	Mid larval	
	let-455[c]	2	2	*s1447*	EMS	Early larval	
				s1511	EMS	Early larval	
	let-466[c]	2	2	*s990*	EMS	2nd generation Mel	Early larval lethal in F_3 generation
				s1063	EMS	Mel	Sterile adult in F_2 generation
9A	*let-344*	2	2	*s376*	EMS	Egg lethal	
				s1555	EMS	Mid larval	
	let-348	3	4	*s1436*	EMS	Early larval	
				s998	γ-ray	Mid larval	
				s1448	EMS	Mel (egg lethal)	Slow developing
				s1622	EMS	Early larval	
	let-430	1	1	*s1042*	EMS	Sterile adult	
	let-459[c]	2	2	*s1432*	EMS	Mid larval	
				s1615	EMS	Mel (egg lethal)	
9B	*let-341*	7	8	*s1031*	EMS	Egg lethal	
				s1415	EMS	Early larval	
				s1421	EMS	Early larval	
				s1454	EMS	Early larval	
				s1516	EMS	Hatches	
				s1534	EMS	Egg lethal	
				s1571	EMS	Early larval	
				s2118	For	ND	
	let-342	5	5	*s1442*	EMS	Early larval	Complements *sDf50*
				s1029	EMS	Mid larval	Sterile adult over *sDf50* (cross-fertility not tested)
				s1487	EMS	Early larval	Sterile adult over *sDf50* (cross-fertile)
				s1549	EMS	Early larval	Sterile adult over *sDf50* (cross-fertile)
				s1616	EMS	Mid larval	Sterile adult over *sDf50* (cross-fertility not tested)
	let-345	4	5	*s578*	EMS	Mid larval	
				s1452	EMS	Mid larval	
				s1509	EMS	Late larval	
				s1510	EMS	Mid larval	
				s1690	For	ND	
	unc-62	0	2	*e644*	EMS	Unc	Viable *unc* allele
				s472	Spo	Egg lethal	
10	*let-331*	1	1	*s427*	EMS	Mid larval	Slow, cold sensitive
	let-350	1	2	*s250*	EMS	Sterile adult	
				s2126	For	ND	
	let-415	2	3	*s1525*	EMS	Early larval	
				s129	EMS	Late larval	
				s1505	EMS	Late larval	
	let-417	2	4	*s204*	EMS	Early larval	
				s1313	EMS	ND	In same strain as *rol-3(s1030)*
				s1424	EMS	ND	In same strain as *let-453(s2167)*
				s1679	For	ND	
	let-419	3	3	*s219*	EMS	Mid larval	
				s1483	EMS	Early-mid larval	
				s1539	EMS	Early-mid larval	
	let-420	6	7	*s1046*	EMS	Sterile adult	Vulva protrudes

TABLE 1—Continued

Zone	Gene	No. of EMS-induced alleles[a]	Total No. of alleles	Mutation	Mutagen[b]	Phenotype	Comments
				s723	γ-ray	Sterile adult	Vulva protrudes
				s1058	EMS	Sterile adult	Vulva protrudes
				s1478	EMS	Sterile adult	Vulva protrudes
				s1573	EMS	Sterile adult	Vulva protrudes
				s1584	EMS	Sterile adult	Vulva protrudes
				s1603	EMS	Sterile adult	Vulva protrudes, abnormal tail
	let-428	2	2	*s1490*	EMS	Late larval	Dumpy, extrudes viscera
				s1070	EMS	Sterile adult	
	let-440[c]	5	5	*s1411*	EMS	Hatches	
				s1440	EMS	Hatches	
				s1552	EMS	Hatches-early larval	
				s1560	EMS	Hatches-early larval	
				s1589	EMS	Hatches-early larval	
	let-452[c]	1	1	*s1434*	EMS	Hatches	
	let-477[c]	1	1	*s1608*	EMS	Slow	
	let-481[c]	1	1	*s1636*	EMS	Hatches	
	unc-46	0	3	*e177*	EMS	Unc	
11A1	*let-443*[c]	1	1	*s1417*	EMS	Early larval	
11A2	*let-401*	0	1	*s193*	EMS	Mid larval	
11B1	*let-349*	2	3	*s217*	EMS	Early larval	
				s502	γ-ray	Early larval	
				s572	EMS	Late larval	Cold sensitive
11B2	*let-429*	2	2	*s584*	EMS	Sterile adult	
				s1597	EMS	Slow	Morphological abnormalities
	let-439[c]	4	4	*s1407*	EMS	Early larval	
				s1503	EMS	Early larval	
				s1522	EMS	Early larval	
				s1524	EMS	Early larval	
	let-462[c]	3	3	*s1594*	EMS	Early-mid larval	Blocks earlier over *sDf36* than over *sDf's 20, 26, 30* or *nDf32*
				s1481	EMS	Mid larval	Molting problem
				s1590	EMS	Mid larval	
	let-473[c]	1	1	*s1602*	EMS	Early larval	Trapped in old cuticle
	let-479[c]	1	1	*s1576*	EMS	Sterile adult	Lays unfertilized eggs
11B3	*let-329*	1	1	*s575*	EMS	Early larval	
	let-463[c]	1	1	*s2168*	EMS	ND	In same strain as *rol-3(s1473)*
11B4	*let-418*	1	1	*s1045*	EMS	Sterile adult/Mel	Vulva protrudes at 24°
				s1617	EMS	Sterile adult	Vulva protrudes, partial rescue at 15°
	let-421	4	4	*s1477*	EMS	Egg lethal	
				s288	EMS	Leaky	
				s1460	EMS	Mid larval	Dumpy
				s1632	EMS	Hatches	Dumpy, curls, escapers to late larval with vulva protrusion
11B5	*let-422*	4	7	*s194*	EMS	Early larval	
				s738	γ-ray	Early larval	
				s739	γ-ray	Early larval	
				s1312	EMS	ND	In same strain as *let-337(s382)*
				s1563	EMS	Early larval	
				s1578	EMS	Mid larval	Permissive temperature of 15°
				s1548	EMS	Early larval	
12	*unc-83*	0	11	*e1408*	EMS	Unc	
12A	*let-402*	1	3	*s1526*	EMS	Early larval	
				s127	EMS	Mid larval	
				s500	γ-ray	Early larval	
	let-444[c]	3	3	*s1418*	EMS	Early larval	
				s1459	EMS	Mid larval	
				s1569	EMS	Mel (egg lethal)	
12B	*let-403*	2	4	*s1482*	EMS	Early larval	
				s120	EMS	Mid-late larval	
				s246	EMS	ND	In same strain as *lin-40(s1704)*
				s498	γ-ray	Late larval	

