THE GENETIC ANALYSIS OF A RECIPROCAL TRANSLOCATION, *eT1(III; V)*, IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

The *Caenorhabditis elegans* mutation *e873*, which results in a recessive uncoordinated phenotype (formerly named Unc-72) and which had been isolated after ^{32}P treatment (BRENNER 1974), has now been found to act as a crossover suppressor and to be associated with a translocation between linkage groups (*LG*'s) *III* and *V*. The translocation has been named, *eT1(III; V)*; *eT1* acts as a dominant crossover suppressor for both the right half of *LGIII* and the left half of *LGV*, providing a balancer for a total of 39 map units. The uncoordinated *e873* phenotype has been shown to be a consequence of an inactive *unc-36III* gene. It was possible to demonstrate that, in translocation heterozygotes, *eT1* chromosomes marked with either *sma-3* or *dpy-11* segregate from normal *LGIII*, while those marked with *bli-5*, *sma-2* or *unc-42* segregate from normal *LGV*. Since *bli-5* and *sma-2* are normally on *LGIII*, and *dpy-11* is normally on *LGV*, it is concluded that: (a) *eT1* is a reciprocal translocation; (b) there is a breakpoint between *sma-3* and *sma-2* in *LGIII* (the region containing *unc-36*) and one between *dpy-11* and *unc-42* in *LGV*; (c) there is no dominant centromere between *sma-2* and *bli-5* on *LGIII*, since in *eT1* these genes are not linked to a *LGIII* centromere. Similarly, it is highly unlikely that there is a centromere to the left of *dpy-11* on *LGV*. The new gene order in *eT1* was determined by measuring recombination rates between markers in *eT1* homozygotes. It is concluded that the new order is: *dpy-1 sma-3* (*break*) *dpy-11 unc-60*, and *bli-5 sma-2* (*break*) *unc-42 unc-51*.——This is the first analysis of a *C. elegans* translocation with respect to reciprocity, breakpoints and new gene order.

IN the first part of this paper, we identify a new crossover suppressor for the nematode *Caenorhabditis elegans* and show it to be associated with a translocation. We then characterize this translocation with respect to its reciprocity, its breakpoints, and its new gene order.

The genetic analysis of *C. elegans* was begun 16 years ago by BRENNER (reviewed by BRENNER 1974; RIDDLE 1978; HERMAN and HORVITZ 1980). Previous studies have shown that region-specific dominant crossover suppressors are inducible in *C. elegans* (HERMAN, ALBERTSON and BRENNER 1976; HERMAN 1978). Crossover suppressors have been used successfully as balancers for the detection and maintenance of unconditional recessive lethal mutations (HERMAN 1978; MENEELY and HERMAN 1979). Initially three suppressors were obtained by HERMAN: *mnDp1 (X;V)* (HERMAN, ALBERTSON and BRENNER 1976), *C1* and *C2* (HERMAN 1978). Subsequently, four more have been identified: three by FODOR

and DEAK (personal communication), and one by FERGUSON and HORVITZ (personal communication). These seven suppressors provide balancers for about half the *C. elegans* genome. The present report adds a new balancer for a previously unbalanced region: the right half of linkage group *(LG)III.*

In *Drosophila melanogaster,* region-specific dominant crossover suppressors are generally associated with chromosomal inversions or translocations (reviewed by ROBERTS 1976). HERMAN showed that, in *C. elegans,* pseudolinkage data, taken together with egg survival data, can be used to determine whether a given crossover suppressor involves a translocation (HERMAN 1978). Using such data, three of the seven above suppressors have been shown to be associated with translocations: *mnT1(II;X)* (formerly C2, HERMAN 1978), *szT1(I;X)* (DEAK and FODOR, personal communication and *nT1(IV;V)* (FERGUSON and HORVITZ, personal communication).

This paper reports an analysis of the mutation *e873,* which results in a recessive uncoordinated phenotype. The mutation had originally been isolated after ^{32}P treatment as a presumptive point mutation near *dpy-11 V* and had been assigned to a new gene, *unc-72* (BRENNER 1974). We now demonstrate that *e873* is associated with a crossover suppressor for the right arm of *LGIII* and the left arm of *LGV* and that its uncoordinated phenotype is due to defective *unc-36 III* gene activity, rather than to a mutation on *LGV.* The crossover suppression properties are shown to be associated with a translocation between *LGIII* and *V.*

Simple translocations may be of two types: reciprocal or insertional (ROBERTS 1976). Cytological mapping makes it possible to distinguish these translocations from each other in Drosophila, where a large number of them have been analyzed with respect to their reciprocity, breakpoints and new gene orders. Detailed cytological mapping is not possible in *C. elegans,* and, as yet no such analyses are available for any of the previously identified *C.* elegans translocations. Therefore, we use genetic analysis to demonstrate the reciprocity of our translocation, to localize its breakpoints and to determine its new gene order.

The locations of centromeres in *C. elegans* are still unknown. An incidental result of this study is the identification of regions on the normal linkage groups *III* and *V* that we believe to be free of any dominant centromeres.

MATERIALS AND METHODS

General: Nematodes were maintained and mated on petri plates of nematode growth medium (NGM), streaked with *Eschericia coli,* strain OP50 (BRENNER 1974). Crosses were carried out on 10 × 35 mm petri plates. Since a recent study (ROSE and BAILLIE 1979) has shown that temperature and parental age affect recombination in *C. elegans,* experiments involving recombination frequencies were carried out at 20°, and all the progeny of an individual worm were scored. Overcrowding was avoided by transferring the parental worm to a fresh plate every 12 to 16 hr. The nomenclature follows the uniform system adopted for *Caenorhabditis elegans* (HORVITZ *et al.* 1979).

