The Unusual Spectrum of Mutations Induced by Hybrid Dysgenesis at the 
Triplo-lethal Locus of Drosophila melanogaster

Douglas R. Dorer and Alan C. Christensen
Department of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, 
Philadelphia, Pennsylvania 19107–6799

ABSTRACT

The Triplo-lethal locus (Tpl) is unique in its dosage sensitivity; no other locus in Drosophila has been 
identified that is lethal when present in three doses. Tpl is also haplo-lethal, and its function is 
still a mystery. Previous workers have found it nearly impossible to mutationally inactivate Tpl other 
than by completely deleting the chromosomal region in which Tpl resides (83DE). We have utilized 
P-M hybrid dysgenesis in an effort to obtain new mutations of Tpl. We recovered 19 new duplications 
of Tpl, 15 hypomorphic mutations of Tpl (a previously rare class of mutation), and no null mutations. 
Surprisingly, 14 of the 15 hypomorphic alleles have no detectable P element sequences at the locus. 
The difficulty in recovering null mutations in Tpl suggests that it may be a complex locus, perhaps 
consisting of several genes with redundant functions. The relative ease with which we recovered 
hypomorphic alleles is in sharp contrast to previous attempts by others to mutagenize Tpl. A higher 
mutation rate with hybrid dysgenesis than with radiation or chemicals also suggests a peculiar genetic 
organization for the locus.

In Drosophila melanogaster there is only one locus that is triplo-lethal; this locus, Tpl, is also haplo-
lethal and is located on the third chromosome at cytological location 83D4.5-E1.2 (Lindsley et al. 
1972; Denell 1976; Keppy and Denell 1979). Individually carrying three doses or one dose of Tpl die 
either as late embryos or early first instar larvae (Denell 1976). No morphological abnormalities have 
been reported in the dying embryos. Extensive mutational analysis has not revealed the function of Tpl; 
however, the results of mutagenesis have suggested that Tpl may be complex or unusual (Keppy and 
Denell 1979; Roehrdanz and Lucchesi 1980). Selection of new mutations at Tpl depends on the existence 
of balanced stocks carrying a duplication of Tpl on one homolog and a deficiency on another. When 
these stocks are crossed to wild-type flies, all progeny will die due to aneuploidy for Tpl. If the wild-type 
parents are mutagenized, then exceptional progeny may result; these should carry either a new duplication 
or a new deficiency of Tpl. In general when ionizing radiation is used as a mutagen, chromosomal aberrations 
are expected, and in the case of Tpl this is what is observed. Keppy and Denell (1979) used γ-rays as 
a mutagen and recovered several deficiencies of Tpl at a mutation rate of approximately $1.8 \times 10^{-4}$. No 
inversion or translocation breakpoints that inactivate Tpl were recovered. In the same study they also used 
the chemical mutagens ethyl methanesulfonate (EMS) and formaldehyde, and recovered inactivations of Tpl 
at the surprisingly low rate of $2.3 \times 10^{-5}$. Of the inactivations recovered following EMS mutagenesis the 
majority were cytologically visible deficiencies. Keppy and Denell (1979) interpreted these results 
to indicate that single base-pair substitutions that inactivate Tpl function are very rare, or even non-
existent.

Roehrdanz and Lucchesi (1980) also carried out EMS mutagenesis of Tpl and found a very low mutation 
rate ($5 \times 10^{-5}$). Of the seven mutations they recovered, four behaved as complete inactivations of 
Tpl and were apparently large deficiencies, supporting Keppy and Denell's (1979) suggestion that single 
base-pair changes that inactivate Tpl are rare or non-existent. The remaining three mutations recovered 
by Roehrdanz and Lucchesi (1980) were of a new class. These were recovered in the selection because 
they rescued flies carrying the duplication bearing chromosome, and they are homozygous lethal, but 
unlike deletions they are viable when heterozygous with a wild-type chromosome. These three alleles 
(Tpl10, Tpl17 and Tpl18) behave, therefore, as hypomorphs. They are cytologically normal and do not 
complement one another for the recessive lethality (Roehrdanz and Lucchesi 1980). Although they are 
cytologically normal, they do not behave as complete inactivations of the locus, again suggesting that simple 
lesions do not readily inactivate Tpl function. In addition, these three hypomorphic alleles are site-specific 
hotspots for recombination (Dorer and Christensen 1989), which led us to suggest that they were
due to transposon insertions rather than single base-pair changes.