TABLE 1—Continued

Zone	Gene	No. of EMS-induced alleles[a]	Total No. of alleles	Mutation	Mutagen[b]	Phenotype	Comments
13	*dpy-11*	0	10	*e224*	EMS	Dpy	
	let-337	4	5	*s1426*	EMS	Early larval	
				s382	EMS	ND	In same strain as *let-422(s1312)*
				s825	EMS	Mid larval	
				s1018	EMS	Mel (egg lethal)	
				s1024	EMS	Mel (egg lethal)	
	let-476[c]	1	1	*s1621*	EMS	Sterile adult	Permissive temperature of 15°
14	*let-410*	1	2	*s815*	EMS	Mid larval	
				s1565	EMS	Mid larval	
	let-469[c]	1	1	*s1582*	EMS	Sterile adult	
	let-471[c]	1	1	*s1570*	EMS	Mel (early larval)	
	let-472[c]	1	1	*s1605*	EMS	Mid larval	Morphological abnormalities
	unc-70	5	8	*e524*	EMS	Unc	
				s115	EMS	Mid larval	Most *unc-70* recessive alleles are dominantly weak Uncs
				s1406	EMS	Early larval	
				s1502	EMS	Hatches	
				s1532	EMS	Early larval	
				s1557	EMS	Hatches	
				s1639	EMS	Hatches	
15	*let-332*	8	8	*s234*	EMS	Egg lethal	
				s369	EMS	Egg lethal	
				s1021	EMS	Early larval	Escapers
				s1441	EMS	Egg lethal	
				s1464	EMS	Egg lethal	Well developed worms in eggs
				s1475	EMS	ND	
				s1498	EMS	Egg lethal	
				s1567	EMS	Egg lethal	
	let-339	3	3	*s1444*	EMS	Early larval	
				s1019	EMS	Mel	Cold sensitive
				s1469	EMS	Early-mid larval	Some egg lethals
	let-343	5	6	*s1025*	EMS	Egg lethal	
				s816	EMS	Early-mid larval	
				s1410	EMS	Early larval	
				s1428	EMS	Early-mid larval	
				s1465	EMS	Slow	Cold sensitive
				s1579	EMS	Early larval	
	let-346	6	7	*s1619*	EMS	Mid larval	
				s373	EMS	Late larval	
				s1026	EMS	Late larval	
				s1575	EMS	Mid-late larval	
				s1580	EMS	Mid-late larval	Most have protruding vulvae
				s1630	EMS	Sterile adult	
				s2166	For	ND	
	let-404	0	1	*s119*	EMS	Mid larval	
	let-425	1	1	*s385*	EMS	Sterile adult	
	let-438	0	1	*s2114*	For	ND	
	let-442[c]	3	3	*s1416*	EMS	Early-mid larval	
				s1430	EMS	Early-mid larval	Sterile adults at 15°
				s1535	EMS	Early-mid larval	
	let-468[c]	1	1	*s1533*	EMS	Early larval	
	mec-1	0	50			Mec	
	unc-68	0	8	*e540*	EMS	Unc	
16	*let-335*	6	6	*s1439*	EMS	Early larval	
				s232	EMS	Mid larval	
				s1412	EMS	Late larval	
				s1476	EMS	Early larval	Tends to coil
				s1520	EMS	Early larval	Escapers (sterile adults with vulva protrusion)
				s1523	EMS	Early larval	
	let-405	1	3	*s116*	EMS	Early larval	
				s829	EMS	Mid larval	

TABLE 1—Continued

Zone	Gene	No. of EMS-induced alleles[a]	Total No. of alleles	Mutation	Mutagen[b]	Phenotype	Comments
				s388	EMS	Mid larval	
	let-406	0	1	*s514*	γ-ray	Mid larval	
	let-411	4	4	*s1595*	EMS	Mid larval	
				s223	EMS	Late larval	
				s1453	EMS	Mid larval	
				s1553	EMS	Mid larval	
	let-423	1	2	*s818*	EMS	Early larval	
				s1550	EMS	Hatches/escapers	Some mid larval lethals
	let-449	0	1	*s1343*	Tc1	Early larval	
	let-474[c]	1	1	*s1577*	EMS	Early larval	
	let-480[c]	1	1	*s1607*	EMS	Sterile adult	
17	*let-408*	0	2	*s827*	EMS	Egg lethal	
				s195	EMS	Late larval	
	let-413	3	4	*s128*	EMS	Egg lethal	
				s1431	EMS	Egg lethal	
				s1451	EMS	Egg lethal	
				s1455	EMS	Early larval	
	let-414	0	2	*s114*	EMS	Mid larval	
				s207	EMS	Mid larval	
	let-424	3	3	*s384*	EMS	Sterile adult	
				s248	EMS	Sterile adult	
				s1587	EMS	Sterile adult	
	let-436[c]	1	1	*s1403*	EMS	Early larval	
	let-445[c]	1	1	*s1419*	EMS	Mel (larval lethal)	Tight coiler
	let-456[c]	1	1	*s1479*	EMS	Early larval	
18	*let-412*	2	2	*s1598*	EMS	Sterile adult	Roller
				s579	EMS	Mel (egg lethal)	
	let-464[c]	2	2	*s1504*	EMS	Early larval	Approximately ½ do not hatch
				s1530	EMS	Early larval	
	rol-3	8	13	*e754*	EMS	Roller	
				s126	EMS	Early larval	
				s422	EMS	Mid larval	
				s501	γ-ray	Early larval	
				s742	γ-ray	Mid larval	
				s833	EMS	Mid larval	
				s1030	EMS	ND	In same strain as *let-417(s1313)*
				s1040	EMS	Mid larval	Temperature sensitive, roller
				s1408	EMS	Early larval	
				s1409	EMS	Early larval	
				s1473	EMS	ND	In same strain as *let-463(s2168)*
				s1494	EMS	Early larval	
				s1519	EMS	Early larval	
19	*let-334*	2	2	*s908*	EMS	Early larval	
				s383	EMS	Mid larval	
	let-340	2	2	*s1508*	EMS	Early larval	
				s1022	EMS	Mid larval	
	let-409	5	7	*s1480*	EMS	Hatches	Tends to curl
				s823	EMS	Early larval	
				s206	EMS	Early larval	
				s1507	EMS	Hatches	
				s1528	EMS	Hatches	
				s1546	EMS	Early larval	
				s1547	EMS	Hatches	
	let-416	0	1	*s113*	EMS	Late larval	
20A	*let-460*[c]	0	1	*s1664*	For	ND	
	let-467[c]	1	1	*s1521*	EMS	Hatches	
	unc-23	0	8	*e25*	EMS	Unc	
20B	*let-407*	1	4	*s1631*	EMS	Hatches	
				s830	EMS	Early larval	
				s118	EMS	Early larval	
				s2122	For	ND	
	let-441[c]	1	1	*s1414*	EMS	Early larval	Escapers, permissive temperature of 15°