Marker mutations: The wild-type strain N2 and some of the mutant strains of *C. elegans* var. Bristol were originally obtained from the MRC stock collection at Cambridge, England. Others were received from the Caenorhabditis Genetics Center at the University of Missouri, Columbia. Many of the mutations were outcrossed to N2 and allowed to resegregate before use. Mutant genes and alleles utilized are listed below:

LGI: *unc-11(e47)*; *unc-67(e713)*; *dpy-5(e61)*; *unc-13(e51)*; *unc-54(e190)*. *LGII*: *unc-4(e120)*. *LGIII*: *dpy-1(e1)*; *dpy-17(e164)*; *sma-3(e491)*; *unc-36(e251)*; *sma-2(e502)*; *unc-32 (e189)*; *unc-16(e109)*; *unc-47(e307)*; *unc-49(e382)*; *unc-50(e306)*; *dpy-18(e364)*; *unc-64(e246)*; *bli-5(e518)*; *eDf2(e1555)*. *LGIV*: *unc-5(e152)*. *LGV*: *unc-60(e677)*; *unc-46(e177)*; "*unc-72*" (*e873*; this name has now been discontinued; see RESULTS); *dpy-11(e224)*; *unc-42(e270)*; *unc-41(e268)*; *unc-51(e369)*. *LGX*: *unc-6(e78)*.

The map positions of the markers used are shown in Figure 1 and are based, for the most part, on the updated map of HERMAN and HORVITZ (1980). Three markers, *unc-36*, *bli-5* and *unc-67*, have been shifted on the basis of the three-factor mapping shown in Table 1. The distance between *bli-5* and *unc-64* has been estimated to be less than 0.6 map units (m.u.) as measured by progeny testing Unc-64 F_1's from *unc-64/bli-5 trans*-heterozygotes. Of 85 F_1's tested, none segregated any Bli progeny. Other relevant distances mentioned in the text were rechecked using *cis*-heterozygotes (BRENNER 1974).

Mutagenesis and screening: P_0 hermaphrodites of the appropriate gentotype were suspended for 4 hr at room temperature in 4 ml M-9 buffer (BRENNER 1974) containing ethyl methanesulfonate (EMS). Single young adult P_0's were placed on 15 × 100 mm NGM plates and were twice transferred to fresh plates at 12- to 24-hr intervals.

Screen for visible mutations linked to eT1e873: One hundred *eT1e873* P_0,s were treated with 0.0125 M EMS, and their F_1 progeny were left on the plates until approximately 24 hr after egg-laying had begun. These F_1's were then washed off with a gentle stream of distilled water, leaving behind the F_2 eggs. The F_2 adults were screened for obvious visible mutants. For any one P_0 plate, only one mutant F_2 worm was kept. Putative *dpy* and *sma* mutations were

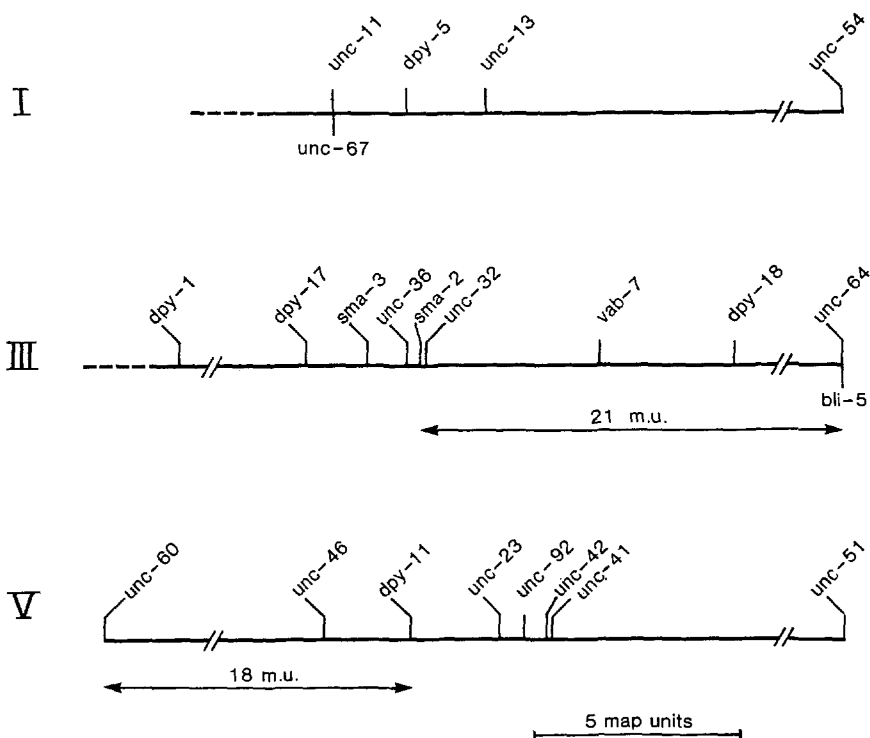

FIGURE 1.—The relative positions of the genes on *LGI*, *LGIII* and *LGV* used in this work (modified from HERMAN and HORVITZ 1980). Genes labeled under the line have not been left-right positioned with respect to the marker above them.

