Chapter 5

Premeiotic instability of the $Dp(D305)$ duplication
Introduction

In this Chapter I describe my studies on the unusual behaviour in a sexual cross of the chromosome segment duplication \( Dp(III\text{R} \rightarrow X; III\text{R}; VIL)D305 \) [hereafter called \( Dp(D305) \)]. \( Dp(D305) \) strains can be derived from crosses between the translocation strain \( T(III\text{R} \rightarrow X; III\text{R}; VIL)D305 \) and normal sequence strains. This is a complex translocation in which a segment of IIIR appears to be inserted in an unidentified chromosome and VIL is also involved (Perkins, 1997). The \( Dp(D305) \) duplication fully covers the segment duplicated by the \( Dp(AR17) \) duplication (see Chapter 3). My motivation to examine this duplication was the report that whereas \( Dp(AR17) \) is stably barren in crosses, crosses with \( Dp(D305) \) have a semi-barren phenotype despite the fact that \( Dp(D305) \) includes the segment duplicated in \( Dp(AR17) \). We found that the frequency of RIP in the duplicated segment in \( Dp(D305) \) was exceptionally low and, unlike other large duplications tested (see Chapter 3), its presence failed to suppress RIP in a smaller duplication. Our results indicate that the unusual behaviour of \( Dp(D305) \) could be the result of an instability of the duplication during the sexual stage.

Results and discussion

\( Dp(D305) \) covers \( erg-3 \)

A cross was performed between the strains \( T(III\text{R} \rightarrow X; III\text{R}; VIL)D305 \) \( A \) and \( dow \ erg-3 \ a \) and we scored the phenotypes of 125 progeny. The distribution of phenotypes was 72 Dow\(^+\) Erg-3\(^+\), 44 Dow\(^-\) Erg-3\(^-\), 7 Dow\(^+\) Erg-3\(^-\), and 2 Dow\(^-\) Erg-3\(^+\). If \( erg-3 \) is covered by \( Dp(D305) \) the duplication progeny should be found amongst the Dow\(^+\) Erg-3\(^+\) segregants, otherwise they should be amongst the Dow\(^+\) Erg-3\(^-\) progeny. Furthermore, crosses of the duplication segregants with the wild type should produce Dow\(^-\) Erg-3\(^-\) progeny. Thirty Dow\(^+\) Erg-3\(^+\) segregants were crossed with the wild type strains 74-\( OR23 \)-1 \( A \) or \( OR8-1 \) \( a \). We were able analyze progeny.
from 26 crosses and of these five yielded Dow Erg-3' segregants. These results indicate that \(Dp(D305)\) covers \(erg-3\). The remaining four crosses could not be analyzed because of their poor fertility. The proportion of duplication progeny amongst the Dow\(^+\) Erg-3\(^+\) segregants (5/26) was much below the expected one-half. This is consistent with earlier observations that \(Dp(D305)\) progeny are under-represented amongst segregants obtained from the cross of translocation with normal sequence strain (Perkins, 1997).

\(Dp(D305)\) covers dow as well as phe-2, tyr-2, un-17 and het-7. \(Dp(AR17)\) covers dow but not phe-2, tyr-2, un-17 and het-7 all of which are proximal to dow. \(Dp(AR17)\) also does not cover erg-3 which is distal to dow. Thus the \(Dp(AR17)\) duplication appeared to be smaller and fully included within the \(Dp(D305)\) duplication. Despite this, \(Dp(D305)\) when heterozygous in a cross is semi-barren whereas \(Dp(AR17)\) is stably barren.