TABLE 1—Continued

Zone	Gene	No. of EMS-induced alleles[a]	Total No. of alleles	Mutation	Mutagen[b]	Phenotype	Comments
	let-454[c]	1	1	*s1423*	EMS	Mid larval	Double cuticle, no pumping
	let-470[c]	2	2	*s1581*	EMS	Mid larval	
				s1629	EMS	Slow	Permissive temperature of 15°
	let-475[c]	1	1	*s1606*	EMS	Sterile adult	

[a] Only the alleles isolated from screening the entire eT1 balanced region are included here. EMS-induced alleles from screens over selected regions of LGV(left) are not included.

[b] For = formaldehyde; Spo = spontaneous; Tc1 = mobilization of the transposon Tc1; UV = ultraviolet irradiation; γ-ray = γ-ray irradiation.

[c] Essential genes first identified in this paper.

[d] Ges = gut esterase.

[e] Sel = supressor of *lin-12* (lin = lineage defective).

[f] Lag = *lin-12* and *glp-1* double mutant phenotype (glp = germline proliferation defective). ND = not done.

at lower temperatures. No mutations had a permissive temperature of 25°, but at 25° *let-343(s1465)* (zone 15) blocked development at a later stage than at 20°.

Table 2 has the results from testing for sperm or production-of-sperm defects by mating hermaphrodites containing homozygous mutations that resulted in sterile adults, maternal-effect lethals or slow developers with wild-type males. Hermaphrodites containing nine of the mutations produced apparently normal progeny when mated with wild-type males. *let-471(s1570)* (zone 14) is a maternal effect-larval lethal that is cross-fertile. *let-444(s1569)* (zone 12A) and *let-346(s1630)* (zone 15) are two weak alleles of genes whose canonical alleles block development at an early stage (*let-444*) or a mid-larval stage (*let-346*). Two more mutations, *let-470(s1629)* (zone 20B), and *let-476(s1621)* (zone 13) (both of these genes are represented by only one allele), are both putative sperm defective and temperature sensitive. *let-470(s1629)* also causes slow development at 20°. Two mutations, *let-479(s1576)* (zone 11B2) and *let-469(s1582)* (zone 14), are cross-fertile sterile adults; *let-479(s1576)* lays unfertilized oocytes.

Five of the 96 essential genes on LGV(left) whose mutant phenotypes have been noted, have canonical alleles with maternal effect lethal phenotypes [*let-445* (zone 17), *let-447* (zone 1B1), *let-459* (zone 9A), *let-466* (zone 8A2) and *let-471* (zone 14)]. Three were tested for cross-fertility: *let-447* and *let-459* were not fertile but *let-471* was. Therefore, at least two genes appeared to be pure maternal effect genes.

Testing for cross-fertility by mating with wild-type males is not only useful for identifying *mel* genes but also for identifying mutations that cause defective spermatogenesis (Spe). Mutations in *spe* genes are ones that result in hermaphroditic worms becoming sterile adults that make functional oocytes. We have identified nine mutations that are cross-fertile, eight of which have been mapped to genes on LGV(left) (Table 2). Two of these genes, *let-479* (zone 11B2) and *let-469* (zone 14), are good candidates to be *spe* genes.

Four of the 10 genes on LGV(left), that were first identified for their recessive nonlethal phenotype (*i.e.*, Rol, Unc), now have recessive lethal alleles. They are: (1) the first recessive lethal allele (*s1586*) of *unc-60* (zone 4A) (predicted by McKim *et al.* 1988); (2) one spontaneous recessive lethal allele of *unc-62* (zone 9B) was isolated by Rosenbluth *et al.* (1988); (3) six recessive lethal alleles of *unc-70* (zone 14) (predicted by Park and Horvitz 1986a), one from Rosenbluth *et al.* (1988) and five new alleles (*s1406*, *s1502*, *s1532*, *s1557* and *s1639*). Worms heterozygous for most of the recessive lethal alleles of *unc-70* appear to be weakly uncoordinated. Worms homozygous for most of the alleles are unable to move and are developmentally arrested in the first larval stage. Occasionally, worms homozygous for *unc-70(s1502)* escape. They mature and give early-larval blocked progeny with an occasional escaper. Our lethal alleles were not complementation tested against *e524* (the visible allele). They were tested against *s115* (an early recessive lethal allele) which had been tested against *e524* (Rosenbluth *et al.* 1988); (4) 12 recessive lethal alleles of *rol-3* (zone 18). We have identified five alleles (*s1408*, *s1409*, *s1473*, *s1494* and *s1519*), the other seven were identified by Rosenbluth *et al.* (1988). All of our alleles (with the possible exception of *s1473*) are recessive for an early-larval lethal phenotype. *s1473* is in a strain with another recessive lethal mutation [*let-463(s2168)* (zone 11B3)], and the developmental blocking stages of the individual mutations were not determined. One of the *rol-3* alleles, *s1040*, which was identified by Rosenbluth *et al.* (1988), is temperature sensitive. At 20° *rol-3(s1040)* is a recessive lethal but at 15° it is a fertile roller. Our lethal alleles were complementation tested against *s442* and/or *s126* (both recessive lethal mutations). Rosenbluth *et al.*