TABLE 1

Three-factor mapping

Gene	Genotype of heterozygote	Phenotype of recombinant	Frequency and genotype of recombinant chromosome
unc-67	*dpy-5 unc13/unc-67*	Dpy-5	3/3 *dpy-5* +
	unc-11 dpy-5/unc-67	Dpy-5	8/8 *unc-67 dpy-5*
	unc-67 dpy-5/unc-11	Dpy-5	23/23 *unc-11 dpy-5*
unc-36	*unc-36 dpy-18/unc-32*	Unc-36	28/30 *unc-36* + 2/30 *unc-36 unc-32*
		Dpy-18	1/31 + *dpy-18* 30/31 *unc-32 dpy-18*
	unc-36 unc-32/sma-2	Unc-36	1/4 *unc-36* + 3/4 *unc-36 sma-2*
	dpy-17 unc-36/sma-3	Unc-36	9/13 + *unc-36* 4/13 *sma-3 unc-36*
bli-5	*dpy-18 unc-64/bli-5*	Dpy-18	15/15 *dpy-18 bli-5*
		Unc-64	17/17 + *unc-64*

then tested for allelism to *dpy-11* (BRENNER 1974). Non-*dpy* mutations were tested for linkage to *e873* by outcrossing them to N2 males and scoring the progeny of the resulting heterozygotes. The mutations that showed linkage were tested for allelism with mutations in known genes exhibiting a similar phenotype that are located in the crossover-suppressed regions of *LGIII* and *V*.

Screen for lethals on LGIII *and* LGV: Ten P_0's (see RESULTS for genotype) were treated with 0.025 M EMS. From these, 500 wild-type adult F_1's were placed individually on 10 × 35 mm plates and removed after 24 hr. From plates showing no Dpy Unc progeny, two putative lethal-bearing wild-type F_2's were retested. Any lineage that continued to show no Dpy Unc's for two more generations was considered to carry a lethal mutation.

Mapping of lethals on LGIII *and LGV*: To map the lethals on one of the two marked chromosomes (see RESULTS), wild-type hermaphrodites from each lethal strain were crossed to N2 males. The F_2 progeny from lethal-bearing *dpy-18/+*; *unc-46/+* F_1's were then scored. A control experiment with no lethal present gave 488 wild-type: 180 Dpy: 157 Unc: 61 Dpy Unc, indicating that, as expected, the two phenotypic ratios (Dpy: wild-type and Unc-46: wild-type) were each approximately 1:3. A lethal was assigned to the right arm of *LGIII* if the number of F_2 Dpy-18's was considerably less than one-third the number of wild types. If the Unc-46: wild-type ratio was less than 1:3, the lethal was assigned to the left arm of *LGV*. The map distance between a *LGIII* lethal and *dpy-18* was then calculated as $100(1 - \sqrt{1 - 3D/D + W})$, where D = the number of F_2 Dpy's and W = the number of F_2 wild types. Similarly, the distance between a *LGV* lethal and *unc-46* was based on the number of F_2 Unc's and wild types.

RESULTS

e873 *acts as a region-specific dominant crossover suppressor*: When first studied, the uncoordinated mutation *e873* was thought to define a new gene, named *unc-72* mapped to within 1.0 m.u. of *dpy-11 V* (BRENNER 1974). This position was based on the inability to recover dumpy-uncoordinated individuals from *dpy-11/e873* (BRENNER, personal communication). While working with other mutations on *LGV*, we wanted to position *e873* precisely with respect to *dpy-11*. Right-left positioning was therefore attempted using *unc-46 dpy-11/e873* and *dpy-11 unc-42/e873* heterozygous hermaphrodites. Unexpectedly, *e873* acted

as though it were associated with a dominant crossover suppressor. Table 2 (top section) compares the phenotypic frequencies of recombinants from the above heterozygotes with those from controls lacking *e873*. The experimental and control values should have been the same if no suppression occurred. It can be seen that the presence of *e873* suppressed recombination between *unc-46* and *dpy-11* and between *dpy-11* and *unc-42*.

In order to determine whether this suppression occurs throughout the *C. elegans* genome, we checked the effect of *e873* on crossing over in the rest of *LGV* and in a region of another linkage group, *LGI*. The middle of Table 2 gives the results for the regions *unc-60 dpy-11 V*, *dpy-11 unc-51 V*, and *dpy-5 unc-54 I*. The data show that *e873* suppressed crossing over only in the *unc-60 dpy-11* region. Thus, *e873* does not act as a crossover suppressor for the whole genome, and in *LGV* the suppression is limited to the left half of the chromosome. The boundary of suppression lies between *dpy-11* and *unc-42*.

e873 *is associated with a translocation*: Suppression of crossing over in specific regions of a eucaryotic genome is generally associated with chromosomal rearrangements such as translocations or inversions (ROBERTS 1976; HERMAN 1978). To determine whether *e873* is associated with a translocation involving a second chromosome, we tested genes on other linkage groups for pseudolinkage to *dpy-11 V* (see ROBERTS 1976). Heterozygous *dpy-11* + / + *e873* males were crossed with homozygous *dpy-11* + ; *unc-x* hermaphrodites, where *unc-x* was either *unc-13 I*, *unc-4 II*, *unc-32 III*, *unc-5 IV* or *unc-6 X*. The progeny from wild-type F_1 hermaphrodites were scored. If the *unc-x* gene were linked to an *e873*-associated translocation, it would have shown pseudolinkage to *dpy-11 V*. If it were not linked to such a translocation, the ratio of Dpy Unc : Dpy non-Unc should have been 1:3. One gene, *unc-36 III*, did show pseudolinkage; among a total of 558 individuals, there were 102 Dpy Unc, but no Dpy non-Unc progeny. Thus, *e873* appears to be associated with a translocation involving linkage groups *III* and *V*.