**RIP in the duplicated segment of \(Dp(D305)\) is undetectable**

We examined the efficiency of RIP in \(Dp(D305)\) by measuring the \(erg-3\) mutation frequency in crosses that were heterozygous for the duplication. A cross was performed between the strain \(T(D305)\ a\) and dow A. Fifty-nine segregants were examined of which 17 were Dow\(^-\) and 42 were Dow\(^+\). Twenty-eight Dow\(^+\) segregants were crossed with the wild type strains 74-OR23-1 A or OR8-1 a. Of the 28 crosses, five (involving segregants #6, 8, 12, 28 and 30) yielded Dow\(^-\) progeny. Thus these 5 segregants represent \(Dp(D305)\ dow^+/dow\) strains. The proportion of duplication progeny (5/28) amongst the phenotypically Dow\(^+\) progeny was less than the expected 50% and was similar to the proportion of duplication progeny (5/26) amongst the phenotypically Dow\(^+\) Erg-3\(^+\) segregants from the cross of \(T(D305)\) A with dow erg-3 a.
Progeny were examined from the crosses of each of the five Dp(D305) dow+/dow and the results are summarized in Table 5.1. The RIP frequency appeared to be exceptionally low; only one cross yielded a single erg-3 mutant. Southern analysis of Dpn II and Sau 3AI digested genomic DNA from the mutant did not show any evidence of cytosine methylation (Fig. 5.1). This suggested that the erg-3 sequences probably were only lightly altered by RIP (Singer et al., 1995). This was consistent with the results of Perkins et al. (1997), showing that RIP in large duplications is milder than in smaller gene-sized duplications.

We also examined the frequency of dow mutants resulting from RIP in Dp(D305). Two of the Dp(D305) dow+*/dow strains (#12 and #28) were each crossed with erg-3 a. Six phenotypically wild-type progeny from the cross of #12 and nine from the cross of #28 were then crossed with the wild-type strains 74-OR23-1 A or OR8-1 a. Of the fifteen crosses, one cross, involving segregant #28-4, segregated erg-3 mutants which indicated that this segregant was a Dp(D305) erg-3+/erg-3 strain. None of the 100 Erg-3+ and 107 Erg-3' progeny examined from this cross was mutant in dow. Thus the frequency of RIP-induced dow mutants from this cross, which was nominally heterozygous for Dp(D305), was less than 1/207 (<0.5%). This is much lower than frequency of dow mutants recovered from crosses heterozygous for Dp(AR17) (see Table 3.1; Perkins, 1997).

Dp(D305) does not suppress RIP in erg-3

We had shown in the previous chapter that large duplications suppress RIP in a smaller duplication. We tested whether Dp(D305) also suppressed RIP in a small duplication using the erg-3 test system which has been described earlier. Crosses were performed between Dp1.3<sup><sup>ec</sup></sup>hph and the five Dp(D305)dow+*/dow strains described above. The erg-3 mutation frequencies ranged from 3.2% to 4.4% (Table 5.2). These frequencies are higher than the <0.46% to 1.33% obtained in comparable heterozygous crosses of Dp1.3<sup>ec</sup>hph with Dp(AR17) strains (see Table 3.4). Thus
Table 5.1: *erg-3* mutation frequencies in *Dp(D305)dow*/dow

<table>
<thead>
<tr>
<th>Cross</th>
<th>ascospores examined</th>
<th>erg-3 mutants</th>
<th>% erg-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 x OR A</td>
<td>1200</td>
<td>0</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>8 x OR A</td>
<td>1414</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>12 x OR A</td>
<td>1331</td>
<td>0</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>28 x OR A</td>
<td>2122</td>
<td>0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>30 x OR A</td>
<td>1491</td>
<td>0</td>
<td>&lt;0.07</td>
</tr>
</tbody>
</table>
Figure 5.1: The *erg-3* mutant obtained from RIP in *Dp(D305)* does not show evidence of methylation. Genomic DNA of a rare RIP-induced *erg-3* mutant from a cross of a *Dp(D305)dow^+/dow^−* with 74-OR23-I A was digested with *DpnII* (D) and *Sau3AI* (S) and probed with the complete *erg-3* sequence. Both the enzymes recognize the same restriction site (GATC), however, *Sau3AI* does not cut if the C is methylated. Presence of identical restriction pattern in both lanes in the above blot is indicative of absence of methylation.
Table 5.2: \textit{erg-3} mutation frequencies in crosses of $Dp(D305) \ dow^+/dow$ with $Dp1.3^{ec}hph$

