Suppression of a Lethal Trisomic Phenotype in Drosophila melanogaster by Increased Dosage of an Unlinked Locus

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ABSTRACT

One of the most extreme examples of gene dosage sensitivity is the Triplo-lethal locus (Tpl) on the third chromosome of Drosophila melanogaster, which is lethal when present in either one or three copies. Increased dosage of an unlinked locus, Isis, suppresses the triplo-lethal phenotype of Tpl, but not the haplo-lethal phenotype. We have mapped Isis to the X chromosome region 7E3-8A5, and shown that the suppression is a gene dosage effect. Altered dosage of Isis in the presence of two copies of Tpl has no obvious effects. By examining the interactions between Isis dosage and Tpl we suggest that Isis does not directly repress Tpl expression, but acts downstream on the triplo-lethal phenotype of Tpl.

A NEUPLIOIDY is often accompanied by developmental defects or lethality, which is generally assumed to be due to the simultaneous imbalance of many genes, some with major effects and others with minor effects. In humans, mice and Drosophila the severity of the phenotype is a function of the amount of the genome that is unbalanced, although some specific regions appear to play a disproportionately large role (Epstein 1988; Lindsley et al. 1972). The best example of such a region is the Triplo-lethal locus (Tpl) of Drosophila melanogaster, which causes lethality when it is present in either three copies or one copy (Denell 1976; Lindsley et al. 1972). Attempts by several groups to mutagenize Tpl have revealed that it is possible to eliminate Tpl function by deleting the region, but it may be impossible to completely eliminate Tpl function by point mutations or transposon insertions (Dorer and Christensen 1990; Keppy and Denell 1979; Roehrdanz and Lucchesi 1980). One possible explanation for these results is that Tpl consists of a cluster of functionally redundant transcription units, perhaps like the previously described Achaete-Scute Complex (Alonso and Cabrera 1988) or the Enhancer of split locus (Delidakis et al. 1992). It has been suggested that Tpl is involved in the sex determination pathway as an autosomal element in the X:autosome ratio (Baker and Belote 1983; Denell 1976; Lucchesi and Manning 1987), but experiments reported here and elsewhere have shown that this is unlikely (Christensen and Lucchesi 1988).

Previous genetic studies of Tpl have exploited the existence of stocks carrying a tandem duplication of Tpl on one homolog and a deficiency for Tpl on the other (Dorer and Christensen 1990; Keppy and Denell 1979; Roehrdanz and Lucchesi 1980). When these are crossed to normal flies, all progeny die because they have either three copies of Tpl or one. Although deficiencies for Tpl are easily recovered from this cross because they block the lethal effect of a duplication of the locus, extensive efforts to obtain point mutations that have the same phenotype as deficiencies (null mutations) have been unsuccessful. A second class of mutation was recovered in two of the studies (Dorer and Christensen 1990; Roehrdanz and Lucchesi 1980). These mutations are tightly linked to the known location of Tpl (polytene bands 83E1.2 on the third chromosome), block the lethal effect of a duplication for Tpl, and are recessive lethals, but they are not haplo-insufficient. Although these were previously thought to be hypomorphic mutations of Tpl, we now believe them to be dominant mutations in a tightly linked, but functionally distinct gene called Suppressor of Triplo-lethal (Su(Tpl)) that suppress the triplo-lethal phenotype of Tpl (our unpublished data).

