The *Triplo-lethal* Locus of Drosophila: Reexamination of Mutants and Discovery of a Second-Site Suppressor

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ABSTRACT

In the genome of *Drosophila melanogaster* there is a single locus, *Triplo-lethal* (*Tpl*), that causes lethality when present in either one or three copies in an otherwise diploid animal. Previous attempts to mutagenize *Tpl* produced alleles that were viable over a chromosome bearing a duplication of *Tpl,* but were not lethal in combination with a wild-type chromosome, as deficiencies for *Tpl* are. These mutations were interpreted as hypomorphic alleles of *Tpl.* In this work, we show that these alleles are not mutations at *Tpl;* rather, they are dominant mutations in a tightly linked, but cytologically distant, locus that we have named *Suppressor-of-Tpl (Su(Tpl)). Su(Tpl)* mutations suppress the lethality associated with three copies of the *Triplo-lethal* locus and are recessive lethal. We have mapped *Su(Tpl)* to the approximate map position 3–46.5, within the cytological region 76B–76D.

IN most diploids, single gene deletions and duplications rarely have an obvious phenotype (MULLER 1950), yet aneuploids often have severe developmental defects. It is usually assumed that the defects in aneuploids are due to the additive effects of simultaneous imbalance of many genes, most of which have only minor effects by themselves (LINDSLEY *et al.* 1972; EPSTEIN 1988). The *Triplo-lethal* locus on chromosome 3R of *Drosophila melanogaster* is an exception to this general rule. When this locus is present in either three copies or one copy, the animal dies late in embryogenesis (LINDSLEY *et al.* 1972; DENELL 1976).

Several groups have attempted to mutagenize the *Triplo-lethal* locus (*Tpl*) with peculiar results. These studies have used a simple genetic selection for alterations of *Tpl:* mutagenized flies are simply mated to flies that carry a tandem duplication of *Tpl* on one homologue balanced by a deficiency of *Tpl* on the other. When this cross is performed, all nonmutant progeny will die because they have either three copies of *Tpl* or one, while survivors represent new alterations of *Tpl.* Duplications and deficiencies of *Tpl* are readily isolated following such experiments (KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980; DORER and CHRISTENSEN 1990), but only two cytologically normal mutations that have the same phenotype as deficiencies have been recovered, and these two could simply be small deletions that do not remove the entire polytene chromosome band. This suggests that although it is easy to eliminate *Tpl* function by deleting the locus, it is difficult or impossible to eliminate *Tpl* function by point mutations or transposon insertions. One possible explanation of this is that *Tpl* consists of a cluster of functionally redundant transcription units. Alternatively, *Tpl* may function as a DNA site or a nonprotein-coding RNA molecule and thus be less sensitive to single base changes than a conventional protein-coding gene.

Of the three large-scale mutational analyses carried out on *Tpl,* two resulted in the isolation of a novel class of mutation. ROEHRDANZ and LUCCHESI (1980) used EMS as a mutagen and recovered three interesting mutations. These three mutations were cytologically normal and rescued flies carrying a duplication of *Tpl,* just as a deficiency would, but were not dominant lethals as *bona fide* deficiencies of *Tpl* are. A small-scale mapping study showed that these mutations mapped very close to *Tpl,* so ROEHRDANZ and LUCCHESI (1980) suggested that these mutations represented hypomorphic alleles of *Tpl.* Consistent with this idea, these mutations are also homozygous lethal, and all three failed to complement one another for the recessive lethal phenotype. When we selected for *Tpl* mutations after mobilization of *P* elements, we recovered 15 mutations with the same phenotype. Furthermore, these 15 mutations all failed to complement the recessive lethal phenotype of ROEHRDANZ and LUCCHESI's original mutation (DORER and CHRISTENSEN 1990).