TABLE 2

Effect of e873 *on recombination*

Region tested	Frequencies of recombinants (total adult progeny). Dpy or Sma (non-Unc)	
	Without *e873*	With *e873* in *trans*
unc-46 dpy-11 V	0.011 (1894)	0 (1178)
*dpy-11 unc-42 V**	0.018 (2248)	0.0016 (2447)
unc-60 dpy-11 V	0.086 (2436)	0.0026† (1539)
dpy-11 unc-51 V	0.133 (3112)	0.136 (1143)
dpy-5 unc-54 I	0.136 (2884)	0.134 (904)
dpy-1 unc-32 III	0.095 (1094)	0.15 (866)
sma-3 unc-64 III	0.095 (1288)	0.004 (1543)
sma-2 unc-64 III	0.079 (1741)	0 (2942)

* Unc-42 recombinants were also scored: in the absence of *e873*, their frequency was 0.016; with *e873*, the frequency was 0.0037.

† The four Dpy individuals recovered were not normal recombinants: two were sterile and the other two carried a homozygous lethal dumpy chromosome.

To determine whether egg-survival data and segregation ratios from *e873* heterozygous hermaphrodites were consistent with the presence of a translocation, the following assumptions were made: (1) There are two abnormal chromosomes; (2) in translocation heterozygotes, these segregate from normal linkage groups *III* and *V*, respectively (*i.e.*, there is no adjacent-2 disjunction); (3) the chromosomes segregate independently (*i.e.*, alternate and adjacent-1 disjunction occur randomly); (4) euploid and aneuploid gametes, both egg and sperm, are formed at equal frequencies and fuse; and (5) aneuploid zygotes are laid as eggs, but only euploid zygotes survive to adulthood. These assumptions are the same as those used by HERMAN (1978) to interpret his *mnT1*(*II*;*X*) data. Table 3 (column 2) gives the percentage of eggs laid by hermaphrodites that developed to adulthood. For eggs from *e873*/+ hermaphrodites, this is expected to be 37.5%. If adjacent-2 disjunction occurs as well, the expected value is 22%. It can be seen that the actual egg survival from *e873*/+ hermaphrodites was 36%, which agrees well with the predicted value and indicates that adjacent-2 disjunction does not occur.

The segregation ratio from *unc-46 dpy-11*/*e873* hermaphrodites supports this conclusion. The phenotypic ratio of Unc-46 Dpy-11 : wild type : Unc(*e873*) should be 1:4:1, but if adjacent-2 disjunction also occurs, the ratio should be 1:6:1. Among a total of 1178 progeny, there were 221 Unc-46 Dpy-11, 763 wild type and 194 Unc (*e873*) individuals. These numbers do not differ significantly from a 1:4:1 ratio ($\chi^2=3.8$), and fit neither a normal 1:2:1 nor a 1:6:1 ratio. Thus, all data agree with the interpretation that *e873* is associated with a translocation in which no adjacent-2 disjunction occurs. The translocation has now been named *eT1*(*III*;*V*) or *eT1*. It should be noted that the homozygote is viable in both sexes, and its fertility in hermaphrodites is shown in Table 3, the third column of which gives the mean number of adult progeny per hermaphrodite. For *e873*/+ heterozygotes, this was almost 36% of the homozygous wild-type value, indicating roughly that a normal number of zygotes were produced (36% of which reached adulthood). *e873*/*e873* hermaphrodites, on the other hand, apparently produce only about half the normal number of zygotes.

Crossover suppression in LGIII: We next determined the effect of *eT1* on crossing over in *LGIII*. From heterozygous hermaphrodites, carrying two *cis* markers (with and without *e873* in the *trans* position) the frequency of Dpy non-

TABLE 3

Egg survival and total adult progeny

Genotype of parental hermaphrodite	Percentage of eggs reaching adulthood*	Mean number of adult progeny per hermaphrodite†
+/+	100 (252)	319 (6)
e873/*e873*	96 (189)	163 (7)
+/*e873*	36 (571)	103 (15)

* Determined as described by HERMAN (1978). The total number of eggs counted is in parentheses.

† The numbers of parent hermaphrodites are in parentheses.

Unc or Sma non-Unc recombinant progeny was measured. In the course of this work, we found that *unc-67* is not at the right end of *LGIII*, as had been previously reported (BRENNER 1974), but maps about 3 m. u. to the left of *dpy-5* on *LGI*. In addition, *bli-5 III* was found to be within less than 0.6 m.u. from *unc-64 III*. Previously, it had been placed about half-way between *dpy-18* and *unc-64* (BRENNER 1974). Consequently, *unc-64* and *bli-5* are now the most distal right-hand markers on *LGIII*. Going from left to right on the *LGIII* linkage map, *eT1* affects crossing over as follows (Table 2, bottom section):

dpy-1 to *unc-32*, no suppression
sma-3 to *unc-64*, partial suppression
sma-2 to *unc-64*, total suppression.

Therefore, *eT1* is a dominant crossover suppressor for the right half of *LGIII*, as well as for the left half of *LGV*. The boundary of suppression in *LGIII* is between *sma-3* and *sma-2*. These suppressions occur not only in heterozygous hermaphrodites, but also in heterozygous males.