In addition to these mutations which suppress the triplo-lethal phenotype of Tpl, it has been shown that the viability of Tpi hyperploids can be enhanced by increased dosage of the X chromosome. Normally flies with three copies of Tpl die as late embryos or early first-instar larvae, but if these individuals also have
three X chromosomes they may survive into the third larval instar, or possibly longer (ROEHRDANZ and LUCCHESI 1981). This corresponds to a lengthening of the life span from 18-24 hr to 4-5 days. Roehrdanz and Lucchesi also used X;Y translocations to show that trisomy for the entire X chromosome was not necessary for the effect. When they studied the distal end of the X chromosome they found that the trisomy must extend at least as far proximal as cytological region 8A in order to prolong the survival ofTpl hyperploids, and when they used proximal translocations they found that the trisomic region must extend at least as far distal as region 6D in order to have the effect. Since the minimal proximal and distal translocations included a common region, they concluded that there is a locus between 6D and 8A that interacts withTpl in a dosage-dependent manner. An alternative interpretation of these data is that the increased viability ofTpl hyperploids is a function of the amount of the X chromosome that is duplicated; as larger and larger terminal translocations are used, an overlap near the middle of the X is an inevitable consequence of the experimental design.

We have further characterized this phenomenon, and have shown that a small region of the X chromosome (7E-8A) is sufficient to rescueTpl hyperploids. We call the genetic material responsible for suppressing the triplo-lethal phenotype Isis, after the Egyptian goddess who resurrected her brother Osiris after he was killed. Using small duplications of the Isis region we have recovered fertile adults of both sexes who bear three copies ofTpl. This demonstrates that suppression of the triplo-lethal phenotype by increased dosage of Isis can result in virtually normal adults, and also provides further evidence thatTpl is not involved in the sex determination pathway. Altered dosage of Isis itself has no obvious phenotype, nor does it have any effect on the haplo-lethal phenotype ofTpl. We have also attempted to map Isis using translocations, duplications, deficiencies, and a deletion constructed by the method of COOLEY, THOMPSON and SPRADLING (1990).

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 translocation stocks T(1;Y)Y16 and T(1;Y)B170 are described in LINDSLEY and ZIMM (1992) and were obtained from John Merriam (University of California, Los Angeles).Tpl(1;2)zn-724 f car; bu0 has been described (CRAYMER and ROY 1980; LEFEBVRE 1981; LINDSLEY and ZIMM 1992) and consists of a transposition of the region 7A8-8A5 from the X chromosome into the second chromosome; the two elements of this transposition will also be referred to separately as Df(1)zn-724 f car and Dp(1;2)zn-724. This stock as well as Df(1)C128, Df(1)GE202 and Dp(1;3)zn-131 were provided by TERRY JOHNSON and ROB DENELL (Kansas State University). Df(1)RA2 was supplied by the Indiana University Drosophila Stock Center. Descriptions of the above markers and chromosome rearrangements and their origins can be found in (LINDSLEY and ZIMM 1992). The p; and Oregon-R stocks used for hybrid dysgenesis, and Dp(3;3)Tpl121 are described in (DORER and CHRISTENSEN 1990). TheTpl duplication/deficiency stock used predominately in this paper has been previously described (DORER and CHRISTENSEN 1990; KEPPE and DENELL 1979). Its full genotype is YXYY, In(1)EN y/YXYY, In(1)EN y : Dp(3;3)Tpl121 p'/Df(3R)1817777 p', but for simplicity we will refer to it as Dp(Tpl)/Df(Tpl). A shortened derivative of Dp(1;2)zn-724, called Dp2, and the deficiency DfTpl were originally created by F. FORQUIGNON and M. GANS, are described by LINERUTH (1987), and were kindly provided by STEPHAN ANDERSSON of the University of Umea, Sweden. Dp2 contains a deletion of the distal end of Dp(1;2)zn-724, and is both c' and sn-.

The cytological limits of DfTpl were described by ANDERSSON and LAMBERTSSON (1990) as being approximately TCl.2-7D19-22.

Hybridization in situ to polytene chromosomes: Salivary gland squashes for in situ hybridization were prepared essentially as described in ENGELS et al. (1986), except that slides were not pretreated with Denhardt's solution. Plasmids p25.7 wc (KARESS and RUBIN 1984) and Carnegie20 (SPRADLING and RUBIN 1983) were used as probes. DNA was biotinylated by nick translation with bio-16-dUTP (Bethesda Research Laboratories) substituted for dTTP. Hybridizations were carried out at 39°C in a buffer containing 4 X 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).