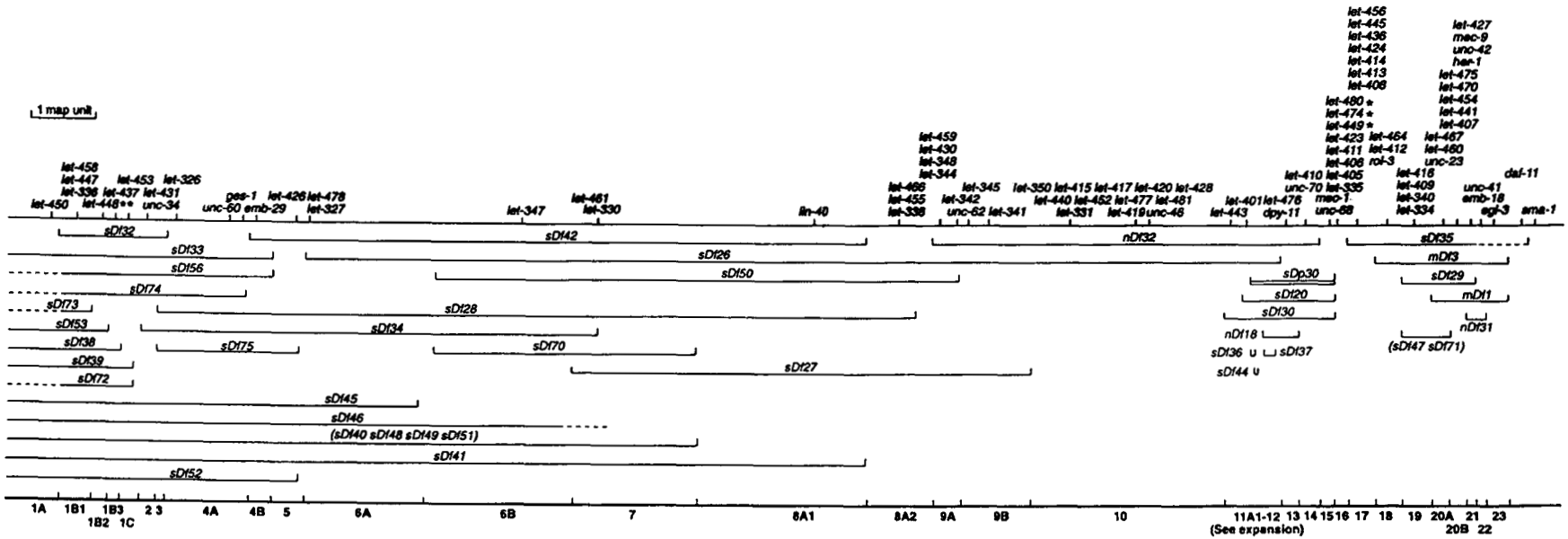

FIGURE 1.—Genetic map of the *eT1* balanced region of LGV(left). Descriptions of the deficiencies' origins (except *sDf56*) may be found in ROSENBLUTH *et al.* (1988), JOHNSEN and BAILLIE (1988), CLARK *et al.* (1990) or STEWART, ROSENBLUTH and BAILLIE (1990). * May not be in zone 16. ** The left end of *sDf73* could be within *sDf32* and then *let-448* could be to the left of *let-336*, *let-447* and *let-458*.

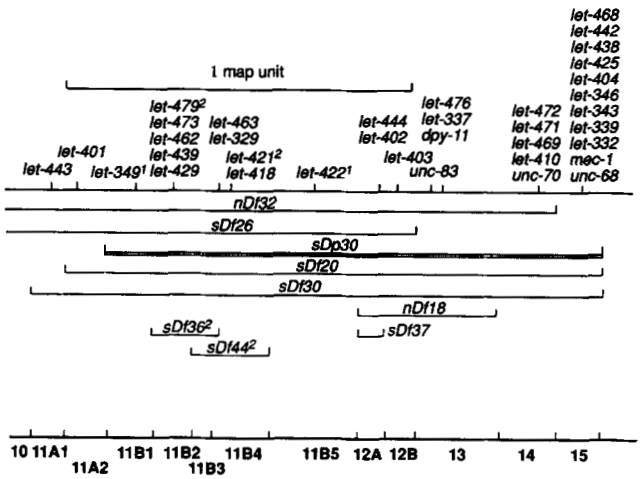

FIGURE 2.—Expansion of zones 11A1–15. *unc-83* was mapped by J. THOMAS (personal communication). [1]*let-349* and *let-422* could be in either zone 11B1 or 11B5, their locations on the map are based on two-factor mapping distances from *unc-46* (zone 10). [2]*sDf36* could overlap *sDf44* to the right, if so 11B1 would be to the right of 11B2 and 11B3 would be to the left of 11B2.

(1988) had shown that *s126* fails to complement *e754* (the allele with the roller phenotype), as well as the lethal allele *s422*.

Some of the genes on LGV(left) deserve comment.

***let-447* (zone 1B1):** *let-447* has two alleles (*s1457* and *s1654*). *s1654* was isolated from a screen of formaldehyde treated chromosomes (JOHNSEN and BAILLIE 1988). Both alleles show a recessive maternal effect egg lethal phenotype. Generally, hermaphrodites homozygous for either allele lay very few eggs, but both *s1457/Df* and *s1654/Df* hermaphrodites consistently lay many eggs. The developmental blocking stages of the eggs in all four cases (homozygous or hemizygous *s1457* or *s1654*) appear to be the same: a multicellular mass of fewer than 550 cells [they did not reach the lima bean (start of morphogenesis) stage, see VON EHRENSTEIN and SCHIERENBERG 1980; SULSTON *et al.* 1983]. We can propose two possible explanations for these results: (1) *let-447* has two functions: (a) it is involved with the production of germ cells; and (b) it has a maternal effect on egg development. Both alleles of *let-447* would be hypermorphic for the first function and probably null for the second. (2) The increased number of eggs is not due to the hemizygosity of *let-447* but due to the hemizygosity of *let-336*, which is deleted by all the currently known deficiencies that delete *let-447*. *let-336(s1413)* dominantly effects brood size. Nematodes heterozygous for *s1413* produce an average of 20% more progeny than wild-type hermaphrodites (our unpublished results). A deficiency that deletes *let-447* but not *let-336* would help in choosing between the two explanations.