Our continuing molecular and genetic analysis of *Tpl* has called into question the interpretation that these mutations are hypomorphic alleles of *Tpl.* Here we present data showing that they are actually dominant mutations in a distinct locus that suppress the lethal effects of trisomy for *Tpl,* have no effect on the haplo-lethal

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TABLE 1

Single *P*-element strains and deficiencies used for mapping

Genotype	Cytological location of insert
A. Single *P* element strains	
w^{1118};; P{w[aRsLTR]}21/TM3	81F
mwh^1 P{hsneo}125 red^1 e^1/TM3	82D
mwh^1 P{hsneo}l(3)neo33 red^1 e^1/TM3, ry^{RK} Sb^1 e^1	83C
mwh^1 P{hsneo}ms(3)neo5 red^1 e^1/TM3	83D

Genotype	Cytology of deficiency
B. Deficiencies	
Df(3L)VW3/TM3	76A3; 76B2
Df(3L)kto^2/TM6B, Tb Hu Hn e	76B; 76D
Df(3L)rdgC, th st in ri p^p/TM6C, Tb Sb cu e^s	77A1; D1
Df(3L)ri79C/TM3	77B-C; 77F-78A
Df(3L)Pc-MK/TM3	78A3; 79E1, 2

phenotype of *Tpl*, and are also recessive lethals. We call this locus *Suppressor of Triplo-lethal*, abbreviated *Su(Tpl)*. We also provide explanations for the long-standing interpretation of these as hypomorphic mutations of *Tpl* and discuss the implications for the unusual genetics of *Tpl*.

MATERIALS AND METHODS

Drosophila stocks: Drosophila stocks were maintained on Formula 4-24 Instant Drosophila medium obtained from the Carolina Biological Supply Company, supplemented with live yeast. The mutation Tpl^{10} was described in ROEHRDANZ and LUCCHESI (1980) and was provided by JOHN LUCCHESI (Emory University); *Dp(3;3)Tpl* (previously referred to as *Dp(3;3)E6*, *Dp(3;3)21l73*, and *Dp(3;3)Tpl21*) and *Df(3R)18i77* [erroneously described as Tpl^{rv2} in LINDSLEY and ZIMM (1992)] were described in KEPPY and DENELL (1979) and were provided by ROB DENELL (Kansas State University). Tpl^{J34} was previously described (DORER and CHRISTENSEN 1990) and is from our collection. Several single *P*-element strains were obtained from the Bloomington Drosophila Stock Center (BDSC) and are described in Table 1. Also obtained from the BDSC were the mapping strains *ru h th st cu sr e^s ca* and *th st in ri p^p*, and the deficiencies described in Table 1. Descriptions of these rearrangements and mutations can be found in LINDSLEY and ZIMM (1992).

Hybridization *in situ* to polytene chromosomes: Salivary gland squashes for *in situ* hybridization were prepared essentially as described by ENGELS *et al.* (1986), except that slides were not pretreated with Denhardt's solution. DNA was biotinylated by nick translation with bio-16-dUTP (BRL-Gibco) substituted for dTTP. Hybridizations were carried out at 39° in a buffer containing 4× SSPE, 40% formamide and 1 μg/ml salmon sperm DNA (Sigma). The Enzo Biochemicals Detek-1-HRP kit was used for peroxidase staining as described in ASHBURNER (1989).

Recombinant DNA: The cDNA and genomic clones of RM62 have been described (DORER *et al.* 1990). Construction of a library from Tpl^{J34}/*Dp(3;3)Tpl* and screening of the library were as previously described (DORER *et al.* 1990).