We have carried out a selection for new mutations ofTpl using hybrid dysgenesis. When mutations are isolated following hybrid dysgenesis, it is usually found that they are due to insertions of the P transposable element (Engels 1989). We have obtained a large number of new duplications ofTpl, as well as 15 new hypomorphic alleles. Surprisingly, we did not recover any null mutations ofTpl. This again suggests that it is not a simple matter to completely inactivateTpl function without removing the entire locus from the genome. Also surprisingly, we found that the hypomorphic alleles induced by hybrid dysgenesis do not appear to be due to insertions of the P element as assayed by in situ hybridization to polytene chromosomes. The implications of these results for the genetic structure of theTpl locus are discussed.

MATERIALS AND METHODS

Drosophila media, stocks and culture conditions: Stocks were maintained and fed as described inDorer and Christensen (1989). TheTpl duplication over deficiency stock used in this study was C(1)M3 ybb//y;Dp(Tpl)p*/Df(Tpl)p*. In(1)EN y;Dp(3;3)2117 p*/Df(3R)18i77 p* . The origin of this stock was described in Dorer and Christensen (1989) and Keppy and Denell (1979). The Dp(3;3)2117 is also referred to as Dp(3;3)E6, Dp(3;3)83D-6, and most recently as Dp(3;3)Tpl21 (Lindsley and Zimm 1987). In this work we will abbreviate Dp(3;3)2117 as Dp(Tpl) and Df(3R)18i77 as Df(Tpl). Alterations ofTpl produced in this study will be referred to specifically by name, or generically by the superscript ‘hd’ for hybrid dysgenesis. Other genetic markers are described in Lindsley and Grell (1968) or Dorer and Christensen (1989), except the y2 stock which was described inEngels and Preston (1979), and was obtained from J. Lucchini (University of North Carolina).

Genetic crosses: The selection for new mutations atTpl was carried out as described in Figure 1 and below. The dysgenic hybrids were raised at 23°, while the subsequent crosses were at 20°. For the selection cross, vials were set to females mated to their male siblings were counted. For the selection cross, vials were set to multiply by two to account for the inviable females estimated by counting the total progeny from 15 crosses were at 20°. For the selection cross, vials were set to multiplying by two to account for the inviable survivors each had aTpl p chromosome, presumably resulting from recombination between the Dp(Tpl)p and Df(Tpl)p chromosomes (Denell 1976). Neither of these kinds of progeny resulted from cross 2. As can be seen in Table 1, we recovered a large number of new duplications ofTpl from these crosses. The 19 duplications listed were recovered from 12 different matings, and thus represent at least 12 independent mutational events. Some of these had very poor viability and have been lost. The breakpoints of the rearrangements in those duplications we were able to analyze are illustrated in Figure 2. Of the duplications that we were able to characterize, all but one appear to be direct tandem repeats with one or both breakpoints often corresponding to the location of P elements in the parental y2 stock (Figure 3, A, B, C, and D, and other data not shown). The one exception, Dp(3;3)Tpl23, appears to be a reverse tandem duplication. As would be expected, tandem direct duplications are not completely stable and many of these were also lost subsequent to the cytological analysis.