Designer deletion: A deficiency for X chromosome bands 7D1-7D14-17 was constructed using the method of COOLEY, THOMPSON and SPRADLING (1990). For target P elements, we chose the pair of P elements tandemly inserted in the singed locus at 7D1, sn- (ROIHA, RUBIN and O'HAIRE 1988), and an insertion of a P(y) element at 7D14-17. A sn-; P(y) stock was obtained from SCOTT HAWLEY (University of California, Davis) and 2a(1)PrO007D-AS334; +/P(y) (formerly called R403.1 (SPRADLING and RUBIN 1983)), hereafter called P(y);+; +/P(y)

This corresponds to a lengthening of the life span from 18-24 hr to 4-5 days. Roehrdanz and Lucchesi also  used X;Y translocations to show that trisomy for the entire X chromosome was not necessary for the effect. When they studied the distal end of the X chromosome they found that the trisomy must extend at least as far proximal as cytological region 8A in order to prolong the survival ofTpl hyperploids, and when they used proximal translocations they found that the trisomic region must extend at least as far distal as region 6D in order to have the effect. Since the minimal proximal and distal translocations included a common region, they concluded that there is a locus between 6D and 8A that interacts withTpl in a dosage-dependent manner. An alternative interpretation of these data is that the increased viability ofTpl hyperploids is a function of the amount of the X chromosome that is duplicated; as larger and larger terminal translocations are used, an overlap near the middle of the X is an inevitable consequence of the experimental design.

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RESULTS

Suppression of Triplo-lethal by overlapping (X;Y) translocations: Since the suppression of the triplo-lethal phenotype of Tpl observed by Roehrdanz and Lucchesi (1981) was mediated by very large portions of the X chromosome, we set out to test this effect using hyperploidy for a much more limited region of the X chromosome. If the suppression of Tpl were merely a nonspecific effect of X hyperploidy, we would expect that reducing the duplicated region would reduce the suppression, while if a specific locus were involved, then reducing the hyperploidy for irrelevant regions of the X should improve the viability of the rescued flies. A synthetic duplication of the cytological regions 6E-8A was created by using two different (X;Y) translocation stocks: T(1;Y)B170, broken in 8A and y^*, and T(1;Y)V16, broken in 6E1-3 and y^*. For T(1;Y)B170, the distal portion of the X chromosome is marked with B^S, and the proximal with y^*. For T(1;Y)V16, the distal fragment of X is marked with y^* and the proximal with B^S (see Figure 1). By crossing these two stocks together and choosing progeny with the appropriate markers, we obtained females carrying one complete X, the distal portion of T(1;Y)B170, and the proximal portion of T(1;Y)V16. These females are essentially disomic for the X chromosome, except that they have three copies of the region between the translocation breakpoints. They were then crossed to Dp(Tpl)/Df(Tpl) males. A small number of female progeny were observed, all carrying the appropriate markers to conclude that they were of the genotype indicated in Figure 1, having three copies of Tpl and three of the 6E-8A region. Some of these females eclosed, but were feeble and died within a few hours after eclosion. A number of other females were seen as dead pharate adults. We conclude from this experiment that flies with three doses of Tpl are rescued to a limited extent by the presence of three copies of the region of the X chromosome from 6E to 8A, and this effect does not require hyperploidy for any other region of the X chromosome. This observation supports the hypothesis that the duplication of a small region of the X chromosome, possibly even a single X-linked locus, suppresses the triplo-lethality of Tpl. This locus was not named by Roehrdanz and Lucchesi, but we call it Isis (see Introduction).