***let-461* (zone 7):** This gene was called *lag-2* by J. KIMBLE and *sel-3* by J. THOMAS. *let-461* has alleles that have the same phenotype as *lin-12 glp-1* double mutants (J. KIMBLE and E. LAMBIE, personal communication) and alleles that are semi-dominant suppressors of *lin-12* dominant alleles (J. THOMAS, personal communication). One allele, (*s1486*), is a recessive EMS-induced lethal mutation and results in the Lag (*lin-12 glp-1*) phenotype at 20° but blocks development at a more advanced stage at 15°, where it causes mid-larval lethality. This suggests that *s1486* is not a null.

TABLE 2

Results of testing mutations for cross-fertility by mating with wild-type males

Zone	Gene	Allele	Blocking stage (20°)	Cross-fertile
1B1	*let-447*	*s1457*	Maternal egg lethal	No
8A1	*lin-040*	*s1611*	Sterile adult, vulva protrudes	No[a]
9A	*let-348*	*s1448*	Maternal egg lethal, slow	No
9A	*let-459*	*s1615*	Maternal egg lethal	No
10	*let-420*	*s1478*	Sterile adult, vulva protrudes	No[b]
11B2	*let-429*	*s1597*	Slow, some morphological abnormalities	No
11B2	*let-479*	*s1576*	Sterile adult, lays unfertilized eggs	Yes
11B4	*let-418*	*s1617*	Sterile adult, vulva protrudes (partial *ts*)	No
12A	*let-444*	*s1569*	Maternal egg lethal (not canonical)	Yes
13	*let-476*	*s1621*	Sterile adult (*ts*)	Yes
14	*let-469*	*s1582*	Sterile adult	Yes
14	*let-471*	*s1570*	Maternal larval lethal	Yes
15	*let-346*	*s1630*	Sterile adult, not canonical allele	Yes
16	*let-480*	*s1607*	Sterile adult	No
17	*let-424*	*s1587*	Sterile adult	No
18	*let-412*	*s1598*	Sterile adult, roller	No
20B	*let-470*	*s1629*	Slow, not canonical allele	Yes

[a] *lin-40(s1634)*, another sterile adult allele, was not male rescued.
[b] Three other alleles of *let-420(s1573, s1584,* and *s1603)* were not male rescued. Note: The strains that contained more than one lethal mutation were not tested for cross-fertility.

***let-330* (zone 7):** *let-330* is a large gene with respect to EMS mutagenesis, with an EMS forward mutation rate of 1.1×10^{-3}. This is greater than the rate of 6.4×10^{-4} for *unc-22* (unpublished results cited in MOERMAN and BAILLIE 1981). *unc-22* spans 47 kb of genomic DNA (about 19 kb of which is coding DNA) and encodes the muscle protein twitchin which has some homology to the vertebrate striated muscle protein titin (BENIAN *et al.* 1989). Twitchin has a molecular weight of approximately 700,000 daltons (BENIAN *et al.* 1989). From Figure 1, one can see that *let-461* has not been separated from *let-330*. Two alleles of *let-330* (*s1497* and *s1518*) are less severe over *sDf34* than over *sDf*s 26 or 42. Worms heterozygous for either *s1497* or *s1518* over *let-330(s573)*, *sDf26* or *sDf42* block as early-mid larvae, but over *sDf34*, mature to adulthood and give occasional larval progeny. Therefore *sDf34* probably breaks within *let-330*. *let-330(s1634)/let-330(s1433)* is a Mel-larval lethal (implying possible interallelic complementation) but *let-330(s1638)/sDf*s 27, 34 or 42 are larval lethal. These

data imply that: (1) *let-330* is a large gene; (2) *let-461* is either internal to *let-330* or to its left; and (3) *let-330* is a multidomain gene and/or encodes for proteins that function as multimers. If *sDf34* does break within *let-330*, then the molecular identification of the right breakpoint of *sDf34* could lead to the cloning of *let-330*.

***lin-40* (zone 8A1):** The developmental blocking stages of the 16 recessive *lin-40* alleles range from early-mid larval lethality to maternal effect early-larval lethality. Worms homozygous for eight of the alleles develop into adults (sterile or maternal effect lethal) with protruding vulvae. The results of interallelic complementation tests show that *lin-40* is a complex locus with at least five classes of alleles (unpublished results). Alleles in the same complementation class cause similar phenotypes. Some alleles of *lin-40* and deficiencies that delete *lin-40*, dominantly cause an increase (approximately 40%) in the number of self-progeny from *C. elegans* hermaphrodites. This implies that some *lin-40* alleles not only affect the vulva-lineage but also delay the switch from spermatogenesis to oogenesis in hermaphrodites.

***let-341* (zone 9B):** *let-341* is the fourth biggest target for EMS mutagenesis on LGV(left). The canonical allele of *let-341(s1031)*, is a recessive early-larval lethal. Some *let-341* alleles have been shown to be dominant suppressors of multivulva *lin-15(X)* mutations (S. G. CLARK and H. R. HORVITZ, personal communication). In addition, mutation in *let-60(IV)*, a *C. elegans ras* gene (HAN and STERNBERG 1990), suppress the lethality of some alleles of *let-341* including *s1031* (S. G. CLARK and H. R. HORVITZ, personal communication). Therefore *let-341* is probably in the *lin-15–let-60* vulva induction pathway.

***let-342* (zone 9B):** There are five alleles of *let-342*, all of which are EMS-induced. Three of the alleles block development at the early-larval stage (*s1442*, *s1487* and *s1549*), the other two (*s1029* and *s1616*) block at the mid-larval stage. Occasionally worms homozygous for *s1616* mature and have internal larval progeny. All of the alleles over *sDf27*, *nDf32* or *let-342(s1029)* appear to block at early- (or mid-) larval stages. *s1442* complements *sDf50*, but worms heterozygous for any of the other alleles and *sDf50* block as sterile adults. Worms heterozygous for either *s1487* or *s1549* over *sDf50* were cross-fertile (*s1029* and *s1616* were not tested for cross-fertility). To explain these data we have two proposals (1) *sDf50* breaks near the promoter region of *let-342* and modifies its level of transcription or (2) *let-342* is transcribed right to left and *sDf50* breaks within *let-342* near the three prime end. In both proposals the chromosome containing *sDf50* produces almost normal levels of *let-342* product or almost normal product. This allows worms containing the *sDf50* chromosome over a putative null *let-342* allele, to mature and become cross-fertile sterile adults. If *sDf50* does break very close to or in *let-342*, then by molecularly identifying the right breakpoint of *sDf50* one should be able to clone *let-342*.