Fifteen of the new Tpl mutations were found to complement duplications of the locus. These 15 mutations represent at least 10 independent events. All of these alleles appear to have normal cytology, and are viable when heterozygous with wild-type third chromosomes. They are thus genetically similar to the previously described class of hypomorphic mutations which includes the alleles Tpl15, Tpl12 and Tpl38 (Roehrdanz and Lucchini 1980). None of the 15 new hypomorphic mutations complement the Tpl19 allele for the recessive lethal phenotype. Of the 6.3 × 106 and 8.1 × 106 zygotes estimated for crosses 1 and 2, respectively, only half (those carrying Dp(3;3)21173) would allow the recovery of new hypomorphic mutations. The recovery of 15 hypomorphic alleles gives an estimated mutation rate of 2
Triplo-Lethal Mutations

Figure 1.—Genetic selection for mutations of Tpl. Males from the r~ stock were mated to Oregon-R virgin females. Both male and female dysgenic F1 progeny were used in reciprocal crosses to Dp(Tpl) p+/Df(Tpl) p~ flies as shown. The occasional p~ F2 survivors were backcrossed to Dp(Tpl) p~/Df(Tpl) p~. The p~ F3 progeny were tested by crossing to ri Tpl<sup>hp</sup> Ki p~/TM3, Ser flies. When the mutagenized chromosome carried a new duplication, then only Dp(Tpl)~<sup>hp</sup> p~/ri Tpl<sup>hp</sup> Ki p~ progeny survived in the test cross, and a stock was established from the F3 progeny. If the mutagenized chromosome had carried a new deficiency or null mutation, then only Df(Tpl)<sup>hp</sup> p~/Dp(Tpl) p~ would have survived and been used to establish a stock (we did not recover this class). If a new hypomorphic allele resulted, the test cross resulted in both Dp(Tpl) p~/ri Tpl<sup>hp</sup> Ki p~ progeny which were discarded, and Tpl<sup>hp</sup>/TM3, Ser progeny which were used to establish a balanced stock. The second and fourth chromosomes are all wild type and are not shown.

### Table 1

<table>
<thead>
<tr>
<th>Genotypes of surviving progeny</th>
<th>Number of survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp(Tpl)&lt;sup&gt;hp&lt;/sup&gt; p~</td>
<td>7</td>
</tr>
<tr>
<td>Dp(Tpl)&lt;sup&gt;hp&lt;/sup&gt; p~</td>
<td>12</td>
</tr>
<tr>
<td>Tpl&lt;sup&gt;hp&lt;/sup&gt; p~</td>
<td>2</td>
</tr>
<tr>
<td>Tpl&lt;sup&gt;hp&lt;/sup&gt; p~ (recombinants)</td>
<td>19</td>
</tr>
<tr>
<td>Triploids (females or intersexes)</td>
<td>4</td>
</tr>
<tr>
<td>Unknown (infertile or lost)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
</tr>
</tbody>
</table>

Crosses were carried out as described in the text and Figure 1. The first three rows indicate new mutational events involving Tpl; the next three rows are other genetic events that led to exceptional progeny.

× 10<sup>−4</sup>, significantly higher than the rate of recovery of hypomorphic mutations following chemical mutagenesis (Keppy and Denell 1979; Rohrdanz and Lucchesi 1980). We have previously shown that Tpl<sup>hp</sup>, Tpl<sup>hp</sup> and Tpl<sup>hp</sup> are all associated with a site-specific increase in recombination frequency and with premeiotic recombination in males (Dorer and Christensen 1989). These observations led us to propose that insertions of transposable elements could be responsible for the phenotypes of these mutations. That we have now recovered new hypomorphic alleles at a high rate following hybrid dysgenesis supports this hypothesis.

