Analysis of lethal mutations induced in a mutator strain that activates transposable elements in *Caenorhabditis elegans*

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Corresponding Editor: P. B. Moens

Received June 19, 1989

Accepted September 18, 1989

CLARK, D. V., JOHNSEN, R. C., MCKIM, K. S., and BAILLIE, D. L. 1990. Analysis of lethal mutations induced in a mutator strain that activates transposable elements in *Caenorhabditis elegans*. Genome, 33: 109–114.

A screen was conducted for lethal mutations in the nematode *Caenorhabditis elegans* in a strain containing the mutator *mut-4 (st700)I* to examine the nature of mutator-induced lethal mutations within two large chromosomal regions comprising a total of 49 map units (linkage group IV (right) and linkage group V (left)). The genetic analysis of 28 lethal mutations has revealed that the mutator locus *mut-4(st700)I* causes both putative single-gene mutations and deficiencies. We have identified lethal mutations in three different genes, in addition to seven deficiencies. There is a mutational hot spot on linkage group V (left) around the *lin-40* locus. Six mutations appear to be alleles of *lin-40*. In addition, 5 of 7 deficiencies have breakpoints at or very near *lin-40*. All seven deficiencies delete the left-most known gene on linkage group V (left) and thus appear to delete the tip of the chromosome. This is in contrast to gamma ray and formaldehyde induced deficiencies, which infrequently delete the closest known gene to the tip of a chromosome.

Key words: *Caenorhabditis elegans*, mutator, deficiencies, lethal mutations.

CLARK, D. V., JOHNSEN, R. C., MCKIM, K. S., et BAILLIE, D. L. 1990. Analysis of lethal mutations induced in a mutator strain that activates transposable elements in *Caenorhabditis elegans*. Genome, 33 : 109–114.

Une source du nématode *Caenorhabditis elegans*, qui contient l'agent mutagène *mut-4(st700)I*, a fait l'objet d'un tamisage des mutations létales en vue d'examiner la nature des mutations induites par l'agent mutagène à l'intérieur de deux grandes régions chromosomiques, comprenant un total de 49 unités cartographiées, soit la LGIV (à droite) et la LGV (à gauche). L'analyse génétique de 28 mutations létales a révélé que le locus de l'agent mutagène *mut-4(st700)I* cause à la fois des mutations liées à un seul gène probable ainsi que des déficiences. Nous avons identifié des mutations létales dans trois gènes différents en plus de sept déficiences. Il existe un point chaud mutationnel sur la LGV (à gauche) autour du locus *lin-40*. Six mutations semblent être des allèles de *lin-40*. De plus, 5 déficiences sur 7 ont des points de coupe au niveau de *lin-40* ou près de ce dernier. Les sept déficiences éliminent toutes le gène connu le plus à gauche sur la LGV (à gauche) et semblent donc éliminer l'extrémité du chromosome. Ceci contraste avec les déficiences induites par les rayons gamma et le formaldéhyde, lesquelles éliminent rarement le gène connu le plus près de l'extrémité du chromosome.

Mots clés : *Caenorhabditis elegans*, agent mutagène, déficiences, mutations létales.

[Traduit par la revue]

Introduction

In the nematode *Caenorhabditis elegans*, various mutator strains have been characterized that have a high rate of transposable element mobility relative to the standard wild-type strain N2 (var. Bristol) (Moerman and Waterston 1984; Mori *et al.* 1988*b*; Collins *et al.* 1987). The transposable element that is responsible for an increase in mutation rates is called Tc*1* (Emmons *et al.* 1983). Other transposable elements, Tc*2* (Levitt and Emmons 1989) and Tc*3* (Collins *et al.* 1989), have been identified and found to be mobile in some *C. elegans* strains.