The localization of the Unc(e873) *defect*: With the finding that *e873* is associated with a crossover suppressor and a translocation, the Unc(*e873*) phenotype could be the result of a defect caused by a break or an additional mutation anywhere in the suppressed regions of the two chromosomes. It could, therefore, be a defect in an already identified *unc* gene (outside the *dpy-11* region to which it had originally been mapped). Since the *e873* heterozygotes used in the above experiments were phenotypically wild type, we already knew that it was not a defect in the *unc-32*, *unc-64*, *unc-60*, *unc-46* or *unc-42* genes. Furthermore, we found that *eDf2(III)*, which is a deficiency of the right arm of *LGIII* from *vab-7* to *unc-64* (HODGKIN, personal communication), also complements the Unc (*e873*) phenotype. We then checked several mutations in genes mapping around *unc-32 III*, to see if any fail to complement *e873*. The mutations tested were *unc-16(e109)*, *unc-36(e251)*, *unc-47(e307)*, *unc-49(e382)* and *unc-50(e306)*. All except *e251* complement *e873*. The failure of *e251* to do so indicates that the Unc (*e873*) phenotype is due to defective *unc-36* gene activity. Consistent with this, we found that, as homozygotes, *e873* and *e251* have very similar visible phenotypes and that both produce only about half the normal number of zygotes. However, in contrast to *e873*, *e251* does not behave like a translocation: as a heterozygote 100% of its eggs reach adulthood, it shows no pseudolinkage to *dpy-11*; and it does not suppress crossing over.

The *unc-36 III* gene had previously been mapped near *unc-32 III* (BRENNER 1974). We decided to determine its position more precisely. By means of three-factor mapping (Table 1), we first positioned it to the left of *unc-32* (using *unc-36 dpy-18/unc-32*). We further placed it to the left of *sma-2* (using *unc-36 unc-32/sma-2*) and to the right of *sma-3* (using *dpy-17 unc-36/sma-3*). Finally, two-factor mapping established its distance from *sma-3* and *sma-2*. *sma-3 unc-36/+ +* heterozygotes segregated 2821 wild-type, 785 Sma Unc, 15 Sma non-Unc and 19 Unc non-Sma, giving a distance of 0.9(0.6–1.2) m.u. between *sma-3* and *unc-36*. *unc-36 sma-2/+ +* heterozygotes segregated 3021 wild-type, 681 Sma Unc, 4 Sma non-Unc and 8 Unc non-Sma, giving a distance of 0.3(0.2–0.5) m.u. between *unc-36* and *sma-2*.

Is eT1 (III;V) *a reciprocal translocation?* The data described so far did not indicate whether the translocation is reciprocal. All we knew was that *eT1* consists of two aberrant chromosomes, which will be referred to as *eT1(III)* (segregating from normal *LGIII*) and *eT1(V)* (segregating from normal *LGV*). In order to test *eT1* for reciprocity, we decided to determine whether any *LGIII* genes are on *eT1(V)*, and, conversely, whether any *LGV* genes are on *eT1(III)*. If both types of genes exist, the translocation is reciprocal.

We therefore needed *eT1* chromosomes marked with mutations in known genes. These were obtained by both recombination and mutagenesis.

By crossing over, an existing mutation in a gene closely linked to the crossover-suppressed regions was recombined onto an *eT1* chromosome. For example, Unc-42 recombinants from *dpy-11 unc-42/e873* heterozygotes were recovered (see footnote, Table 2). These recombinants segregated phenotypically Unc-36 Unc-42 individuals (*i.e.*, homozygous *e873 unc-42*) in their progeny, and a strain of homozygous *e873 unc-42* hermaphrodites was established. To confirm that the *eT1* translocation was also present, these hermaphrodites were crossed to N2 males. Eggs laid by the resulting wild-type heterozygotes were counted. Less than 37% of these survived to adulthood, indicating the presence of *eT1*. Thus, we now had a strain in which one of the two translocation chromosomes was marked with *unc-42*. Similarly Sma-3 recombinants from *sma-3 unc-64/e873* (Table 2) provided an *eT1* strain marked with *sma-3*.

In addition, mutations were induced in genes lying within the crossover-suppressed regions. For this purpose, *eT1e873* hermaphrodites were treated with 0.0125 M EMS and the F_2 progeny were screened for new visible mutations. Of 82 putative *dpy* or *sma* mutations that were retained, one, *s287*, failed to complement *dpy-11(e224)V*, and another, *s262*, failed to complement *sma-2(e502) III*. In addition, we obtained a *bli* mutation, *s277*, which failed to complement *bli-5 (e518)III*. To confirm that these mutations were in the crossover-suppressed regions, mutant hermaphrodites were crossed to N2 males, and wild-type F_1 hermaphrodites were picked. As expected, no recombinants were found among the F_2 progeny from *dpy-11(s287) eT1e873/+ +* (1,108 F_2's), *sma-2(s262) eT1e873/+ +* (1,728 F_2's), and *bli-5(s277) eT1e873/+ +* (851 F_2's) heterozygotes.