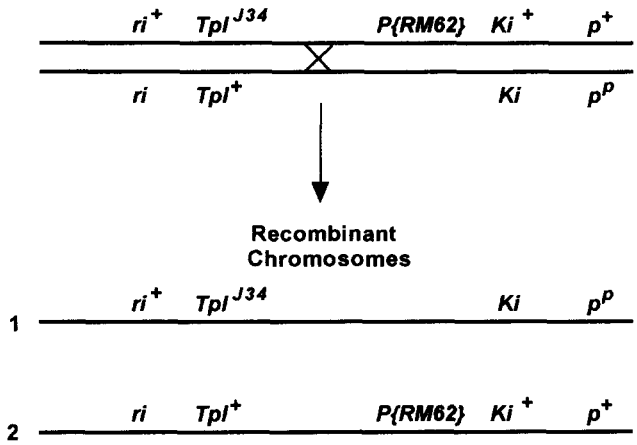

FIGURE 1.—Crossovers between the Tpl^{J34} mutation and the *P* element in RM62. The heterozygous females indicated at the top were mated to *l(3)DTS2 Sb/TM3, ri p^p Ser* males. Serrate progeny were scored for radius incompletus, Kinked and pink-peach. Recombinants in the *ri* to *Ki* interval were scored for Tpl^{J34} by testcrossing to *Dp(3;3)Tpl/Df(3R)Tpl18i77* and looking for survivors. Presence or absence of the *P* element was scored by *in situ* hybridization and Southern blotting (data not shown). *P{RM62}* designates the *P* element found in the RM62 gene, as previously described (DORER *et al.* 1990). Recombinant chromosomes were recovered that correspond to the chromosomes labeled *1* and *2*. Note that we use the prior nomenclature and position for the Tpl^{J34} mutation even though we subsequently show that it is located to the left of *ri* and is an allele of a different gene, *Su(Tpl)* (see RESULTS).

Polymerase chain reaction: PCR was carried out on crude extracts of individual adult flies as described (GLOOR and ENGELS 1992), using primers corresponding to nucleotides 89–104 (5′-GGAAAGGTTGTGTGCGGACG-3′) and 433–414 (5′-GTACTCCCACTGGTATAGCC-3′) of the *P*-element sequence. This amplification results in a 344-bp product that was detected on 2% agarose gels stained with ethidium bromide (SAMBROOK *et al.* 1989).

RESULTS

Paradox between genetic and molecular mapping of *Tpl*: After hybrid dysgenesis we recovered 15 new mutations with the same phenotype as the three mutations called Tpl^{10}, Tpl^{17}, and Tpl^{38} by ROEHRDANZ and LUCCHESI (1980). Since these mutations all failed to complement Tpl^{10}, we assumed that they were also hypomorphic alleles of *Tpl* (DORER and CHRISTENSEN 1990). Of these 15 mutations, only one, called Tpl^{J34}, had a *P* element at the known cytological location of *Tpl*, 83E1,2. This *P* element was cloned and found to reside within the transcription unit of an RNA helicase gene, RM62 (DORER *et al.* 1990). However, two lines of evidence suggested that the *P* element in RM62 was not responsible for the mutation affecting *Tpl*. One was the recovery of recombinants from ri^+ Tpl^{J34} Ki^+ p^+/*ri* Tpl^+ *Ki* p^p females. The relevant markers in these crosses are *ri, radius incompletus*, which maps at 46.8, and is located in cytological region 77EF and *Ki, Kinked*, which maps at 47.6 and 83E (LINDSLEY and ZIMM 1992) (Figure 1).

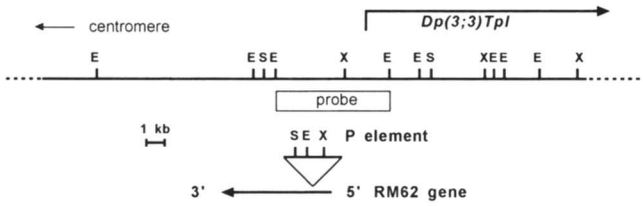

FIGURE 2.—Molecular map of the region near the RM62 gene. The restriction map shows sites for *Eco*RI (E), *Sal*I (S) and *Xho*I (X). The locations of the RM62 gene and the *P*-element insertion in it have been previously described (DORER *et al.* 1990) and are shown. The *Eco*RI fragment used as a probe to clone the *Dp(3;3)Tpl* breakpoint is indicated, and the location of the breakpoint is also shown.