Suppression of Triplo-lethal by Dp(1;2)sn13a1: We confirmed Roehrdanz and Lucchesi's (1981) results with Dp(1;3)sn13a1 (data not shown) which does not suppress the triplo-lethal phenotype of Tpl. We then used another available rearrangement involving this region, T(p;2)sn^*72d. This rearrangement is a transposition of the genetic material between 7A8 and 8A5 from the X to the second chromosome (see Figure 2). When T(p;2)sn^*72d males were mated to Dp(Tpl)/Df(Tpl) females, a number of progeny survived to adulthood. These adult flies were males with yellow body color and wild-type eyes. Female progeny were not observed in this cross, since they carried the X-linked deficiency for 7A8-8A5 and therefore could not have had a net duplication for that region. While many of the survivors lived for only a few days, some of them lived long enough to mate and proved to be fertile. Test crosses verified that they carried Dp(1;2)sn^*72d and Dp(3;3)Tpl21. The simultaneous occurrence of the Dp(1;2)sn^*72d and Dp(3;3)Tpl21 chro-
The extents of some of the rearrangements tested for the presence of Isis are shown aligned with a portion of Bridge's (1935) map of the X chromosome. Df(1)RA2 is shown according to our revised mapping (see text). The locations of a number of nearby loci, including the P(r7) element described in the text, are indicated above the map. The gray regions at the ends of rearrangements indicate the limits of certainty for the breakpoint position. The duplications are tested by asking for suppression of the mapping (see text). The locations of a number of nearby loci, Minute including the indicated the limits of certainty for the breakpoint position. The triplo-lethal phenotype of progeny from a nesis: males. The ing the salivary gland chromosomes of a surviving were mated to Minute technique of cross are viable and totaled usually die, since they have only one copy of the severe mosomes in a single fly was also observed by examin- and Minute, and thus were probably rare survivors were recovered. Of these, 85 were Minute (DORER, ANANE-FIREMPONG and CHRISTENSEN 1991; LEJEFM and JOHNSON 1973). From an estimated 17,000 Df(1)sn+/+, car/+, yb0/+ zygotes (the three other possible genotypes from this cross are viable and totaled 51,253 adults), 87 adult survivors were recovered. Of these, 85 were infertile and Minute, and thus were probably rare Df(1)sn+/ + survivors. The other two survivors were not Minute. Both of these have duplications which were observed genetically and cytologically to be X-linked. Dp(1;1)GH65 is a tandem duplication of 6A to 8A, and is semiviable in males. Dp(1;1)GH95 covers a larger region, and therefore has breakpoints which are less informative about the location of Isis. Dp(1;1)GH95 is very poorly viable in males.

Dp(1;1)GH65 and Dp(1;1)GH95 were both tested for the presence of Isis. Dp(1;1)GH65/FM7c or Dp(1;1)-GH95/FM7c females were mated to Dp(3;3)Tpl21/Df(3R)18is77 or Dp(3;3)Tpl24'/Df(3R)18is77 males. Dp(3;3)Tpl21 is a duplication of 83E-84B (DENELL 1976) while Dp(3;3)Tpl24' (DORER and CHRISTENSEN 1990) is a duplication of 83A-83E. As shown in Table 1, a significant number of adult survivors are recovered with all four combinations of Isis and Tpl duplications, demonstrating that the suppression of Tpl is a gene-dosage-dependent phenomenon and not due to a position effect of a particular rearrangement.

**Suppression of Triplo-lethal by shortened derivatives of Dp(1;2)sn724.** The experiments described above show that duplication of the region 7A8-8A5 suppresses the triplo-lethal phenotype of Tpl, but that the region from 7A8-7C9 is probably not necessary for the effect (this region is included in Dp(1;3)sn13al, see Figure 2). To further localize the essential genetic material necessary for this effect we tested for the presence of Isis in shortened duplications derived from Dp(1;2)sn724. The most significant duplication that we tested is known as Dp6 (see MATERIALS AND METHODS). The region of the X chromosome which is duplicated in Dp6 has the same proximal breakpoint (8A5) as Dp(1;2)sn724 and the distal breakpoint is probably located between 7D1 and 7D5,6 (LINERUTH 1987; our unpublished data). This duplication suppresses Tpl and must therefore include Isis. Similar results were obtained with Dp(1;2)ct7907, which was created in our laboratory by γ-irradiation of Dp(1;2)sn724 (DORER, ANANE-FIREMPONG and CHRISTENSEN 1991). These experiments have ruled out any