We now had five separate *eT1* strains, each carrying a marker in a known gene. To determine on which of the two translocation chromosomes, *eT1(III)* or *eT1(V)*, these genes are localized, the experiments presented in Table 4 were done. Appropriate heterozygous translocation genotypes were constructed, and the crosses shown in column 2 were made. From these, single wild-type F_1 hermaphrodites were selected and allowed to "self." The genotypes of these F_1's were expected to be heterozygous for the two translocation chromosomes (both unmarked except for *e873*) and also heterozygous for one diagnostically marked normal homologue. The diagnostic chromosome was expected to have either a single or double marker. In the case of each cross, the ratio of F_1's carrying a single to those carrying a doubly marked chromosome was predicted for the two possibilities: either the gene being tested is on *eT1(III)* (column 3) or it is on *eT1(V)* (column 4). The actual F_1 genotypes obtained were deduced from the F_2 pheno-

TABLE 4

Mapping of genes in eT1 *to either* eT1(III) *or* eT1(V)

Gene tested	Parental genotypes	Wild-type F_1 hermaphrodites No. carrying single: no. carrying double diagnostic marker — Predicted ratios: If gene is on *eT1(III)*	Predicted ratios: If gene is on *eT1(V)*	Actual numbers	Gene location
sma-3	*sma-3 unc-64/eT1sma-3* ⚥ × *sma-3 +/eT1* ♂	0:1	1:1	0:21	*eT1(III)*
	sma-3 +/eT1sma-3 ⚥ × *sma-3 unc-64/eT1* ♂	1:0	1:1	26:0	
dpy-11	*+ dpy-11/eT1dpy-11* ⚥ × *unc-60 dpy-11/eT1* ♂	1:1	1:0	5:13	*eT1(III)*
sma-2	*sma-2 unc-64/eT1sma-2* ⚥ × *sma-2 +/eT1* ♂	0:1	1:1	9:8	*eT1(V)*
bli-5	*+ bli-5/eT1bli-5* ⚥ × *dpy-18 bli-5/eT1* ♂	1:0	1:1	18:20	*eT1(V)*
unc-42	*dpy-11 unc-42/eT1unc-42* ⚥ × *+ unc-42/eT1* ♂	1:1	0:1	0:15	*eT1(V)*
	+ unc-42/eT1unc-42 ⚥ × *dpy-11 unc-42/eT1* ♂	1:1	1:0	14:0	

types, and the actual F_1 numbers are given in column 5. In making the predictions, we assumed that the pattern of disjunction in heterozygous *eT1* males is similar to that found for the hermaphrodites and that aneuploid male sperm are viable. The results from the crosses localizing *sma-2*, *bli-5* and *dpy-11* indicate that heterozygous *eT1* males do produce viable sperm arising from both alternate and adjacent-1 disjunction. Our predictions were therefore valid, and column 6 gives the translocation chromosome on which the tested gene must be localized. [If heterozygous *eT1* males underwent only alternate disjunction, the same results would have been expected in the case of the crosses testing *sma-3* and *unc-42*, regardless of whether these genes were on *eT1 (III)* or *eT1 (V)*.] Thus *sma-3* and *dpy-11* were found to be on *eT1(III)*, while *sma-2*, *bli-5* and *unc-42* are on *eT1(V)*. Since *dpy-11* is normally on *LGV*, and *sma-2* and *bli-5* on *LGIII*, we conclude that *eT1 (III;V)* is a reciprocal translocation.

Locating the translocation breakpoints and establishing the new gene order in eT1(III;V): The fact that the Unc-42 recombinants from *dpy-11 unc-42/e873*

carried *eT1* (see previous section) told us that a *LGV* breakpoint must lie to the left of *unc-42*. Since translocation breakpoints are not necessarily at the boundaries of the crossover-suppressed regions (ROBERTS 1970 and 1972; HAWLEY 1980), this meant that the breakpoint could be anywhere in the left half of *LGV*. Similarly, the Sma-3 recombinant data indicated a *LGIII* breakpoint anywhere to the right of *sma-3*. The results presented in Table 4 now allowed a more precise localization of these breakpoints. That is, *sma-3* and *sma-2*, which are normally on the same chromosome (*LGIII*), were found to be on different *eT1* chromosomes. Therefore, a linkage group *III* breakpoint must exist between *sma-3* and *sma-2*, *i.e.*, in a region of about 1.2 m.u. containing *unc-36* and the *LGIII* crossover-suppression boundary (Table 2). Similarly, a linkage group *V* breakpoint must lie between *dpy-11* and *unc-42*, a region of about 3.5 m.u., containing the *LGV* suppresion boundary (Table 2). Thus, both *eT1* breakpoints do, in fact, lie close to the crossover-suppression boundaries.

We now asked, what is the new gene order in the two translocation chromosomes? From both the crossover suppression data and the Sma-3 and Unc-42 data above, as well as from the data in Table 4, we drew the simplest conclusions, assuming only one breakpoint in each chromosome: *eT1(V)* consists of the right half of *LGV*, and joined to it, at the *LGV* breakpoint, the right half of *LGIII*. Similarly, *eT1(III)* consists of the left half of *LGIII*, with the left half of *LGV* joined to it at the *LGIII* breakpoint. However, we did not know the orientation of the right half of *LGIII*: is it joined at its distal (*bli-5*) or medial (*sma-2*) end? Nor did we know the orientation of the left half of *LGV*.

In order to answer these questions, we measured the rates of recombination between various markers in hermaphrodites homozygous for *eT1*. We assumed that recombination was not suppressed in these hermaphrodites. [ROBERTS (1970) had found a homozygous viable Drosophila translocation, *T(2,3)C49*. The *C49* homozygotes exhibited almost normal crossing over in a region that is greatly suppressed in heterozygotes.]