Both reciprocal recombinants were recovered, and *in situ* hybridization of the recombinant chromosomes with a *P*-element probe showed that the ri^+ Tpl^{J34} Ki p^p chromosome, which still had the mutant phenotype, had lost the *P* element at 83E1,2 (DORER and CHRISTENSEN 1990), while the reciprocal recombinant chromosome, *ri* Tpl^+ Ki^+ p^+, retained the *P* element. The second observation is that we recovered revertants of the Tpl^{J34} mutant that retained the *P* element in the RM62 gene (data not shown). These data suggested that these mutations all arose by some mechanism other than *P*-element transposition in to the *Tpl* locus, and that the insertion of the *P* element into the RNA helicase gene was a coincidental event. These data also suggest that the mutation maps proximal (left on the standard Drosophila genetic map) to the RM62 gene.

In situ hybridization of cloned DNA from either side of RM62 to the *Tpl* duplication chromosome *Dp(3;3)Tpl* showed that RM62 is proximal to the duplication breakpoint and allowed us to orient the molecular map with respect to the chromosome as shown in Figure 2 (DORER *et al.* 1990). Genomic Southern blots of *Dp(3;3)Tpl* probed with RM62 clones and subclones revealed a duplication-associated polymorphism just to the right (centromere distal) of the RM62 gene (data not shown). We rescreened our Tpl^{J34}/*Dp(3;3)Tpl* genomic library with the *Eco*RI fragment indicated in Figure 2 and isolated clones corresponding to the breakpoint in the *Dp(3;3)Tpl* chromosome. *In situ* hybridization of one of these clones (called λMargo25) to wild-type polytene chromosomes shows simultaneous hybridization at 83E1,2 and 84B (Figure 3), indicating that it is a clone of the new junction formed by the duplication.

These two observations result in a paradox. The recombination results show that Tpl^{J34} maps to the left of the *P* element in RM62, while the breakpoint mapping data show that *Tpl* maps to the right of RM62.

Hypothesis to explain the paradox: Although it could be supposed that *Tpl* is in fact located on both sides of RM62, a more parsimonious hypothesis is that Tpl^{J34} and the other so-called hypomorphic alleles are not actually mutations in *Tpl* at all, but are mutations in a closely linked locus that suppresses the triplo-lethality of *Tpl*. These would be dominant mutations that have no effect on the viability of flies with two doses of *Tpl*, no effect on the lethality of flies with one dose of *Tpl*, but allow survival of flies with three doses of *Tpl*. These mutations would also be recessive lethals. This hypothesis does not contradict the mapping data of ROEHRDANZ and LUCCHESI (1980), as their mapping of Tpl^{10} was based on a small number of progeny. We decided to test this hypothesis by doing much larger scale mapping of these mutations. We chose to use the original Tpl^{10} mutation of ROEHRDANZ and LUCCHESI (1980) for several reasons. Tpl^{10} was isolated on chromosomes carrying the visible markers *ri*, *Ki*, and p^p, whereas our hybrid-dysgenesis-induced mutations were in otherwise wild-type chromosomes. The hybrid-dysgenesis-induced mutations are also *P* strains, which would have made it impossible to use single *P*-element insertions as genetic markers (see below), and they occasionally revert to wild type, which we have never observed with Tpl^{10} (DORER and CHRISTENSEN 1990). Finally, all of the other mutations have been tested for complementation of the recessive lethal phenotype with Tpl^{10}. Hereafter, we refer to these mutations by their revised names as alleles of *Suppressor of Triplo-lethal*, abbreviated *Su(Tpl)*. Tpl^{10} thus becomes $Su(Tpl)^{10}$, Tpl^{J34} becomes $Su(Tpl)^{J34}$, and so on.