***let-420* (zone 10):** Worms homozygous for any of the seven alleles of *let-420* are sterile adults with protruding vulvae. *let-420* appears to be a large target for EMS mutagenesis. One allele was induced by gamma-irradiation and six were induced by EMS. This EMS-induction frequency is approximately four times higher than the average hit frequency for essential genes on LGV(left).

***let-418* (zone 11B4):** Both alleles of *let-418* show temperature sensitive phenotypic effects. The terminal phenotypes of worms homozygous for *let-418(s1045)* include mid-larval lethality at 15°, maternal effect early-larval lethality at 20° and at 24° the adults are sterile and have protruding vulvae (R. E. ROSENBLUTH, personal communication), interestingly the middle temperature is the most permissive. Worms homozygous for *let-418(s1617)* at 15° have a maternal effect lethal phenotype, giving a few larval progeny but at 20° or 25° are sterile adults with protruding vulvae.

Three genes [*let-418* (zone 11B4), *let-420* (zone 10) and *lin-40* (zone 8A1)] have recessive alleles that cause adult worms to be sterile and to have protruding vulvae and thus these genes putatively affect the vulva-lineage.

Zone 16: This is a region that has not been deleted by any deficiencies. It is possible that there is a haploinsufficient gene in the region which prevents the isolation of deficiencies. The location of most genes to the zone 16 region has been confirmed by 3-factor mapping (ROSENBLUTH *et al.* 1988), but 3-factor mapping has not been used to confirm the position of *let-449*, *let-474* or *let-480*. From 2-factor mapping data of *let-449* and *let-474* to *unc-46* (zone 10), we got distances of 4.3 (2.1–9.1) and 4.8 (2.7–7.6) m.u., respectively. These distances are consistent with both genes being in zone 16. Two-factor mapping of *let-480* (an adult sterile) was not done. As all three genes are represented by only one allele each, it is possible that one or more of those alleles are hypermorphic (generally rare alleles) such that worms heterozygous for the appropriate allele over a deficiency appear normal. If such is the case then the mutations of this type could be anywhere on LGV(left).

***rol-3* (zone 18):** We have identified five EMS-induced recessive lethal alleles of *rol-3* (*s1408*, *s1409*, *s1473*, *s1494* and *s1519*). This increases the number of recessive lethal alleles of *rol-3* to 12 (the other seven were identified by ROSENBLUTH *et al.* 1988). All of the new alleles (with the possible exception of *s1473*) are recessive for an early-larval lethal phenotype. *s1473* is in a strain with another recessive lethal mu-

tation [*let-463(s2168)* (zone 11B3)] and the individual developmental blocking stages due to these two mutations individually were not determined. One of the *rol-3* alleles, which was identified in an earlier screen (see ROSENBLUTH *et al.* 1988), is temperature sensitive. At 20° it is a recessive lethal but at 15° it is a fertile roller. The existence of the temperature sensitive allele that causes rolling at the permissive temperature allows us to argue that weak alleles cause rolling but stronger alleles cause lethality. Interestingly *let-412* (zone 18), which has *not* been separated from *rol-3* by a chromosomal rearrangement breakpoint, has an allele (*s1598*) which is recessive for a sterile adult roller phenotype. *s1598* has only been complementation tested against two *rol-3* alleles, *s833* and the "visible" allele *e754*, and complemented both. The possibility that *rol-3–let-412* is a complex locus is being examined by W. B. BARBAZUK (personal communication). Another possibility is that *rol-3* and *let-412* are neighboring genes that affect the same or related developmental pathway(s).

DISCUSSION

The results presented in this paper have brought to 101 the number of essential genes identified on LGV(left). For 89 of these, there are EMS-induced mutations isolated by screening the whole *eT1* region for recessive lethals. We have used these data and a truncated Poisson equation to derive minimum estimates for the total number of essential genes on LGV(left) and then for the total number in the *C. elegans* genome. From the 89 identified genes we determined m (the mean number of mutations per essential gene). Knowing that f (the fraction of genes represented by more than one allele) was 47/89, we got a m value of 1.35 hits per gene from a graph of m *vs.* f where $f = (1 - e^{-m} - me^{-m})/1 - e^{-m}$. With this we calculated $1 - e^{-m}$ (the frequency of genes with at least one mutation) to be approximately 0.74, giving a minimum estimate of 120 essential genes on LGV(left).

To estimate the number of essential genes in the *C. elegans* genome from the number of essential genes identified on LGV(left), one must know what fraction of the total number of genes are on LGV(left). EDGLEY and RIDDLE (1990) list 830 genes in the *C. elegans* genome, 244 are *lets* (essential genes) and most come from screens of specific regions [for LGI, see ROSE and BAILLIE (1980), HOWELL *et al.* (1987) and HOWELL and ROSE (1990); LGII: SIGURDSON, SPANIER and HERMAN (1984); LGIV: ROGALSKI, MOERMAN and BAILLIE (1982), ROGALSKI and BAILLIE (1985), CLARK *et al.* (1988) and ROGALSKI and RIDDLE (1988); LGV: ROSENBLUTH *et al.* (1988), JOHNSEN and BAILLIE (1988) and CLARK *et al.* (1990); LGX: MENEELY and HERMAN (1979, 1981)]. Ignoring the *lets* there are 586 genes, 77 of which are on LGV. Assuming that there are no biases in the distribution, and that the essential genes follow the same distribution, then 13% of all *C. elegans'* genes are on LGV. Of the 77 genes on LGV 21 are on LGV(left), 45 are on LGV(right), and 11 have not yet been mapped to position. Ignoring the 11 genes not positioned, 32% of the genes on LGV are on LGV(left) and therefore LGV(left) contains 4.2% of the essential genes in the genome. Using the minimum estimate of 120 essential genes on LGV(left), we calculate that there are a minimum of 2850 essential genes in the *C. elegans* genome. BRENNER's (1974) original estimate was 2000, HOWELL and ROSE (1990) estimated 3300 and CLARK (1990) estimated 3500.