The distances measured are shown in Table 5. For any pair of markers, P_0 *trans*-heterozygotes (homozygous for *eT1*) were constructed. Single mutant F_1 hermaphrodites (*i.e.*, those that showed the phenotype of one of the markers) were progeny tested for the presence of the other marker. Let c equal the number of F_1's that carried both markers. The map distance between the markers was then calculated as $100\ p$, where $p = c/[2(\text{total No. mutant } F_1\text{'s tested}) - c]$. For *bli-5* to *unc-42*, the distance was large, indicating that recombination does occur in homozygous *eT1*. However, *sma-2* to *unc-42* was only about 1.6 m.u. The gene order on *eT1(V)* is, therefore, *bli-5 sma-2 (breakpoint) unc-42 unc-51*. (We assume that there is no breakpoint in the regions in which no crossover suppression occurs.) On *eT1(III)*, the distance from *sma-3* to *dpy-11* was also calculated to be small, about 2.7 m.u., indicating that here the gene order is . . . *.dpy-1 sma-3 (breakpoint) dpy-11*. . . . Thus, each of the translocation chromosomes appears to have been made by the joining of the respective medial parts of the original chromosomes.

eT1(III;V) *as a balancer for lethals on* LGIII *and* LGV: The crossover sup-pressors *C1* (HERMAN 1978) and *mnDp1* (MENEELY and HERMAN 1979) have

TABLE 5

Map distances between markers on eT1 *chromosomes*

P_0 genotype	Phenotype* of mutant F_1	Fraction of mutant F_1's carrying *trans*-marker	Map units
bli-5 eT1/eT1 unc-41,2†	Unc-41,2†	22/47	31
sma-2 eT1/eT1 unc-42	Unc-42	1/32	1.6
sma-3 eT1/eT1 dpy-11	Dpy-11 and Sma-3	9/174	2.7

* Phenotype beside that of Unc-(*e873*).
† Data combined from experiments using *unc-42* and *unc-41*. *eT1 unc-41* was made by recombination from *dpy-11 unc-41/eT1*. *unc-42* and *unc-41* are only 0.1 map units apart.

been shown to be useful as balancers for the detection and maintenance of recessive lethal mutations ("lethals") on the right arms of *LGII* and *LGX*, respectively. We decided to check whether *eT1(III;V)* would act as a balancer for lethals on both the right arm of *LGIII* and left arm of *LGV*.

Heterozygous *dpy-18 III*; *unc-46 V/ eT1* hermaphrodites (P_0's) were treated with 0.025 M EMS. The F_2 progeny from 429 single, fertile, wild-type F_1's were screened for lethal mutations. Since *dpy-18* and *unc-46* are in the crossover-suppressed regions, they showed pseudolinkage, and the only F_2 phenotypes produced were wild type, Unc-36 and Dpy Unc. A recessive lethal mutation on either the *dpy-18*- or the *unc-46*-marked chromosomes (anywhere in the crossover-suppressed region) was detected by an absence of Dpy Unc F_2's. Of 38 such lethals obtained and maintained as heterozygous strains, 21 carried a lethal linked to *dpy-18*, 15 carried a lethal linked to *unc-46* and 2 carried lethals linked to both *dpy-18* and *unc-46*. The range of map distances between the lethals and their respective markers (see MATERIALS AND METHODS) was approximately 0 to 16 on *LGIII* and 0 to 20 on *LGV*. It is therefore evident that *eT1(III;V)* is an efficient balancer for both regions.

DISCUSSION

We have demonstrated that the mutation *e873* is associated with a translocation, now named *eT1(III;V)*, that acts as a crossover suppressor for linkage groups *III* and *V*. This provides a balancer for a new section of the *C. elegans* genome: the right half of *LGIII*. On each of the linkage groups, the suppressed region extends from the most distal marker at one end, to a boundary in the middle. On *LGV*, the region is similar to that suppressed by *mnDp1(X;V)* (HERMAN, ALBERTSON and BRENNER 1976) and by *nT1(IV;V)* (FERGUSON and HORVITZ, personal communication). Since no Sma recombinants were found among 2,942 progeny from *sma-2 unc-64/e873* heterozygotes, recombination is reduced in *LGIII* from a normal value of 21% to less than 0.2%. Similarly the absence of Dpy recombinants among 1539 progeny from *unc-60 dpy-11/e873* heterozygotes reduces recombination in *LGV* from 18% to less than 0.3%. Thus, *eT1* balances a total of 39 map units (about 15% of the entire genome).

The value of crossover suppressors for the detection and maintenance of unconditional recessive lethal mutations in *C. elegans* has been discussed by others (HERMAN 1978; MENEELY and HERMAN 1979). HERMAN's group used *C1* and *mnDp1 (X;V)* to obtain lethals in the right halves of *LGII* and *X*, respectively. The present paper shows that, in a single screen, *eT1* can be used to obtain *LGIII* and *LGV* lethals. In spite of the fact that *eT1* is viable as a homozygote, we found that the maintenance of these lethals does not require the selection of heterozygotes at every generation. This is presumably due to the fact that the Unc *eT1* homozygote develops slowly and produces only slightly more progeny than does the phenotypically wild-type heterozygote. Lethals on *LGV* are of particular interest to us. Our laboratory is involved in analyzing the regions adjacent to genes affecting muscle development. One of these, *unc-60*, lies in the balanced half of *LGV* (WATERSON, THOMSON and BRENNER 1980), a region in which few other visible mutations have been found. The region, therefore, cannot be balanced by the use of closely linked markers, as was done in *LGI* by ROSE and BAILLIE (1980). We plan to use *eT1* to screen for lethal mutations around the *unc-60* gene.

Crossover suppressors are also of value in *C. elegans* when one wishes to introduce a multiply marked chromosome into a cross from a male. Since many homozygous mutant males have difficulty in mating, it is usually necessary to use a heterozygote. In contrast to those of *D. melanogaster*, *C. elegans* males undergo recombination (BRENNER 1974). Thus, markers may become separated unless they are balanced by a crossover suppressor.