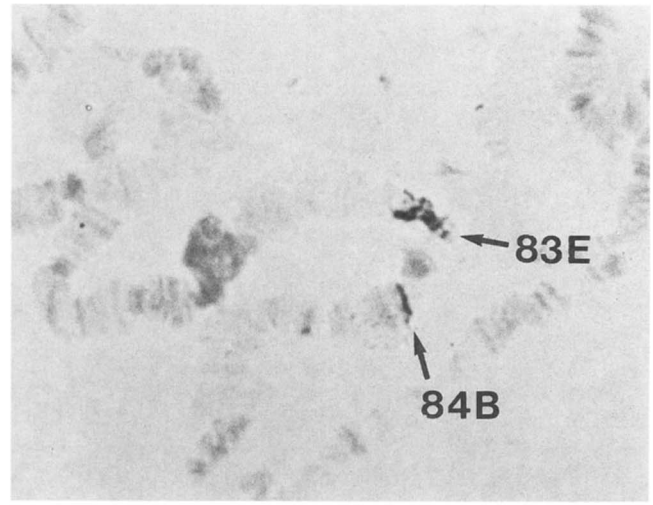

FIGURE 3.—*In situ* hybridization of a clone of the *Dp(3;3)Tpl* breakpoint to wild-type chromosomes. The breakpoint clone described in the text was hybridized to polytene chromosomes from the wild type strain Oregon-R. The two bands of hybridization at 83E and 84B are indicated.

Recombination mapping using *P* elements as markers: Four single *P*-element insert lines were used for recombination mapping of *Su(Tpl)*. One carried a *P{w⁺}* element at cytological location 81F, and three carried *P{hsneo}* at 82D, 83C, and 83D, respectively (see MATERIALS AND METHODS for more details on these strains). The principle of these crosses is the same as in the cross described above and in Figure 1, but with *P* elements farther to the left than *P{RM62}*. Females

TABLE 2

Recombination mapping using single *P* elements as markers

Recombinant genotype	Number of recombinants recovered for each *P*-element location			
	81F	82D	83C	83D
Su(Tpl)^{10} ri P Ki$^+$ p$^+$	4	3	1	4
Su(Tpl)$^+$ ri$^+$ Ki p^p	0	3	5	6
Su(Tpl)$^+$ ri$^+$ P Ki p^p	0	1	0	0
Su(Tpl)^{10} ri$^+$ P Ki p^p	0	1	0	0

Females heterozygous for each of the single *P* elements listed in Table 1 and *ri Su(Tpl)^{10} Ki p^p* were crossed. All of the progeny that were recombinants in the *ri* to *Ki* interval and were tested for the presence of *Su(Tpl)^{10}* and the *P* element are listed.

heterozygous for each of the single *P* elements and *ri Su(Tpl)^{10} Ki p^p* were crossed to *l(3)DTS2 Sb/TM3, ri p^p bx^{34e} e Ser* males. At 29° the *l(3)DTS2 Sb* progeny died and the remaining progeny were scored for the recessive marker *ri* and the dominant marker *Ki*. Recombinants in the *ri* to *Ki* interval were collected for further analysis. They were testcrossed to *Dp(3;3)Tpl/Df(3R)18i77* to assay the *Su(Tpl)^{10}* mutation and subjected to PCR to assay for the single *P* element. The results are shown in Table 2. In every case but the two exceptional recombinants shown for the 82D element, all recombinant chromosomes obtained were either *ri Su(Tpl)^{10}* with the *P* element present or *Ki p^p* with the *P* element absent. This means that the crossovers always occurred between the *P* element and *ri*, with *Su(Tpl)^{10}* always segregating with *ri*. These data indicate that *Su(Tpl)^{10}* maps to the left of the *P* elements in these crosses. One of the exceptions is a recombinant chromosome that is *Ki p^p* and also has the *P* element at 82D. The simplest interpretation is that this crossover occurred between the *P* element and *Ki*, with *Su(Tpl)^{10}* again mapping to the left of the crossover. The second exceptional recombinant is *ri$^+$ Su(Tpl)^{10} Ki p^p* and has the *P* element. There are two possible interpretations. If the map order is *ri Su(Tpl)^{10} P{82D} Ki p^p*, then this chromosome represents a triple recombinant, with crossovers occurring in the *ri-Su(Tpl)^{10}*, *Su(Tpl)^{10}-P{82D}* and *P{82D}-Ki* intervals. Alternatively, if the map order is *Su(Tpl)^{10} ri P{82D} Ki p^p*, then this is a double recombinant with crossovers occurring in the *Su(Tpl)^{10}-ri* and *P{82D}-Ki* intervals. Although there is a high coefficient of coincidence in this region (MORGAN *et al.* 1925; GREEN 1975; SINCLAIR 1975; DENELL and KEPPY 1979) and the *Su(Tpl)^{10}* mutation increases recombination rates in its vicinity (DORER and CHRISTENSEN 1989), it is still much more likely to observe a double crossover than a triple, given that the total map distance between *ri* and *Ki* is only 0.8 cM. These results demonstrate that *Su(Tpl)* is not as tightly linked to *Ki* as was previously thought (ROEHRDANZ and LUCCHESI 1980) and may in fact be to the left of *ri*, on the left arm of chromosome *3*.