Tc*1* is present in approximately 30 copies in the N2 strain, while there are approximately 300 copies in the BO (var. Bergerac) strain. Tc*1* is dispersed throughout the genome (Emmons *et al.* 1983). A high frequency of spontaneous mutations in *unc-22* was observed in the BO strain (Moerman and Waterston 1984). The spontaneous mutations in *unc-22* (Moerman *et al.* 1988) and *unc-54* (Eide and Anderson 1985) isolated in BO were all found to be due to Tc*1* insertions. Mutator activities responsible for Tc*1* transposition in BO have been localized to specific regions of the genome (Mori *et al.* 1988*b*). Other mutators identified after ethyl methanesulfonate (EMS) mutagenesis of BO have also been localized (Collins *et al.* 1987).

The structure of Tc*1* consists of a 1.6-kilobase (kb) sequence with a pair of 54 base pair (bp) inverted repeats and two open reading frames (Rosenzweig *et al.* 1983; for review see Herman and Shaw 1987). The nature of the sites of insertion for Tc*1* and the results of its excision in two loci, *unc-22* (Mori *et al.* 1988*a*) and *unc-54* (Eide and Anderson 1988), are well characterized. There is a strong site specificity for Tc*1* insertion, and a 9-bp consensus sequence with an invariant TA at the point of insertion has been identified (Mori *et al.* 1988*a*; Eide and Anderson 1988). Tc*1* excision was often found to be imprecise upon examination of Tc*1* excision events from both *unc-22* and *unc-54*.

Tc*1* has been used as a molecular tag for cloning genetically characterized genes (e.g., Moerman *et al.* 1986; Greenwald 1985). Mutator strains with low copy number and low mobility of Tc*1* relative to BO are used for this purpose. The mutator strain RW7037, which was derived from BO, has 70 Tc*1* elements and the mobility of Tc*1* is approximately one-half that in the BO strain. The mutator activity in RW7037 has been localized to linkage group I (LGI) and named *mut-4(st700)* (Mori *et al.* 1988*b*).

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TABLE 1. Mutator-induced lethal mutations on LGV (left)

Mutation	Distance from *unc-46* (map units)[a]	Lethal phase[b]
let-448(s1363)	16.1(12.8–19.8)	Late larva
let-449(s1343)	4.5(2.1–9.1)	Early larva
lin-40(s1352)	4.7(3.4–6.3)	Early larva[c]
lin-40(s1345,s1351, s1358,s1360,s1373)	ND	
sDf40	0.5(0.1–1.3)	Embryo
sDf41	0.15(0.01–0.81)	Embryo
sDf45	6.4(5.1–8.0)	Embryo
sDf48	ND	Embryo
sDf49	0(0–0.7)	Embryo
sDf51	0(0–0.4)	Embryo
sDf52	1.5(0.8–2.7)	Embryo

[a]Recombination distances were calculated using the formula $1-[1-(4U/\text{total})]^{1/2}$, where U is Unc-46 and total is total number of surviving progeny (see also Materials and methods). The 95% confidence intervals for recombination distances are given in parentheses and were calculated from the tables in Crow and Gardner (1959).
[b]Lethal phases were determined for mutant homozygotes at 20°C (see also Results).
[c]All *mut-4(st700)* induced alleles of *lin-40* have the same early larval lethal phase.

This study was conducted for two reasons. The first reason was to determine the frequency of induced lethal mutations in a large region of the genome in a mutator strain containing *mut-4(st700)I*. Strains containing these mutations could then be examined for Tc*1* polymorphisms. The Tc*1* polymorphisms can potentially be used to clone the essential gene, providing that it can be proven that there was an actual insertion of Tc*1* into the gene. This could be done by showing that a reversion of the mutation is correlated with an excision of Tc*1*. The second reason was to examine the types of recessive lethal mutational events associated with the mutator *mut-4(st700)I*.