**Analysis of hypomorphic alleles:** For 14 of the 15 new hypomorphic alleles, in situ hybridization with a P element probe showed no hybridization signal at 83DE, although numerous other sites of hybridization were clearly visible (Figure 3, E and F; other data not shown). One mutant, Tpl<sup>hp</sup>, shows a positive hybridization signal at 83E (Figure 4A). In an attempt to determine whether the P element at 83E segregated with the hypomorphic phenotype of Tpl<sup>hp</sup>, a recombinant chromosome of the genotype ri<sup>+</sup> Tpl<sup>hp</sup> Ki p~ was recovered from a female of the genotype ri<sup>+</sup> Tpl<sup>hp</sup> Ki<sup>+</sup> p+/ri Tpl<sup>hp</sup> Ki p~. This chromosome still carries the Tpl hypomorphic phenotype, but it no longer has a P element hybridization signal at 83E (Figure 4B). Since we have recovered only one such recombinant, it is difficult to rule out alternative possibilities, but this result suggests that the P element inserted at 83E is probably not responsible for the mutant phenotype.

**Reversion of hypomorphic alleles:** In the course of maintaining the hypomorphic mutations as stocks balanced with TM3, Ser for several generations we occasionally observe flies who do not carry the TM3 chromosome. Further crossing and testing of these exceptional flies reveals that they are heterozygotes carrying one wild-type chromosome and one mutant chromosome. We also made lines from single mutant
flies, which bred true for several generations, and then were able to recover revertants in the stock. The revertant lines are always stable. These revertants therefore must actually arise as spontaneous events rather than being due to a second-site suppressor that segregates. We have also systematically looked for revertants of the hypomorphic alleles by crossing large numbers of Tpl\(^{hd}/TM3,Ser\) flies to \(ri\) Tpl\(^{10}\) \(ki\) p\(^{+}/TM3, Ser\). Any Ki Ser\(^{+}\) progeny that result are tested and generally found to be revertants of the dysgenic hypomorphic allele. Some of our new hypomorphic alleles are quite stable, while others are occasionally seen to revert. We have established lines from several of these revertants, but since we do not know the basis of the mutations, we are as yet unable to determine the basis for reversion. We have never observed revertants among the original three hypomorphs from the ROEHRDANZ and LUCCHESI (1980) study, Tpl\(^{-}\), Tpl\(^{2}\) and Tpl\(^{22}\).

DISCUSSION

In general one expects mutations induced by hybrid dysgenesis to be due to insertions of the \(P\) element into a gene. Although there have been occasional reports of P-M hybrid dysgenesis resulting in transposition of other elements (RUBIN, KIDWELL and BINGHAM 1982; LEWIS and BROOKFIELD 1987), ENGELS has presented data suggesting that P-M hybrid dysgenesis does not mobilize other elements (EGGLESTON, JOHNSON-SCHLITZ and ENGELS 1988; ENGELS 1989). While the \(P\) transposase is probably very specific for \(P\) element sequences, very little is known about the control of transposition of other elements. Thus it is certainly possible that when \(\pi_2\) males are crossed to Oregon-R females, other transposable element systems may also be activated. In this study we used this dysgenic cross to generate mutations and rearrangements involving the \(Triplo-lethal\) locus. Interestingly, although we obtained evidence that \(P\) elements were mobilized, at least one other element was likely to have been mobilized to a high degree in the cross.

The duplications we recovered are consistent with the findings of ENGELS and PRESTON (1984). In that study they showed that most of the rearrangements recovered following hybrid dysgenesis had breakpoints that coincided with the locations of \(P\) elements in the parental strain, and that \(P\) element sequences often remained at the new junctions created by the rearrangement. Many of the duplications we recovered have breakpoints in common with the locations of \(P\) elements in our \(\pi_2\) stock (data not shown). Many of the duplications also had \(P\) element sequences remaining at a breakpoint (Figure 3, A, B, C and D, and other data not shown). The simplest way to explain the genesis of our duplications is by a two-break interchromosomal event, with at least one of the breaks usually occurring at the site of a \(P\) element. The reverse tandem duplication, \(Dp(3;3)Tpl^{J25}\), is most likely the result of a three-break event. A transposition would also have required a three-break event and would have been expected to occur at lower frequencies, so the absence of this class is not surprising. Deficiencies would most likely have been produced by a similar two-break process. However, this would often have produced large deficiencies which would either have uncovered a haplo-inviable region nearby [LINDSLEY et al. (1972) report haplo-inviability for 82C to 83E and 84D through 85D] or uncovered a recessive lethal at one of the breakpoints of \(Dp(3;3)21173\) (KEPPY and DENELL 1979).