<table>
<thead>
<tr>
<th>Cross</th>
<th>ascospores examined</th>
<th>\textit{erg-3} mutants</th>
<th>% \textit{erg-3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>$6 \times Dp1.3^{ec}hph A$</td>
<td>125</td>
<td>4</td>
<td>3.2</td>
</tr>
<tr>
<td>$8 \times Dp1.3^{ec}hph A$</td>
<td>84</td>
<td>6</td>
<td>7.14</td>
</tr>
<tr>
<td>$12 \times Dp1.3^{ec}hph A$</td>
<td>297</td>
<td>12</td>
<td>4.04</td>
</tr>
<tr>
<td>$30 \times Dp1.3^{ec}hph A$</td>
<td>213</td>
<td>9</td>
<td>4.23</td>
</tr>
</tbody>
</table>
\(Dp(D305)\), unlike \(Dp(AR17)\), did not appear to suppress RIP in trans.

We also tested whether \(Dp(D305)\) suppressed RIP in cis by examining the frequency of \(erg-3\) mutants obtained from crosses parented by \(Dp1.3^{ec}hph; Dp(D305)\) double duplication strains. To construct the double duplication strains, a \(Dp(D305)dow^+\) strain (#8) was crossed with a \(Dp1.3^{ec}hph; dow A\). The frequency of \(erg-3\) mutants in this cross was 6/39 (15.4%). Sixty-five \(erg-3^+\) segregants were examined of which 53 were \(dow\) and 12 were \(dow^+\). Of the 10 \(dow^+\) \(erg-3^+\) progeny tested, four (#8-1, #8-3, #8-5 and #8-8) were hygromycin-resistant. Only two (with #8-3 and #8-5) segregated \(dow\) progeny (12/26 and 9/12, respectively) indicating that #8-3 and #8-5 represent \(Dp(D305)dow^+/dow; Dp1.3^{ec}hph\). [From the crosses with #8-1 and #8-8 nineteen and 29 segregants, respectively, were examined and none was \(dow\), therefore it could not be confirmed that #8-1 and #8-8 contained \(Dp(D305)\).] The \(erg-3\) mutation frequencies in the crosses with #8-3 and #8-5 were 27/277 (9.7%) and 18/248 (7.3%). These results indicate that ability of \(Dp1.3^{ec}\) to induce RIP in \(erg-3\) remains unaffected even when if \(Dp(D305)\) is present with in cis.

Is the \(Dp(D305)\) duplication unstable?

The preceding results suggested that RIP in \(Dp(D305)\) was extremely low and that it failed to suppress RIP in a smaller duplication. Moreover it has been described as being semi-barren in crosses (Perkins, 1997); in fact, we found no discernible difference between a cross that was fertile and one that was heterozygous for \(Dp(D305)\). Perkins (1997) also noted that translocation progeny were recovered at a lower frequency than normal sequence from a cross of the \(T(D305)\) translocation with normal. These observations raised the possibility that the duplication was unstable. Instances of breakdown of duplications are not unknown (see Perkins, 1997 for a review). A barren duplication can revert to a fertile euploid condition by deletion of either of the duplicated segments; loss of the duplicated segment from the
translocated position would restore normal sequence whereas loss from the normal position would restore the translocation sequence. For reasons that are not clear, the loss is more common from the translocated segment (Newmeyer and Galeazzi, 1977; Smith et al., 1996).

We tested $Dp(D305)$ erg-3+/erg-3 strains for breakdown during vegetative growth. If the duplication suffered vegetative breakdown, then a subset of the conidia would become tomatine-resistant due to the uncovering of erg-3 (Sengupta et al., 1995). Conidia from three $Dp(D305)$erg-3+/erg-3 strains (#4, #12 and #14) were streaked onto Vogel’s-sorbose plates supplemented with tomatine but no conidia with the tomatine-resistance phenotype was observed. This suggested that $Dp(D305)$ is stable during vegetative growth. In contrast, a subset of conidia from control heterokaryons made between a dow erg-3 strain and the helper-1 strain showed tomatine-resistance.