**FIGURE 2.**—Summary of the cytogenetic region surrounding Isis. The extents of some of the rearrangements tested for the presence of Isis are shown aligned with a portion of Bridge's (1935) map of the X chromosome. Df(1)RA2 is shown according to our revised mapping (see text). The locations of a number of nearby loci, including the P(r7) element described in the text, are indicated above the map. The gray regions at the ends of rearrangements indicate the limits of certainty for the breakpoint position. The duplications are tested by asking for suppression of the mapping (see text). The locations of a number of nearby loci, Minute including the indicated the limits of certainty for the breakpoint position. The triplo-lethal phenotype of progeny from a nesis: males. The ing the salivary gland chromosomes of a surviving were mated to Minute technique of cross are viable and totaled usually die, since they have only one copy of the severe mosomes in a single fly was also observed by examin- and Minute, and thus were probably rare survivors were recovered. Of these, 85 were Minute (DORER, ANANE-FIREMPONG and CHRISTENSEN 1991; LEJEFM and JOHNSON 1973). From an estimated 17,000 Df(1)sn+/+, car/+, yb0/+ zygotes (the three other possible genotypes from this cross are viable and totaled 51,253 adults), 87 adult survivors were recovered. Of these, 85 were infertile and Minute, and thus were probably rare Df(1)sn+/ + survivors. The other two survivors were not Minute. Both of these have duplications which were observed genetically and cytologically to be X-linked. Dp(1;1)GH65 is a tandem duplication of 6A to 8A, and is semiviable in males. Dp(1;1)GH95 covers a larger region, and therefore has breakpoints which are less informative about the location of Isis. Dp(1;1)GH95 is very poorly viable in males.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Generality of rescue of Dp(Tpl)/Tpl* by Isis duplications</th>
<th>X chromosomes of survivors</th>
<th>Tpl duplication</th>
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<tr>
<td>Dp(1;1)GH65 cross:</td>
<td>Dp(3;3)Tpl21</td>
<td>0 0</td>
</tr>
<tr>
<td>Dp(1;1)GH65/FM7c</td>
<td>64 0</td>
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</tr>
<tr>
<td>Df(1;1)GH65/YrX,Yr   x2</td>
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<td>Dp(1;1)GH95 cross:</td>
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<tr>
<td>Df(1;1)GH95/MF7c</td>
<td>0 0</td>
<td></td>
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<tr>
<td>Df(1;1)GH95/YrX,Yr   x2</td>
<td>34 237</td>
<td></td>
</tr>
</tbody>
</table>

Females of the genotype Dp(1;1)/FM7c were mated to YrX,Yr; Dp(3;3)Tpl21/Df(3R)18is77 and YrX,Yr; Dp(3;3)Tpl24'/Df(3R)18is77 males. Small numbers of exceptional progeny resulting from nondisjunction were also recovered but are not shown.

### New Isis duplications induced by hybrid dysgenesis

Two new tandem duplications which include the cytological region 7C were recovered by the antimate technique of Grell (1969). Drosyge genetic female progeny from a py male × Oregon-R female cross were mated to Df(1)sn724, f car/Y; Dp(1;2)sn724/bu0 males. The Df(1)sn724, f car/+; +/bu0 F2 females usually die, since they have only one copy of the severe Minute locus, M(1)7C (DORER, ANANE-FIREMPONG and CHRISTENSEN 1991; LEFEVRE and JOHNSON 1973). From an estimated 17,000 Df(1)sn+/+, car/+; bu0/+ zygotes (the three other possible genotypes from this cross are viable and totaled 51,253 adults), 87 adult survivors were recovered. Of these, 85 were infertile and Minute, and thus were probably rare Df(1)sn+/ + survivors. The other two survivors were not Minute. Both of these have duplications which were observed...
TABLE 2