TABLE 3

Summary of recombinants in the *st* to *ri* interval

Phenotype of recombinant fly	*Su(Tpl)* genotype	
	Su(Tpl)^{10}	*Su(Tpl)$^+$*
th st ri Ki p^p	10	3
th$^+$ st$^+$ ri$^+$ Ki$^+$ p$^+$	3	41

Su(Tpl)^{10} ri Ki p^p/ru h th st cu sr e^s ca females were crossed to *th st in ri p^p* males, and recombinants were scored as described in the text. The recombinants in the *st* to *ri* interval that were able to be scored for all the markers, including *Su(Tpl)^{10}*, are listed here.

Recombination mapping with a multiply marked chromosome: Since it appeared that *Su(Tpl)* might map to the left of *ri*, we did a large-scale mapping cross to determine where it is located. Females of the genotype *Su(Tpl)^{10} ri Ki p^p/ru h th st cu sr e^s ca* were backcrossed to *th st in ri p^p* homozygotes, and recombinants were scored. The mutant phenotypes that can be scored in this cross are th, st, ri, Ki, and p^p. *Su(Tpl)^{10}* can be scored by a test cross to *Dp(3;3)Tpl/Df(3R)18i77*. The relevant map positions are *th* 43.2, *st* 44.0, *ri* 46.8, *Ki* 47.6, and *p^p* 48.0. From a total of 2324 progeny scored, there were 98 recombinants in the *st* to *ri* interval. This corresponds to a map distance of 4.2 cM, slightly higher than the standard map distance of 2.8 cM (LINDSLEY and ZIMM 1992). Of these, 57 were able to be testcrossed to score for *Su(Tpl)^{10}*. The results are shown in Table 3. Of the 13 *th st ri Ki p^p* recombinants, 10 were *Su(Tpl)^{10}*. In the reciprocal recombinant class, *th$^+$ st$^+$ ri$^+$ Ki$^+$ p$^+$*, 41 of them were *Su(Tpl)$^+$* and three were *Su(Tpl)^{10}*. These data indicate that *Su(Tpl)^{10}* is between *st* and *ri*, at an approximate map position of 46.5.

Deficiency mapping: Since *Su(Tpl)* mutations are recessive lethals, we attempted to see if the lethal phenotype could be complemented by a deficiency. The five deficiencies shown in Figure 4 were used. These experiments were done by crossing *Su(Tpl)^{10} ri Ki p^p/TM3, Ser* to each of the deficiencies. The deficiencies were obtained as heterozygous balanced stocks, so the F1

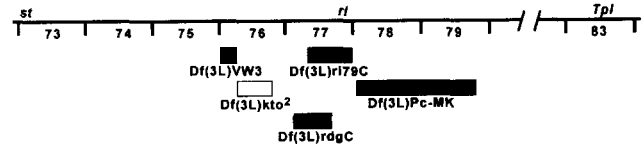

FIGURE 4.—Deficiency mapping of *Su(Tpl)*. The proximal region of chromosome arm 3L is diagrammed, with the numbered divisions of BRIDGES (1935) shown. A portion of the right arm of chromosome *3* is also shown to indicate the location of *Tpl*. The extents of the deficiencies tested for complementation of the recessive lethal phenotype of *Su(Tpl)^{10}* are shown. The locations of the genetic markers *st* and *ri* are also indicated. *Df(3L)kto^2* failed to complement *Su(Tpl)^{10}* and is indicated as a hollow bar.