We have constructed a mutator strain containing *mut-4(st700)I* that recovers recessive lethal mutations in the two large regions balanced by the chromosomal rearrangement *nT1(IV;V)*. Recombination is suppressed over approximately 49 map units on linkage group (LG) IV (right) and V (left) (Ferguson and Horvitz 1985; Clark *et al.* 1988), which is about one-sixth of the genetic map (Edgley and Riddle 1987). Thus, compared with screens selecting for mutations in a specific gene, our target was a large one. We anticipated recovering mutations in single essential genes as well as chromosomal rearrangements that result in the deletion of essential genes. Previous studies (Clark *et al.* 1988; Johnsen and Baillie 1988; Rosenbluth *et al.* 1988) have already characterized a large number of lethal mutations on LGIV (right) and LGV (left). These mutations had been induced by EMS, gamma irradiation, or formaldehyde mutagenesis and thus provide a basis of comparison for the efficacy of *mut-4(st700)*, and presumably Tc*1*, as a mutagen in these chromosomal regions.

Materials and methods

General

The genetic nomenclature in this paper follows the recommendations of Horvitz *et al.* (1979). Nematodes were maintained on Petri plates containing nematode growth medium streaked with *Escherichia coli* OP50 (Brenner 1974).

Stock maintenance and strains

The wild-type strain N2 (var. Bristol) and strains carrying the following mutations used in this study 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: LGI, *dpy-5(e61)*; LGIII, *dpy-18(e364)*; LGIV, *mDf7* (Rogalski and Riddle 1988); LGV, *unc-46(e177)*, *nT1(IV;V)* (Ferguson and Horvitz 1985), *eT1(III;V)* (Rosenbluth and Baillie 1981). The strain carrying the mutator, *mut-4(st700)I;unc-22(st136)IV* (RW7037) (Mori *et al.* 1988*b*), was provided by I. Mori and D. Moerman of Washington School of Medicine, St. Louis, MO. *lin-40(e2173)V* was isolated by S.W. Emmons and supplied by J. Hodgkin (Medical Research Council, Cambridge, England). *mDf1(V)* and *mDf3(V)* (Brown 1984) were obtained from D. L. Riddle's laboratory (University of Missouri, Columbia, MO). *nDf32(V)* (Park and Horvitz 1986), *nDf18(V)*, *nDf31(V)*, and *nDf27(IV)* originated from H. R. Horvitz's laboratory at the Massachusetts Institute of Technology. The deficiencies denoted with the *s* prefix were isolated at Simon Fraser University. Several mapped lethal mutations isolated at Simon Fraser University (Rosenbluth *et al.* 1988; Johnsen and Baillie 1988) were used in the complementation tests. The mutations isolated in this study are listed in Table 1.

Construction of mutator strain BC2643

Our screen for recessive lethal mutations balanced by *nT1(IV;V)*, as described in Fig. 1*b*, was conducted in a genomic background of relatively low Tc*1* mobility and copy number using the mutator *mut-4(st700)* (Mori *et al.* 1988*b*). The *mut-4* locus was derived from *C. elegans* (var. Bergerac) where there is a high copy number and mobility of Tc*1*. It was crossed into the genome of *C. elegans* (var. Bristol) which has a low copy number and no mobility of Tc*1* to make the strain RW7037. Our strain, BC2643, was derived from RW7037.

The genotype of the mutator hermaphrodite strain is *mut-4(st700)I;unc-22(s7)/nT1(IV);unc-46(e177)/nT1(V)*. This strain was constructed by virtue of the fact that *mut-4* and *dpy-5* are closely linked on LGI (Mori *et al.* 1988*b*) and that *nT1* causes pseudolinkage between LGIV and LGV (Ferguson and Horvitz 1985). *nT1* is associated with a recessive vulvaless phenotype. The strain construction is shown in Fig. 1*a*. This strain is maintained by selecting wild-type hermaphrodites.