<table>
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<th>X chromosomes of survivors</th>
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<tr>
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<tr>
<td>FM7c</td>
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<tr>
<td>FM7c/Y;X,Y10</td>
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<tr>
<td>Y;X,Y10</td>
<td>8</td>
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</tbody>
</table>

The number of progeny resulting from crosses involving five different deficiencies of the X chromosome are tabulated. Df(1)/FM7c; Dp(1;2)sn+72d/+ females were mated to Y;X,Y10; In(1)EN y; Dp(Tp1)/Df(Tp1) males. All progeny were Dp(1;2)sn+72d/+; Dp(Tp1)/+. The Y;X,Y10 male progeny result from nondisjunction of the X chromosomes in the Df(1)/FM7c female parent.

region distal to 7D as the possible location for Isi (see Figure 2).

Mapping Isi using X-linked deficiencies: Since the suppression of triplo-lethality seems to be caused by the presence of an extra copy of a locus which maps within Dp(1;2)sn+72d, it follows that the deletion of this locus from the X chromosome in a Dp(1;2)sn+72d/+; Dp(Tp1)/+ male should prevent the suppression. We tested five deficiencies in the 7D-8A region (see Figure 2). Each test cross for these deficiencies included an internal control for the efficiency of suppression using the FM7c balancer X chromosome. These crosses were complicated by the variable, but generally poor fertility of males carrying Dp(1;2)sn+72d, and the incomplete penetrance of the suppression of Tpl by Isi. Females of the genotype Df(1)/FM7c; Dp(1;2)sn+72d/+ were crossed to Y;X,Y10; In(1)EN y; Dp(Tp1)/Df(Tp1) males. Half of the progeny from this cross inherit Df(Tp1) from the male and will die. Of the Dp(Tp1)-bearing progeny that remain, half of them inherit Dp(1;2)sn+72d from the female, and are potential survivors, depending on their X chromosome(s). The recovery of FM7c; Dp(1;2)sn+72d/+; Dp(Tp1)/+ male progeny is an indicator of a fertile cross, and the survival of Df(1)-bearing males indicates that the deficiency does not delete Isi. The results from these crosses are shown in Table 2. Males of the genotype Df(1)GE202; Dp(1;2)sn+72d/+; Dp(Tp1)/+ were easily obtained (Table 2). This clearly rules out the chromosomal region from 7D12-13 to 7E3-4 as being required in an extra copy to suppress Dp(Tp1)/+. In contrast, no survivors were found with Df(1)RA2 (Table 2). Cytological examination of this deficiency suggests that the distal breakpoint of Df(1)RA2 may have been incorrectly assigned to 7D10 (LINDSLEY and ZMM 1992). This deficiency is frequently associated with asynapsis distal to the deficiency in heterozygous females, which makes the breakpoint difficult to determine, but it appears to be in 7D18-22, and it complements Df(1)Des3 which is deleted for 7D1 through 7D14-17 (see below). Figure 2 indicates our revised estimate of its breakpoints, 7D18-22 and 8A4.5. Although we did a large number of crosses with Df(1)RA2, the number of control progeny was still small. The reasons for this are not entirely clear, but we have observed that stocks carrying both Df(1)RA2 and Dp(1;2)sn+72d are not as fecund as those carrying only one of these rearrangements. As further evidence for Df(1)RA2 being Isi5, we also performed a cross using Df(1)RA2/Df(1)RA2; Dp(1;2)sn+72d/bd females and Dp(Tp1)/Df(Tp1) males. There were no survivors from this cross, in contrast to a similar experiment using Df(1)GE202/Df(1)GE202; Dp(1;2)sn+72d/bd which produced many progeny. Taken together, these results suggest that Isi5 is deleted by Df(1)RA2, but not by Df(1)GE202, and is therefore located somewhere in the 7E3-8A5 region. Furthermore, we have never recovered any survivors bearing Dp(Tp1) in these crosses. If deficiencies for Isi5 rescued flies carrying only one dose of Tpl, we would have seen them in these crosses as Df(1)RA2/Y;X,Y10; In(1)EN y; +/-; Dp(Tp1)/+ females, and this did not occur, demonstrating that altered Isi5 dosage has no apparent effect on the haplo-lethal phenotype of Tpl. A similar test cross was found to produce Df(1)C128; Dp(1;2)sn+72d/+; Dp(Tp1)/+ progeny at a very low ratio compared to their FM7c-bearing siblings (see Table 2). However, in spite of the low rate at which flies bearing this deficiency are recovered, the recovery of any of these flies at all suggests that the region 7D1-5,6 is not essential for suppression of triplo-lethality by Isi5. We also tested the X-linked deficiency Df(1)hE. The results with Df(1)hE are similar to those with Df(1)C128. We have seen Df(1)hE; Dp(1;2)sn+72d/+; Dp(Tp1)/+ adults, though they appear at a frequency far lower than the FM7c; Dp(1;2)sn+72d/+; Dp(Tp1)/+ control flies which survive in the same crosses (Table 2). We also tested a new deficiency which we have synthesized called Df(1)Des3. Df(1)Des3 is a "designer deletion" (COOLEY, THOMPSON and SPRADLING 1990) induced between P elements located at 7D1 and 7D14-17 (see MATERIALS AND METHODS). After extensive crosses of Df(1)Des3/FM7c; Dp(1;2)sn+72d/+ to Dp(Tp1)/Df(Tp1) we recovered almost no survivors of any type, including the FM7c control. A total of 251 females were used in this experiment, but the overall viability of this mating appears to be very poor for reasons we do not fully understand (see DISCUSSION). Since Df(1)hE and Df(1)Des3 are deficient for the same region that is missing in Df(1)C128, it is perhaps not surprising that the recovery of survivors is affected in the same negative manner by all three deficiencies. However, the recovery of even small numbers of progeny is good evidence that these deficiencies are Isi5, since the probability of recovering adults with three doses of Tpl is essentially nil (KEPPY and DENELL 1979; ROEHRDANZ and LUCCHESI 1980; DORER and
CHRISTENSEN 1990; our unpublished data).