To calculate our forward mutation rate for the average gene, we removed the highly mutable genes from our sample [as did CLARK (1990) and HOWELL and ROSE (1990)]. Our rate, based on 14,545 chromosomes (determined after removing genes with more than four alleles), is 6.7×10^{-5} mutations per gene per chromosome. To compare this with the rates of others, we have adjusted the forward mutation rates cited by BRENNER (1974), HOWELL and ROSE (1990), and CLARK (1990) to the rates expected with 0.012 M EMS, using a dose-response curve (ROSENBLUTH, CUDDEFORD and BAILLIE 1983). BRENNER (1974) estimated the forward mutation rate for genes in *C. elegans* with visible phenotypes. His estimate becomes 2.4×10^{-4}, adjusted for our EMS dosage. HOWELL and ROSE (1990) noted this estimate was based on a small sample size and a low estimate of the total number of genes and argued that the estimate was too high. They estimated the average forward mutation rate per essential gene in the *hDf6* region of LGI. Their estimate becomes 4.3×10^{-5} when we adjusted for our EMS dosage. CLARK's (1990) estimated adjusted rate for the *sDf2* region of LGIV is 5.8×10^{-5}. Our rate is therefore similar to that of CLARK's; about one and one half times that of HOWELL and ROSE; and three to four fold lower than that of BRENNER. We support HOWELL and ROSE's (1990) argument that BRENNER's (1974) gene number estimate was too low.

We determined the frequency of temperature sensitive mutations among our EMS-induced mutations. We tested 190 recessive lethal mutations for development blocking stage at 15°. Worms containing four (2.1%) of the mutations were fertile and worms with three other mutations blocked development at a later stage than at 20°. Therefore seven (3.7%) were at least partially heat sensitive. SUZUKI *et al.* (1967) found a similar level (6.3%) of all EMS-induced lethal mutations on the *X* chromosome in *D. melanogaster* were temperature sensitive. None of the 177 mutations had a permissive temperature of 25°, although one

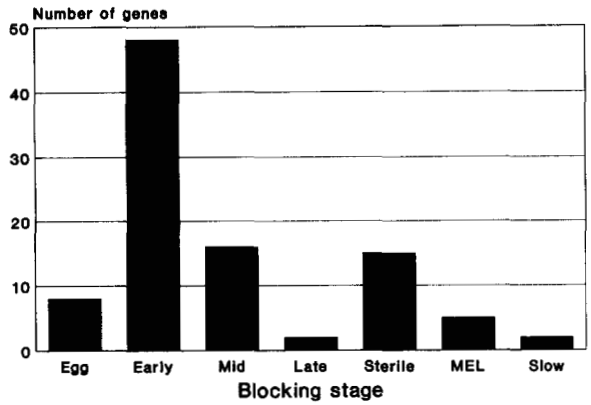

FIGURE 3.—Plot of the number of genes whose most severe alleles block development at the indicated developmental stages. The stages are egg lethal, early- mid- late-larval lethal, sterile adults, MEL (maternal effect lethal) and slow developing but fertile.

blocked development at a later stage at 25° than at 20°. Other genes on LGV(left) that have *ts* alleles are *emb-29* (zone 4B) (CASSADA *et al.* 1981) and *rol-3* (zone-18). *let-327* (zone 6A) has a cold sensitive dominant allele (*s247*).

Figure 3 shows the number of genes identified on LGV(left) whose canonical alleles block development at the indicated stages. If one assumes that LGV(left) is representative of the genome and that the distribution of phenotypes shown in Figure 3 reflects the proportion of genes needed at the different developmental stages, then the proportion and minimum number of genes needed at each stage can be calculated. From these assumptions, wild-type genes needed in the zygote for normal egg development consist of 8% (230) of all the essential genes. To get beyond early-larval development requires 50% (1425) of all the essential genes. To get past the mid-larval stage requires 17% (485) of the essential genes. To get past late-larval development requires 2% (60) of the essential genes. 16% (455) of the essential genes are needed to make fertilized eggs. These include the genes needed for the formation and development of viable oocytes and viable sperm. Our estimate for the number of genes necessary for the formation and development of viable sperm is 60. At least 36 *spe* genes have been identified (L'HERNAULT, SHAKES and WARD 1988 and sources cited therein) in the *C. elegans* genome. L'HERNAULT, SHAKES and WARD used a truncated Poisson equation to calculate that there are a minimum of 14 *spe* genes on LGI (42 m.u.). If LGI is representative of the 300 m.u. in the genome, then their estimate would be a minimum of 100 *spe* genes in the genome. Our estimate (based on two genes) of a minimum of 60 *spe* genes is similar to their estimate. From this it appears that *spe* genes are randomly located in the *C. elegans* genome. A further 5% (140) of the essential genes are needed to make viable progeny, these are the *mel* genes of which at least 2% (60) are purely maternal. KEMPHUES *et al.* (1988) identified 17 genes with strict maternal effect mutations which they divided into two classes: 13 genes that had one or two alleles and four that had four or more alleles. Because the smaller class had a forward mutation rate comparable to BRENNER's (1974) rate, they considered the smaller class to be the pure maternal genes. This led them to an estimate of the number (12) of pure maternal genes in the *C. elegans* genome that is much lower than ours.

It is interesting that half of the essential genes appear to be needed strictly for early-larval development. This may be a misleading result for two reasons: (1) The products of many of the genes may be required much earlier but are also maternally supplied and (2) the early-larval blocking stage could be due to the metabolic demands of rapid growth and cell division or moulting, *i.e.*, sickly worms could live for awhile but not make it through a moult, even if all the genes needed strictly for moulting were wild type. The decreasing number of genes necessary to develop through mid- and late-larval stages seems reasonable, they are probably representatives of a relatively small class of genes involved in the specifics of the later moults. The relatively large number of genes required to get past the mid-larval stage could be related to the fact that a developmental choice is made there. Under conditions of starvation and overcrowding *C. elegans* nematodes can enter a dispersive larval stage called dauer (GOLDEN and RIDDLE 1982, 1984; OHBA and ISHIBASHI 1982). There is a class of dauer-constitutive mutations that cause the worms to get locked into and blocked along the dauer development pathway. Such worms would not grow longer than the length that we assigned as mid-larval and thus be called mid-larval lethal mutations. The large number of genes needed strictly to make fertile animals probably reflects the complexity of the gonad.