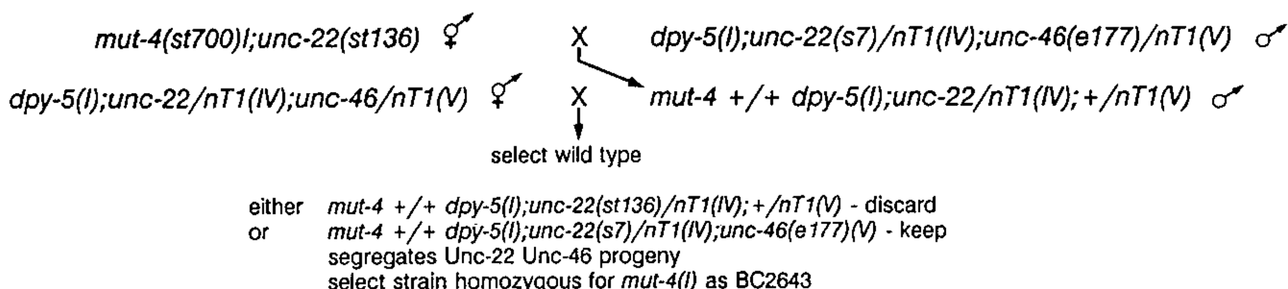
Screen for recessive lethal mutations

Young gravid BC2643 P_0 hermaphrodites were set singly and allowed to lay eggs for 24 h and then were transferred to fresh plates for another 24 h to prevent crowding of the F_1s. The F_1s were examined for the presence of gravid Unc-22 Unc-46s to ensure that all lethal mutations isolated subsequently arose in the germ line of the P_0 hermaphrodites. All wild-type F_1s were then picked individually and allowed to self-fertilize and the F_2s were screened for the absence of gravid Unc-22 Unc-46 individuals (see Fig. 1*b*). At least three wild-type individuals from each strain carrying a putative recessive lethal mutation were selected. The progeny of these were screened for the absence of Unc-22 Unc-46 recombinants to confirm that the mutation lies within the *nT1* balanced region. The translocation *nT1(IV;V)* balances 21 map units on LGIV (right) and 28 map units on LGV (left) (Clark *et al.* 1988). So that the lethal strains could be stably maintained, we removed the *mut-4(st700)* lesion (see Fig. 1*c*).

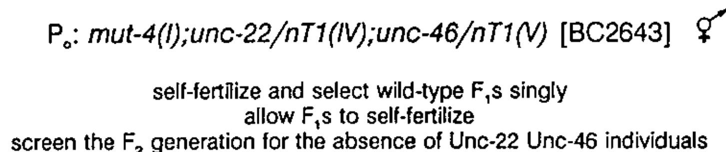
Mapping and complementation tests

Recombination mapping was carried out at a constant 20° following the recommendations of Rose and Baillie (1979). Mapping of lethal mutations isolated in the screen to either LGIV or LGV involved crossing each strain to N2 males and scoring the progeny of one or two hermaphrodites of the genotype *(let-x) unc-22/+ + (IV);unc-46/+ (V)* or *unc-22/+ (IV); (let-x) unc-46/+ + (V)*. A ratio of less than 1:3 for Unc-22:Wild indicated a lethal on LGIV, while a ratio of less than 1:3 for Unc-46:Wild indicated a lethal

A CONSTRUCTION OF BC2643



B SCREEN FOR LETHAL MUTATIONS



C REMOVAL OF MUTATOR ACTIVITY FROM LGI

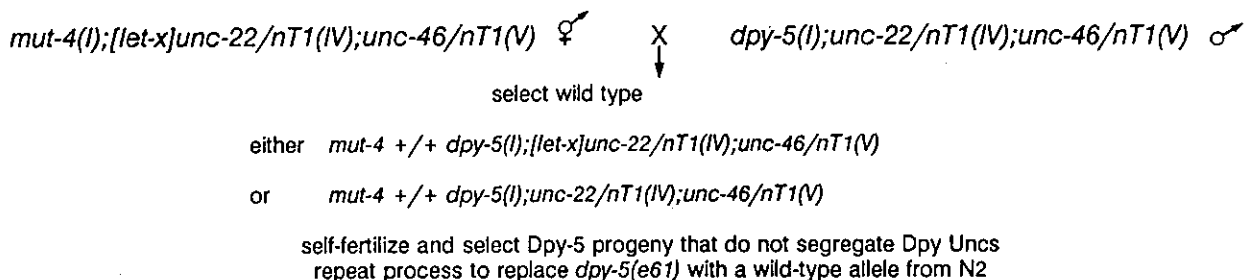


FIG. 1. Major genetic crosses conducted in this study.