We next asked if the duplication was stable through a sexual cross. Crosses were performed between erg-3 A and two $Dp(D305)$ dow+/dow a strains (#12 and #28). For the cross with segregant #12, the frequency of erg-3 progeny was 79/167 (47.3%). For the second cross, with segregant #28, 67/134 (50%) segregants were erg-3. In both crosses the proportion of erg-3 progeny was much higher than the expected 25%. Twenty erg-3 segregants from both crosses were examined of which 7 (35%) from the first cross and 4 (20%) from the second were also dow. Thus the frequency of crossovers between dow and erg-3 appeared to be much greater than the 10% cross-over distance between the two loci. Of the 40 erg-3+ progeny examined from each cross, 11 from the former and five from the latter were dow+. These erg-3+ dow+ progeny would include some that are $Dp(D305)$. Since the proportion of erg-3+ segregants recovered from the cross of the duplication strains with the normal sequence was about 50%, the proportion of duplication progeny is expected to be half of 16/80, i.e., 10%. This was much less than the expected 50% duplication progeny.
from a cross of duplication with normal sequence and suggests that \( Dp(D305) \) is lost during the sexual cycle.

Loss of \( Dp(D305) \) was also observed in a cross that was homozygous for the duplication. The two confirmed \( Dp1.3^{echph}; Dp(D305) \) dow*/dow double duplication strains, designated #8-3 and #8-5 (described above), were of opposite mating types and therefore, they could be crossed with each other. Of the 30 segregants examined from the cross, 26 were dow'. This result, again, suggests that \( Dp(D305) \) is lost from a majority of the progeny and the loss is from the translocated position.

**Timing of the loss of \( Dp(D305) \)**

RIP occurs after fertilization when the dikaryon undergoes multiple rounds of mitoses prior to karyogamy, which is followed by meiosis (Fig. 1.3). Therefore, for \( Dp(D305) \) to be "invisible" to the RIP machinery, it would have to be lost premeiotically rather than during meiosis. We tested this premise by performing crosses between three different \( Dp(D305) \) dow* erg-3+/dow erg-3 a strains with an erg-3 A strain. erg-3 mutants are female sterile; they form only a few protoperithecia which fail to mature into perithecia (Perkins et al., 2001). The female sterility of erg-3 mutants is not rescued in heterokaryons with the helper-1 strain (M. Vyas and D. P. Kasbekar, unpublished results). If the duplicated segment from the \( Dp(D305) \) dow* erg-3+/dow erg-3 parents is indeed lost at the premeiotic stage, then these strains should become female sterile. We found that the three \( Dp(D305) \) dow* erg-3+/dow erg-3 a x erg-3 A crosses were as sterile as erg-3 homozygous crosses whereas control crosses of the duplication strains with the wild type strain 74-OR23-1 A were fertile. Furthermore, a cross of \( Dp(D305) \) dow*/dow with an erg-3 strain is expected to be fertile, and such is indeed the case (see above). These results supported the idea that the loss of \( Dp(D305) \) occurs during the premeiotic stage.
Conclusions

Our results allow us to conclude that although the \( Dp(D305) \) duplication is stable during vegetative growth it is highly unstable during the premeiotic stage of the sexual cycle. Instances of premeiotic loss of DNA sequences are not unknown. Examples include the high frequency of intrachromosomal recombination of repeat sequences (reviewed by Selker, 1990) and changes in nucleolar organizer region during premeiosis (Butler and Metzenberg, 1989). It is possible that the instability of \( Dp(D305) \) is a related phenomenon. At any rate, it could account for the atypical properties of the duplication, \( \textvisiddash \) other large duplications, namely, semi-barrenness in heterozygous crosses, low recovery of duplication progeny from crosses, exceptionally low RIP in the duplication and, failure to suppress RIP in a smaller duplication. We also observed that the frequency of crossing-over between \( dow \) and \( erg-3 \) was much higher in \( Dp(D305) \). However, we do not know whether the increase in crossover is related to the premeiotic instability.