progeny were examined for Kinked flies not carrying the balancer chromosome from the deficiency parent. In four of the cases, *Df(3L)81K19, Df(3L)Cat, Df(3L)VW3, Df(3L)rdgC, Df(3L)ri79C,* and *Df(3L)Pc-MK,* progeny carrying both $Su(Tpl)^{10}$ and the deficiency were obtained, indicating that all of these deficiencies complement the recessive lethal phenotype of $Su(Tpl)^{10}$. In the cross of $Su(Tpl)^{10}$ *ri Ki* p^p/ *TM3, Ser X Df(3L)kto*^{2}/ *TM6B, Tb Hu Hn e,* 209 progeny that were either $Su(Tpl)^{10}$ *ri Ki* p^p/ *TM6B, TM3/ TM6B,* or *Df(3L)kto*^{2}/ *TM3* were obtained, along with only one that was possibly $Su(Tpl)^{10}$ *ri Ki* p^p/ *Df(3L)kto*^{2}. The single exception was infertile, so its genotype could not be tested further. It may have been a rare survivor, or it could have been a $Su(Tpl)^{10}$ *ri Ki* p^p/ *TM6B* fly whose Hu phenotype could not be scored accurately. These results suggest that $Su(Tpl)^{10}$ is located within the boundaries of the *Df(3L)kto*^{2} deficiency, cytological location 76B-D. Since *Df(3L)kto*^{2} appears to be deleted for *Su(Tpl),* it was tested to see if the deficiency could rescue flies carrying three copies of *Tpl;* it did not.

DISCUSSION

We have shown that a class of mutation previously described as alleles of the *Triplo-lethal* locus actually maps to a location distinct from *Tpl.* Due to the very low levels of recombination in the vicinity of the centromere, all loci in this region appear to be very tightly linked, even though the physical distances separating them can be quite large. This also prevented the correct map position of the *Su(Tpl)* locus from being determined in previous studies (ROEHRDANZ and LUCCHESI 1980; DORER and CHRISTENSEN 1989, 1990), which in turn prevented it from being recognized as a separate locus from *Tpl.* Mutations in the *Su(Tpl)* locus have two properties: they are dominant suppressors of the triplo-lethal phenotype of *Tpl,* and they are recessive lethals. They have no effect on the haplo-lethal phenotype of *Tpl.*

Su(Tpl) has an unusual mutagenic history. Although the selection is very powerful, *Su(Tpl)* mutations have been obtained at variable frequencies. KEPPY and DENELL (1979) did a very large scale selection for mutants using a variety of mutagens, including EMS, and did not recover any *Su(Tpl)* mutations. ROEHRDANZ and LUCCHESI (1980) did a smaller scale selection using EMS as a mutagen and recovered three *Su(Tpl)* mutations. In contrast, when we did a hybrid dysgenesis screen we obtained 15 *Su(Tpl)* mutations (DORER and CHRISTENSEN 1990). These three experiments correspond to mutation rates of $<2 \times 10^{-6}$, 2.4×10^{-5}, and 2×10^{-4}, respectively. An explanation of these differences in mutation rate is suggested by the observation that the deficiency, *Df(3L)kto*^{2}, uncovers the lethal phenotype of $Su(Tpl)^{10}$, yet does not suppress *Tpl.* We propose that *Df(3L)kto*^{2} deletes the *Su(Tpl)* locus, but we suggest that the *Su(Tpl)* mutants are not null mutants and that indeed any null mutant does not have the *Tpl* suppressing phenotype. Supporting this model is the fact that none of the *Su(Tpl)* mutations appears to be associated with any chromosomal rearrangements. The *Su(Tpl)* mutations may be alterations in the expression pattern of the gene, perhaps causing it to be expressed at a higher or lower level than normal, or causing it to be expressed at a novel time or location. Transposon insertions are known to alter gene expression in such ways. Indeed, the highest rate of recovery of *Su(Tpl)* mutations was obtained when we deliberately mobilized *P* elements. Perhaps ROEHRDANZ and LUCCHESI's cross mobilized an unknown transposon, while KEPPY and DENELL's did not. This model suggests that our hybrid-dysgenesis-induced mutations should result from the insertion of *P* elements in 76B–D, which would also explain why no *P* elements were found in *Tpl* in these mutants. Although these mutants are all *P* strains, and consequently have many *P* elements in their genomes, a preliminary analysis of the 76B–D region reveals that several of them do have a *P* element there (D. R. DORER and A. C. CHRISTENSEN, unpublished data). Many of the mutants described previously (DORER and CHRISTENSEN 1990) have now been lost or have spontaneously reverted; however, we are currently attempting single-*P*-element mutagenesis of *Su(Tpl)* to test this hypothesis.