**Interactions between Isis and other mutations affecting Tpl:** Duplications of Isis might directly repress the expression of Tpl, or they might act downstream to suppress the lethal effects of overexpression of Tpl. The Su(Tpl) mutations described in the Introduction also permit the survival of flies carrying a duplication of Tpl (DORER and CHRISTENSEN 1990; ROEHRDANZ and LUCCHESI 1980). If either Su(Tpl) or Isis represses Tpl directly we might expect combinations of these to further reduce the effective Tpl dose. The net effect would be to mimic deficiencies of Tpl which should result in lethality. We used one representative mutation, Su(Tpl)^10, [previously called Tpl^10 (ROEHRDANZ and LUCCHESI 1980)], and were able to recover both males and females with the genotype Dp(1;2)m^T2/H; ri Su(Tpl)^10 Ki p^+/+, suggesting that Isis acts downstream on the triplo-lethal effects of Tpl.

**DISCUSSION**

In their original description of the rescue of \(Dp(Tpl)/Tpl^+\) by X chromosome hyperploidy, ROEHRDANZ and LUCCHESI (1981) proposed that duplications of 7DE would be sufficient to effect suppression of Tpl. However, all of the translocations successfully used in their experiments were hyperploid for either the distal or proximal regions of the X in addition to the 7DE area. Therefore, the possibility that suppression of triplo-lethality was caused by the presence of large regions of the X chromosome was not conclusively ruled out prior to the experiments reported here. We have shown that the suppression of Tpl is probably mediated by a single locus in the 7E3–8A5 region of the X chromosome, which we have named Isis. This work is also relevant to the previously suggested model of Tpl as a component of the sex determination pathway (BAKER and BELOTE 1983; DENELL 1976; LUCCHESI and MANNING 1987). The existing data do not support this model for the following reasons. (1) We have obtained sexually normal males and females with a variety of combinations of dosage of both Tpl and Isis (above, and our unpublished data). (2) None of the known genes involved in sex determination (including Sxl) are located in the Isis region (CLINE 1988). (3) Tpl does not interact with known sex-determination genes (CHRISTENSEN and LUCCHESI 1988).