Generally, for genes with more than one allele, the developmental blocking stages are approximately the same, suggesting that most of our lethal mutations are nulls. This is consistent with findings described by others (SHANNON *et al.* 1972; ROSE and BAILLIE 1980; MENEELY and HERMAN 1981; HOWELL and ROSE 1990; CLARK 1990), although CLARK found somewhat more variability. She often found that later arresting mutations blocked earlier as hemizygotes than homozygotes, indicating that they were hypomorphs. Because most lethal mutations appear to cause null phenotypes, genes with alleles of several different phenotypes are good candidates for complex loci. An example is *lin-40* (zone 8A1), where the variation in developmental blocking stages appear to be consistent with the intragenic complementation map (our unpublished results). In most cases we did not note the phenotypes of our mutations over deficiencies, but we did find that some anomalous late blocking alleles

were temperature sensitive, thus possibly indicating that they were hypomorphs.

Information regarding the biological function of six genes [*let-461* (zone 7), *let-341* (zone 9B), *rol-3* (zone 18), *unc-60* (zone 4A), *unc-62* (zone 9B) and *unc-70* (zone 14)] was obtained by virtue of their map positions. Their lethal mutations were found to be allelic to mutations mapped to the same positions by other researchers, but described in terms of phenotypes other than lethality. The *unc* (uncoordinated) genes generally are involved in muscle or in the nervous system controlling muscles. The null phenotype of some identified muscle genes is early-larval lethality. *let-75* (gene encoding myo-1) has early-larval lethal recessive mutations (ROSE and BAILLIE 1980). Some dominant missense alleles in *unc-54* have a recessive lethal phenotype (MACLEOD *et al.* 1977; DIBB *et al.* 1985; BEJSOVEC and ANDERSON 1988). WATERSTON (1989) showed that the minor myosin heavy chain (*mhcA*) also has recessive lethal mutations. WATERSTON demonstrated that worms homozygous for such mutations do not progress beyond two folds in the egg (as the worm elongates in the egg it must fold to accommodate its length, wild-types progress beyond three folds). The worms with *mhcA* recessive lethal mutations hatched but remained in a folded state with no locomotion. The *unc-70* recessive lethal mutations cause a similar phenotype implying that *unc-70* is a gene involved in muscle development.

The mapping of large numbers of essential genes and deficiencies is also of interest with respect to genomic organization and chromosomal behavior. The map positions of the genes have further delineated regions of recombinationally "clustered" genes from gene "sparse" regions. A gene cluster at the center of LGV [*i.e.*, at the right end of LGV(left)] was already evident from the map positions of non-essential genes (BRENNER 1974; EDGLEY and RIDDLE 1990). Now a second smaller cluster is evident at the left end (zones 1–6A), and a gene sparse region, *let-347* (zone 6B) to *lin-40* (zone 8A1), lies between the two clusters (Figure 1). Comparing deficiency sizes in the cluster with those in the gene sparse region, leads us to argue that the difference between gene clustered and gene sparse regions reflects a difference in recombination rates per unit length of chromosome rather than a difference in the physical gene spacings. In the *let-347* (zone 6B) to *lin-40* (zone 8A1) region there are approximately 6 m.u. with six genes (based on four known genes and the fact that the region is 70% saturated for essential genes). Since *C. elegans* has about 2850 essential genes per 300 m.u., the region has a recombination rate at least 10 times greater than the average genome rate. It is interesting to note that two putative complex genes, *let-330* (zone 7) and *lin-40* (zone 8A1), lie within this region. Genetic fine structure analysis and DNA sequencing could determine whether intragenic recombination is indeed high in these genes. If so, then we must consider that the ability to reshuffle the domains of alleles of these genes could be evolutionarily important. Further analysis of these, and genes in other regions of high recombination, should lead to insights into the nature of the nonrandom recombinational distribution of genes first noted in *C. elegans* by BRENNER (1974).

Interestingly, the *let-347* (zone 6B) to *lin-40* (zone 8A1) gene sparse region is highly subject to being mutated by the mobilization of the Tc1 transposable element (CLARK *et al.* 1990; JOHNSEN 1990). It was shown that there is a "hotspot" for Tc1 induced putative point mutations in *lin-40* and a "hot region" for Tc1 induced deficiencies near *lin-40*. The seven deficiencies that CLARK *et al.* (1990) identified are putative tip deletions because they delete the left-most known gene on the chromosome. The tip deficiencies could be explained by the mobilization of resident Tc1 elements, if the excision is not followed by repair of the chromosomal breaks. ENGELS *et al.* (1990) proposed a homolog-dependent model of *P* element loss in Drosophila. In the model they proposed that *P* transposition created a double strand break which was repaired by using the homolog chromosome as a template. A similar mechanism could explain the high incidence of tip deficiencies in the *eT1* translocation region of *C. elegans* where the homolog is not available to effect repair. Tc1 induced mutations were picked up over a translocation (*nT1*) that acts as a crossover suppressor. Such translocations are thought not to pair at meiosis in *C. elegans* (ROSENBLUTH and BAILLIE 1981). Therefore, the homolog would not be available and thus any Tc1 excision would often be accompanied by a tip deficiency. We propose that the Tc1 *lin-40* induced mutations arise from the excision and subsequent insertion of a local Tc1 element. A method to test this hypothesis would be to isolate mobilization of Tc1 induced recessive lethal mutations in a chromosome region both in the presence and absence of a translocation. A substantially higher ratio of putative tip deficiencies to putative point mutations in the screen with the translocation would support the hypothesis.

In conclusion, approximately 70% of the essential genes on LGV(left) have now been identified. It is the largest well characterized genomic region in any higher eukaryote. HOCHMAN (1970) analyzed chromosome *4* in *D. melanogaster* and identified 33 essential loci and estimated that there were approximately 40 in total. Others have attempted to saturate regions in *D. melanogaster*, a recent example is the 17 polytene bands of 73A2–73B7 by BELOTE *et al.* (1990). They identified 18 complementation groups including 13 that had not been previously identified. The complex-

ity of most higher organisms makes the task of mapping all of their essential genes formidable. The ease of maintenance of *C. elegans* and the rapidity of its genetic analysis makes it feasible to saturate the entire genome for essential genes. The mapping and characterization of the majority of essential genes would provide *C. elegans* researchers with a collection of identified genes which can be quickly used to study the various developmental pathways.

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