on LGV. Complementation tests were performed between LGIV mutations and the deficiencies that lie in the regions of interest to our laboratory: *mDf7*, *sDf2*, and *nDf27*. To perform complementation tests between the mutations mapping to LGV and mutations of known genes and deficiencies in strains constructed in our laboratory (Rosenbluth *et al.* 1988), we constructed strains of the following genotype: *dpy-18(e364)/eT1(III);(let-x) unc-46(e177)/eT1(V)*. *eT1* is a reciprocal translocation that balances the left-most 23 map units of LGV as well as LGIII (right) and causes pseudolinkage between *dpy-18* and the LGV lethals (Rosenbluth and Baillie 1981). The mutations were first localized to regions defined by the following set of overlapping deficiencies: *sDf26*, *sDf30*, *sDf33*, *sDf34*, and *sDf35*. Using the genetic maps from Rosenbluth *et al.* (1988) and Johnsen and Baillie (1988), other appropriate deficiencies were then chosen to position our mutations more precisely. Subsequent complementation tests were conducted between our mutations and alleles of all other known genes in the same subregion. A subregion is defined by an interval on the genetic map flanked by the nearest deficiency breakpoints. For mutations that mapped to more than one subregion, complementation tests against alleles of appropriately selected genes were conducted to determine whether the mutations were the result of multiple point mutations or deficiencies and to determine the extent of the deficiencies. Two-factor mapping data between *unc-46* and some of the LGV mutations are shown in Table 1. Progeny of several hermaphrodites of the genotype *dpy-18/+ (III); (let-x)unc-46/+ (V)* were scored.

Determination of developmental lethal phases of mutant homozygotes

The Dpy-18 Unc-46 progeny of the strains carrying the lethal mutation [*dpy-18/eT1(III);(let-x)unc-46/eT1(V)*] were observed for the approximate stage at which they arrested development. Because the larval molts were not observed, the stage could not be described as L1, L2, L3, L4, or adult. Early larval is approximately L1, midlarval is L2 or L3, and late larval is L4. A strain with no apparent Dpy Uncs was presumed to carry an embryonic or early larval lethal mutation. It is not possible to distinguish between these two possibilities in an *eT1* background because *eT1* segregates two classes of aneuploids: embryonic and early larval lethals (L. M. Addison, personal communication). Therefore, for the cases where no arrested Dpy Uncs were apparent, the progeny of *let-x* or *Dfx/+* individuals were observed for the presence of arrested embryos.

Results

A total of 3503 F_1 "chromosomes" (LGIV (right) and LGV (left)) were screened for the presence of lethal mutations (Fig. 1*b*). Thirty-seven lethal mutations were recovered within the 49 map units balanced by *nT1*. The lethal mutation frequency (37/3503 = 1.1% (0.8–1.4) in the *nT1* region of our *mut-4* strain was significantly higher than the spontaneous frequency in a purely Bristol strain (2/1736 = 0.1% (0.02–0.4)) (Clark *et al.* 1988; 95% confidence limits from Crow and Gardner 1959).

Upon mapping 28 of the lethal mutations to either LGIV or LGV, we found that 24 (86%) of them were on LGV, while only 4 (14%) were on LGIV. This result is in contrast to an EMS mutagenesis screen for lethal mutations in the