In view of this evidence that the \(\pi_2 \times\) Oregon-R cross mobilized \(P\) elements, the data on the hypomorphic alleles of \(Tpl\) are surprising. Previous workers have found that the rate of mutation at \(Tpl\) is very low, even in the presence of chemical mutagens such as EMS (KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980). Given that we have recovered hy-
pomorphic mutations in Tpl at a rate significantly higher than that seen in any other study, it seems likely that these mutations were the result of the dysgenesis we induced. Furthermore, the instability of some of these mutations suggests that they are due to transposon insertions. However, none are due to P element insertions that are detectable by in situ hybridization. Other dysgenic systems are known in
Drosophila melanogaster, including the I-R system (FINNEGAN 1989) and hobo (BLACKMAN and GELBART 1989). The \( \pi_2 \times \) Oregon-R cross has apparently not been widely used for \( P \) element hybrid dysgenesis so it is difficult to know whether either of these other dysgenic systems or an as yet undescribed one was activated in the cross.

Finally, our data have implications for the structure of the Triplo-lethal locus. Eighteen years have passed since the locus was first described (LINDSLEY et al. 1972) and the genetics has become only more enigmatic. It seems to be nearly impossible to recover complete inactivations of the locus that are not deletions. Two alleles did result from the mutagenesis carried out by KEPPY and DENELL (1979) which behave genetically as deficiencies but are cytologically normal. It is possible that these are small deficiencies that cannot be seen in polytene preparations, or they may in fact represent the extremely rare occurrence of true point mutations. KEPPY and DENELL (1979) suggested three possible genetic explanations to account for the paucity of null mutations: 1) the locus, and hence the target size for single base-pair changes is very small; 2) the locus may not encode a protein and therefore is not readily disrupted by single base-pair changes; or 3) the locus is redundant in some way, possibly encoding two or more proteins with overlapping or identical functions.

It is paradoxical that deletions are readily selected (KEPPY and DENELL 1979; ROERDANZ and LUCCHESI 1980), and hypomorphs are readily recovered following hybrid dysgenesis, but point mutations are seemingly never recovered. If the locus is redundant, and inactivating one of two or more equivalent genes gives a hypomorphic phenotype, then why should single base-pair changes fail to inactivate one gene and also give a hypomorphic phenotype? If hypomorphs are due to partial inactivations, then why do we fail to recover complete inactivations that are due to transposon insertions? There must be some fundamental difference in the molecular effects of single base-pair changes vs. transposon insertions to which \( Tpl \) is peculiarly sensitive. Point mutations would most likely result in a missense protein while transposon insertions might be more likely to affect the rate of accumulation of a correctly processed and translatable mRNA. This difference might account for the unusual genetics of \( Tpl \). It is our hope that the forthcoming molecular analysis (D. R. DORER, A. C. CHRISTENSEN and D. H. JOHNSON, in preparation) will shed some light on this strange and unique locus.

This work was inspired by the late LARRY Sandler who, in June 1983, suggested to A.C.C. that it would be “easy” to get \( P \) element-induced mutations of \( Tpl \) by hybrid dysgenesis. We are grateful to the inscrutable MARILYN CADDELL for technical assistance, and to JOHN C. LUCCHESI for interesting discussions and for stocks. We thank BRIAN MARIANI for helpful comments on the manuscript, and DANIEL JOHNSON for being enthusiastic about our data. This work was supported in part by National Institutes of Health grant R29-GM38483. D.R.D. was supported in part by a predoctoral fellowship from the Percival E. and Ethel Brown Foerderer Foundation.

LITERATURE CITED


Communicating editor: R. E. DENELL