Our pseudolinkage data have shown *eT1* to be a translocation between linkage groups *III* and *V*. This suggests that ^{32}P can produce chromosomal breaks in *C. elegans*. As in the case of *mnT1* (HERMAN 1978), the translocation chromosomes segregate independently from normal homologues in both types of hermaprodite germ cells, alternate and adjacent-1 disjunction occurring with about equal frequencies. In addition, we have shown that this pattern of disjunction also occurs in the spermatocytes of *eT1* heterozygous males. The fact that we obtained viable progeny from the male adjacent-1 disjunction means that male sperm from *C. elegans* that are aneuploid for autosomal genes can be viable and can fuse to form euploid zygotes. That this is true for the hermaphrodite sperm was already evident from HERMAN's (1978) data.

The translocation *eT1* differs from *mnT1* and many Drosophila translocations in that it produces homozygous viable hermaphrodites and males and that the former are fertile. (Since homozygous *eT1* males are uncoordinated, they do not mate readily and have not been tested for fertility.) This means that the breakpoints do not cause any loss of essential gene activities and that *eT1* can be maintained easily as a homozygous strain. The homozygote, however, does have an Unc phenotype, which is due to a defective *unc-36 III* gene activity. This defect could be due either to one of the translocation breakpoints or to an associated independent mutation. The demonstration that the *LGIII* breakpoint is very near *unc-36* makes the first suggestion a probable one.

The fact that *eT1* is homozygous viable has also made the genetic analysis of its new gene arrangement possible. It was found that *eT1* is a reciprocal transloca-

tion: the right half of *LGIII* (at least *sma-2* to *bli-5*) is linked to the *LGV* centromere while a *dpy-11*-bearing segment of *LGV* is linked to the *LGIII* centromere. A medial breakpoint was found for each of the two chromosomes involved. The new order of the genes indicates that the translocation chromosomes were formed by the reciprocal fusion of the broken ends created by these two breakpoints. This is not surprising if there are no other breakpoints, since in Drosophila, at least, fusion apparently cannot involve chromosomal ends (see ROBERTS 1976). The fact that crossing over is not suppressed between *dpy-1* and *sma-3 III* and to the right of *unc-42 V* makes it unlikely that there are other breakpoints in these regions. The possibility that another breakpoint exists between *unc-60* and *dpy-11 V* cannot be ruled out until we obtain an *eT1* chromosome carrying an *unc-60* mutation. However, in Drosophila rearrangement breakpoints cannot be literally at the ends of chromosomes (see ROBERTS 1976). If this is also true for *C. elegans*, the existence of a breakpoint between *unc-60* and *dpy-11* would require an additional breakpoint to the right of *bli-5* on *LGIII*. The simplest conclusion from the data as they stand is that there are only two breakpoints and that the new gene orders in *eT1* are: *eT1(III)*: *dpy-1 sma-3 (break) dpy-11 unc-60*; *eT1(V)*: *bli-5 sma-2 (break) unc-42 unc-51*.

Crossover suppression in simple reciprocal translocations is believed to be due to the inability of regions associated with a heterozygous translocation breakpoint to pair properly at meiosis (reviewed by ROBERTS 1970). One might suppose, then, that single breakpoints on each of linkage groups *III* and *V* would not affect crossing over in the large regions that are suppressed by *eT1*. However, ROBERTS (1970) found that single breakpoints in the arms of Drosophila autosomal chromosomes can reduce recombination values from about 40% to less than 1%. ROBERTS' data further showed that breakpoints and boundaries of suppression do not necessarily coincide. The fact that both *eT1* breakpoints lie close to suppression boundaries was therefore not necessarily expected. We can only speculate on the chromosomal nature of these boundaries. In the metacentric autosomes of Drosophila, crossover suppression is usually limited to the rearranged arm (see ROBERTS 1976). Thus, centromeres may act as crossover suppression boundaries. In the rod-like *X* chromosome of Drosophila, HAWLEY (1980) found suppression boundaries associated with chromosomal constrictions. HAWLEY suggested that these may be "sites where normal homologous pairing is established or reestablished." It is conceivable, therefore, that the *eT1* suppression boundaries represent pairing initiation sites and/or centromeres. The suppression by *mnT1(II;X)* led HERMAN (1978) to suggest that the *LGX* centromere may be near *unc-6*. As yet, no *C. elegans* centromere has been mapped. However, the segregation of our marked *eT1* chromosomes from their normal homologues (Table 4) provides partial mapping for the *LGIII* and *LGV* centromeres: no dominant centromere can lie between *sma-2* and *bli-5* on *LGIII*, and it is highly unlikely that there is one between *unc-60* and *dpy-11* on LGV. If the crossover-suppression boundary on, say, *LGIII* is indeed the site of a dominant centromere, it should be possible to find a translocation, again with a breakpoint to the right of *sma-3*, but this time one in which *sma-2* would segregate from normal *LGIII*, while *sma-3* would not. The

finding of such a translocation (taken together with the *eT1* data) would then map the *LGIII* centromere between *sma-3* and *sma-2*.

Finally, it is of interest to note that the breakpoint on *LGV* may lie in a region for which a molecular probe is available: HIRSH and co-workers (personal communication) have tentatively mapped an actin gene cluster to the right of and near *dpy-11*. If this mapping proves to be correct, it may be possible to use the actin gene probe with *eT1* DNA as a means of identifying *LGIII* DNA fragments.

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