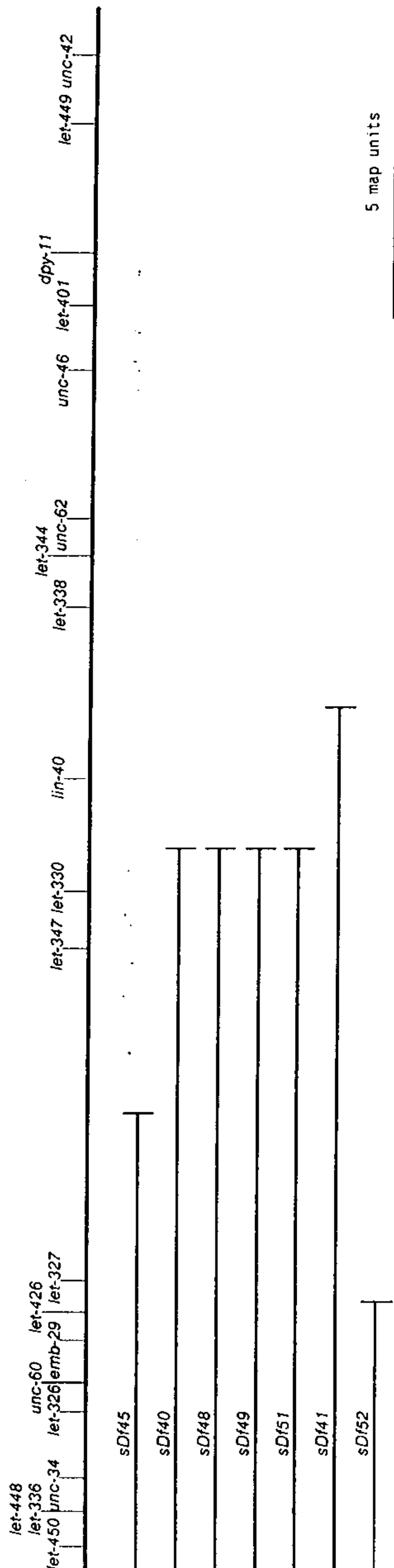


FIG. 2. Partial genetic map of the left end of LGV, showing the set of *mut-4* induced deficiencies and two new complementation groups identified in this study (*let-448* and *let-449*).

nT1 balanced region where 57% of the lethal mutations fell on LGIV and 43% on LGV (Clark *et al.* 1988). The remaining nine lethal mutations all appeared to be essentially unlinked to *unc-22* and *unc-46*. However, the balanced wild-type heterozygotes gave no fertile Unc-22 Unc-46 progeny. The possibility that these strains carry duplications of *unc-22* and *unc-46* or the possibility that the strains contain some other complex rearrangement has not been tested. These nine mutations were not further characterized.

It therefore appeared to us that *mut-4* was active in our strain and that there was at least one mutational hot spot on LGV. In order that the lethal strains could be stably maintained and that there would be no reversion events or subsequent accumulation of additional mutations in the balanced region, we removed *mut-4(st700)* (Fig. 1*c*).

None of the four LGIV mutations fell within the region of interest to our laboratory (the *unc-22* region) when they were tested against the three deficiencies *mDf7*, *sDf2*, and *nDf27* (Edgley and Riddle 1987). These mutations were thus not further characterized. Upon balancing the LGV mutations over *eT1(III;V)* (see Materials and methods), we found that two mutations were not in the *eT1*-balanced region. The remaining 22 LGV mutations were analyzed.

Two mutations define new genes (*let-448* and *let-449*). *let-448* maps between *let-450* and *unc-34*, but it has not yet been positioned relative to *let-336* (see Fig. 2). *let-449* maps 4.5 map units to the right of *unc-46*. Six mutations are alleles of *lin-40*. Thus, it appears that *lin-40* is the hot spot for *mut-4* induced mutational events on LGV (left).

Seven mutations are deficiencies which break in various places on LGV (left). However, they all uncover *let-450*, which is the left-most known gene on LGV (Johnsen and Baillie 1988). Four of these deficiencies have right breakpoints that lie between *let-330* and *lin-40* (see Fig. 2). These four breakpoints have not yet been resolved further. It is important to note that these mutations were of independent origin (see Materials and methods). *let-330* is currently the nearest known gene to the left of *lin-40*. One deficiency (*sDf41*) either breaks within *lin-40* or between *lin-40* and the next known gene (*let-338*) to the right. Therefore, not only is *lin-40* a hot spot for *mut-4* mutagenesis, but the region around *lin-40* is also very susceptible to *mut-4* induced chromosome breakage. This result indicates that there may be a resident Tc*1* element responsible for the mutations in and around *lin-40* (see Discussion).