Previous studies have shown that the triplo-lethal phenotype of *Tpl* can also be suppressed by duplications of an *X*-linked locus, *Isis* (ROEHRDANZ and LUCCHESI 1981; DORER *et al.* 1993). *Isis* differs from *Su(Tpl)* in that only cytologically visible duplications of *Isis* have been seen to cause suppression, rather than the apparent point mutations responsible for mutant alleles of *Su(Tpl).* Also, the rescue of *Dp(Tpl)/ Tpl*$^+$ flies by *Isis* duplications is usually very weak with only a small fraction of flies surviving to eclosion (DORER *et al.* 1993). In contrast, $Su(Tpl)^{10}$ is an efficient suppressor of triplo-lethality, and crosses between $Su(Tpl)^{10}$ and *Dp(Tpl)/ Df(Tpl)* flies appear fully fertile. One might propose that *Su(Tpl)* mutations, as well as *Isis* duplications, suppress triplo-lethality by acting directly to lower the apparent dosage of *Tpl.* However, no lethal dosage-dependent interactions between *Su(Tpl)* and *Isis* have been observed, suggesting that the two loci do not both function in this manner (DORER *et al.* 1993).

These new data also shed light on our previous results showing that the *Su(Tpl)* mutations increased recombination rates six- to 10-fold in the *ri* to *Ki* interval (DORER and CHRISTENSEN 1989). Based on the assumption that these mutations were alleles of *Tpl* and mapped to the *ri* to *Ki* interval, we interpreted the increase in recombination (and premeiotic recombination in males in the same interval) to be due to a hotspot at the site of the mutations. Since we now know that the mutations are not in fact in the same map interval where the increased recombination was occuring, a new explanation is needed. One possibility is that the *Su(Tpl)* mutations cause a local increase in recombination frequencies,

including premeiotic recombination, and that they are responsible for a hot region, rather than a hotspot. Another possibility is that the *Su(Tpl)* mutations somehow cause an increase in recombination near the centromere. We also concluded in our previous study that *Tpl* maps to the left of *Ki*, however that conclusion was based on the assumption that $Su(Tpl)^{10}$, $Su(Tpl)^{17}$ and $Su(Tpl)^{38}$ were alleles of *Tpl*. It is now apparent that the relative map positions of *Tpl* and *Ki* are still undetermined.

We have provided evidence that the mutants $Su(Tpl)^{134}$ and $Su(Tpl)^{10}$ are located some distance to the left of *Tpl*. We have carefully mapped $Su(Tpl)^{10}$ and shown that it is located in cytological region 76B–D at map position 3–46.5. This location is several megabases away from *Tpl*, on the opposite side of the centromere. Since all the known mutations of this type fail to complement $Su(Tpl)^{10}$ for the recessive lethal phenotype, it seems appropriate to rename these mutants *Su(Tpl)* and to recognize that they represent a previously unrecognized gene that interacts with *Tpl*. Further study of this gene and its mutants may lead to greater understanding of the genetic complexities and the function of *Tpl*, as well as possibly providing a model for understanding gene dosage effects and their amelioration. Since *Su(Tpl)* mutations also affect recombination in the vicinity of the centromere, it is possible that the *Su(Tpl)* locus plays a role in chromatin structure and function. Whether these two functions are interrelated remains to be seen.

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