We have also used a variety of duplications of both Tpl and Isis, which serve to narrow down the location of Isis and also demonstrate that the effects seen here are not due to position effects of a particular duplication, but are true gene dosage effects. The effect of Isis on Tpl resembles the “inverse effect” described by BIRCHLER and colleagues, wherein variation in the dosage of chromosomal segments inversely affects the expression of unlinked genes (BIRCHLER 1979; BIRCHLER, HIEBERT and KRIETZMAN 1989; DEVLIN, HOLM and GRIGLIATI 1988; RABINOW and BIRCHLER 1989; RABINOW, NGUYEN-HYUNH and BIRCHLER 1991). However, there are significant differences, especially the failure of Isis deficiencies to suppress Tpl haplo-lethality, and the fact that duplications and deficiencies of Isis are fully viable. Unfortunately, deficiency mapping of Isis has been somewhat complicated. Although the results with \(Df(1)RA2\) and \(Df(1)GE202\) are fairly clear, the other three deficiencies, \(Df(1)b^F\), \(Df(1)Des^5\) and \(Df(1)CI28\) somewhat weaken the conclusion that Isis is a single locus in 7E3–8A4.5. These deficiencies clearly reduce the viability of flies carrying three doses of Tpl and three doses of Isis, but that could be a specific effect on Isis or a nonspecific effect on viability. One possibility is that Isis consists of two or more separate genes acting synergistically to suppress Tpl, and the various deficiencies in this region delete some but not all of the required sequences. As an alternative, the viability of the rescued flies is poor, and as a result they may be exquisitely sensitive to further genetic imbalances. Since we observe variability in the efficiency of the rescue by the various duplications of Isis, as well as from experiment to experiment, we prefer the latter possibility, but we cannot rule out the hypothesis that Isis consists of redundant genetic information. On this point, an example of a repeated sequence in the Isis region was described by WARING and POLLACK (1987). This sequence, XDm, is 372 nucleotides long, occurs in dispersed tandem arrays, and is present a total of 300–400 times in the genome. It appears to be localized almost exclusively to the middle of the euchromatic portion of the X chromosome, primarily between cytological divisions 4 and 11. Interestingly, one of the largest clusters appears to be in the 7E–8A region (WARING and POLLACK 1987). Whether this sequence has any relationship to Isis (or if it has any function at all) is currently unknown, but could be tested as the molecular analysis of Tpl proceeds.

Although the Isis-Tpl interaction is a gene dosage effect, it is asymmetric. Tpl is extremely sensitive to dosage alterations while the Isis region is apparently not. Isis duplications suppress the triplo-lethal phenotype of Tpl, but have no effect on the viability of flies with either one or two doses of Tpl. In addition, the lack of interaction of Isis with the Su(Tpl) mutations suggests that Isis does not act directly to repress Tpl expression. The \(Su(Tpl)^10+/+\) genotype contains two copies of Tpl and a suppressor of Tpl. The successful addition of a duplication of Isis to this genotype suggests that if Isis and \(Su(Tpl)^10\) act directly on Tpl, they do not act additively or synergistically to reduce Tpl expression, since that should mimic a deficiency of Tpl. It seems more likely that Isis acts downstream on the triplo-lethal effects of Tpl. This in turn suggests
that the triplo-lethal and haplo-lethal phenotypes of \( T^{pl} \) may be different from each other. We hope that our continuing analysis of \( T^{pl} \) will help to resolve these questions.

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