Two-factor mapping data for some of the LGV mutations can be found in Table 1. The low values for the recombination distances between most of the deficiencies and *unc-46* are consistent with the phenomenon of recombination suppression associated with other deficiencies (Rosenbluth *et al.* 1988, 1990).

The phenotypes of the various mutants are listed in Table 1. They range from embryonic to late larval developmental blocks. For the deficiency *sDf52*, we examined the progeny of *sDf52*/+ hermaphrodites and found some that arrested in the embryonic stage. Because *sDf52* is the smallest deficiency (see Fig. 2), we presumed that the other deficiencies also have an embryonic lethal phenotype.

The other seven *mut-4* induced mutations on LGV caused low fertility and were difficult to work with. We suspect that they were caused by complex mutational events or were large deficiencies, or that Tc*1* was still mobile in these strains.

A *mut-4* strain has been isolated in the past that contained additional mutator activity linked to LGIV (Mori *et al.* 1988*b*). Therefore, some strains that carry *mut-4* induced lethal mutations could also be carrying mutator activity, in spite of the fact that *mut-4* was removed from the strains.

Discussion

The lethal mutation frequency of the mutator strain for the 49 map units balanced by *nT1(IV;V)* was 1%. This is 10 times higher than the spontaneous lethal mutation frequency for the same region in a purely Bristol strain and one-ninth the frequency produced by 0.012 M EMS in a Bristol strain (Clark *et al.* 1988). The distribution of the 28 *mut-4* induced lethal mutations between LGIV (right) and LGV (left) was strikingly different from the distribution of EMS-induced lethal mutations. Of the *mut-4* induced lethals, 14% were on LGIV and 86% were on LGV, while 57% of the EMS mutations fell on LGIV and 43% fell on LGV (Clark *et al.* 1988). The six alleles of *lin-40* and the five deficiencies that break on either side of *lin-40* account for a large part of this difference in distribution of lethal mutations.

From the analysis of mutator-induced lethal mutations on LGV (left), we can conclude that different types of mutational events can be isolated from strains containing *mut-4(st700)*. Only Tc*1*-induced mutations have so far been recovered from RW7000, from which RW7037 was derived (Moerman *et al.* 1988), which makes it probable that the mutations derived from BC2643 are Tc*1* induced also. There are three basic classes of lethal mutations recovered in this screen. The first class is lethal mutations (7 of 22 LGV lethals) which, in a heterozygote, result in a decrease in fertility and (or) viability below that expected for point mutations and relatively small deficiencies. These are difficult to analyze and most likely the result of complex mutational events, involving more than one chromosomal break (Rosenbluth *et al.* 1985). They could also be large deficiencies that have some dominant effect on viability. The second class is deficiencies (7/22) amenable to analysis. These deficiencies are distinctly different from those isolated in gamma ray mutagenesis screens (Rosenbluth *et al.* 1985) and formaldehyde mutagenesis screens (Johnsen and Baillie 1988) in that they all delete the end of the chromosome on the genetic map. Gamma ray and formaldehyde mutagenesis can produce chromosomally internal deficiencies as well as ones that delete the end of the chromosome on the genetic map. It is not known if any LGV deficiencies actually delete the physical end of the chromosome. The *mut-4* induced deficiencies could be the result of an excision event followed by improper repair of the chromosomal break. They could also be half translocations that were brought about by recombination between homologous transposable or other repetitive elements. Another possibility is that there is a resident Tc*1* element to the left of *let-450*. Together with another Tc*1* element on LGV, the two elements could excise or recombine with each other to produce a deficiency. The third class of mutations (8/22) is the apparent point mutations. We have no formal proof that all of these mutations are Tc*1* insertions and not small deficiencies. LGV (left) is 81% saturated for mutations in essential genes, using a variety of mutagens (Johnsen and Baillie 1988; Rosenbluth *et al.* 1988). Therefore there is a possibility that the apparent point mutations are small deletions that include neighbouring unidentified essential genes. Probing *Eco*RI-digested genomic DNA from the six *lin-40* strains with the transposable element Tc*1* shows that three alleles share a common Tc*1* band not found in wild-type *C. elegans* (var. Bristol) or in the mutator strain BC2643 (R.C. Johnsen and D.L. Baillie, unpublished results). This indicates that the mutations in these three strains may be Tc*1* insertions in *lin-40*, all in the same *Eco*RI fragment. Although all six *mut-4* induced *lin-40* mutations are early larval lethals, *lin-40* was originally identified with a recessive adult sterile mutation (*e2173*) that affected vulva development (J. Hodgkin, personal communication).

Previously, it has been shown that Tc*1* inserts at a high rate in some genes relative to others (for review see Herman and Shaw 1987). It is possible that the lethal mutations that appear to be point mutations are simple insertions of Tc*1* and that *lin-40* is the hot spot for insertion in the *nT1* balanced regions of LGIV (right) and LGV (left). Studies of Tc*1* insertion mutants in the muscle gene *unc-22* have shown that there is a 9-bp consensus insertion sequence with a central completely conserved TA insertion site (Mori *et al.* 1988*a*). Perhaps the high mutation rate of *lin-40* in our *mut-4* strain is due at least in part to the presence of this consensus sequence.

Studies of P element mutagenesis in the zeste–white region of *Drosophila melanogaster* using dysgenic P-M hybrids have shown that P element mobility can result in insertions, deficiencies, and inversions (Simmons and Lim 1980). It was also noted that hybrid dysgenesis induced mutations occurred at preferred sites. Simmons *et al.* (1984) described two major classes of P element mutation mechanisms: (*i*) local P element activity of elements that naturally reside near the mutated locus and (*ii*) P element transposition and insertion at a new site. One explanation for mutational hot spots of P elements comes from studies on a wild-type revertant of a P element induced yellow allele (Geyer *et al.* 1988). A residual P element sequence in the revertant allele appears to be a preferred site for introduction of new P elements not through insertion but through either recombination or gene conversion. If there are Tc*1* sequences within or near *lin-40*, then a similar process may explain the mutational hot spot on LGV. Alternatively, the presence of a Tc*1* element that is resident between *let-330* and *lin-40* and that excises imprecisely could explain the existence of the *lin-40* alleles. The deficiencies with a breakpoint on either side of *lin-40* could also be explained by the mobilization of this resident Tc*1* element if the excision is not followed by proper repair of the chromosomal break or by an interaction with a second element to the left of *let-450*, as discussed above.

We have shown that the recovery of mutations in essential genes (*let-448* and *let-449*) not previously identified by EMS, formaldehyde, or gamma-radiation mutagenesis (McKim *et al.* 1988; Johnsen and Baillie 1988; Rosenbluth *et al.* 1988) is feasible when mutator strains are used as the source of lethal mutations. The LGV (left) region is 81% saturated for mutations in essential genes. Therefore, there is still a possibility that identification of new genes is simply the result of the region not being fully saturated.

We conclude that it is feasible to obtain lethal mutations with the *mut-4* strain used in this study at a frequency of 1% (of chromosomes screened). A significant fraction of these lethals (at least 7/28) are rearrangements. At least 8/28

mutations appear to affect single genes. The three genes (*let-448*, *let-449*, and *lin-40*) with *mut-4* induced alleles are thus three essential genes that can possibly be cloned by Tc*1* tagging. The mutator strain used in this study generated many mutations in and around one gene, *lin-40*. It is possible that the mutation frequency of a particular essential gene could vary among different mutator strains, owing to the activity of the mutator or the type of transposable element that is mobilized.

Acknowledgements

The authors thank Raja Rosenbluth, Terry Starr, and Ann Rose for their valuable comments, and Margaret Rogalski for her technical assistance. The authors extend special thanks to Don Moerman and Ikue Mori for providing RW7037 and mapping information prior to publication. This research was supported by a Muscular Dystrophy Association (MDA) of Canada fellowship to D.V.C., a Medical Research Council (Canada) studentship to R.C.J., a Natural Sciences and Engineering Research Council of Canada (NSERC) summer scholarship to K.S.McK., and MDA of Canada and NSERC grants to D.L.B.

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