Regular Papers

Premeiotic instability of the *Neurospora crassa* duplication Dp(III R) D305

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The duplication Dp(D305) is shown to cover the erg-3 locus (which encodes the ergosterol biosynthetic enzyme C-14 reductase). Additionally, the efficiency of RIP in Dp(D305) is shown to be very low. This low efficiency may be due to the marked instability of the duplication in the premeiotic stage of the sexual cross. Premeiotic instability might also account for the low frequency with which duplication progeny are recovered from Dp(D305) x Normal crosses.

In *Neurospora crassa* the complex translocation, T(III R -> X; III R; VII) D305 (also referred to as T(D305)) translocates a LG III segment into another unidentified chromosome (Perkins 1997 Adv. Genet. 36: 239-397). When this translocation is crossed by normal sequence some of the progeny are duplicated for the translocated III R segment. The duplication progeny are referred to as Dp(D305) (also as Dp(III R) D305). Dp(D305) was reported earlier to cover the LG III markers phe-2, tyr-1, un-17, het-7 and dow but not acr-2, thi-2, trp-1, and ro-2 (Perkins 1997 Adv. Genet. 36: 239-397). We show here that Dp(D305) also covers erg-3 (the structural gene for the ergosterol biosynthetic enzyme C-14 reductase) which is located distal to dow (Perkins et al. 2001 The Neurospora Compendium Chromosomal Loci. Academic Press, San Diego, USA). Additionally, the frequency of mutants generated by RIP in crosses heterozygous for Dp(D305) was found to be unexpectedly low. This apparent low frequency of RIP could be attributed to an instability of Dp(D305) in the premeiotic stage of the sexual cross.

**Dp(D305) covers erg-3:** A cross was performed between T(D305) A (FGSC #2139) and dow erg-3 a (FGSC # 7244) and the following phenotypic classes were obtained amongst 125 progeny: 72 Dow⁺ Erg-3⁺, 44 Dow⁻ Erg-3⁻, 7 Dow⁺ Erg-3⁻, and 2 Dow⁻ Erg-3⁺. It should be noted that one-fourth of the progeny are expected to be inviable because of partial deletion of LG III. If Dp(III R) D305 covers erg-3, the duplication progeny should have the Dow⁺ Erg-3⁺ phenotype, otherwise they should be Dow⁺ Erg-3⁻. Furthermore, crossing the duplication segregants with the wild type should yield Dow⁻ Erg-3⁺ progeny. Thirty Dow⁺ Erg-3⁺ segregants were crossed with the wild type strains 74-OR23-1 A or OR8-1 a, progeny were analyzed from 26 crosses and five yielded Dow⁺ Erg-3⁺ segregants. These results indicate that Dp(III R) D305 covers erg-3. In fact, it is possible that Dp(D305) covers the whole chromosome arm from between ro-2 and phe-3 to TipIII R. The four crosses that were not analysed produced very few ascospores and we could not be sure that they represented non-Dp(D305) segregants. The proportion of confirmed duplication progeny amongst the Dow⁺ Erg-3⁺ segregants (5/26) was lower than the expected 1/2. The lowered recovery of duplication progeny has been noted previously (Perkins 1997 Adv. Genet. 36: 239-397).

**RIP is barely detectable in Dp(III R) D305 heterozygous crosses:** We wanted to determine the frequency with which erg-3 mutants are generated by RIP in crosses heterozygous for Dp(D305). Ascospores mutant for erg-3 generate colonies with a characteristic morphology (Noubissi et al. 2000 Fungal Genet. Biol. 31: 91-97), which makes their identification very easy. A cross was performed between T(D305) a (FGSC #2140) and a dow A laboratory strain. Of 59 segregants examined, 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. Crosses with five segregants (# 6, 8, 12, 28, and 30) yielded dow progeny, thereby confirming that they were Dp(D305), dow⁺/dow⁻ duplication strains. The proportion of confirmed duplication segregants (5/28) was less than the expected 50%, but similar to that from T(D305) x dow erg-3. Only one RIP-induced erg-3 mutant was obtained out of the 7558 progeny examined from the crosses of the five Dp(D305), dow⁺/dow⁻ strains with the wild-type. Thus the frequency of RIP in Dp(D305) appeared to be exceptionally low.

Southern analysis of Sau 3A1 digested genomic DNA from the lone mutant did not show any evidence for methylation of cytosine residues (data not shown). This was consistent with an earlier report that RIP in large duplications is milder than in smaller, gene-sized duplications (Perkins et al. 1997 Genetics 147: 125-136).

We also examined the frequency of RIP-induced mutations in the dow locus. Two of the Dp(D305), dow⁺/dow⁻ strains (#12 and #28) were each crossed with erg-3 A. Six phenotypically wild-type progeny from one cross and nine from the other were then crossed with the wild-type strains 74-OR23-1 A or OR8-1 a. One cross, involving the segregant #28-4, segregated erg-3 mutants in the progeny. This indicated that #28-4 was genotypically Dp(D305), erg-3⁺/erg-3⁻. We examined 100 Erg-3⁺ and 107 Erg-3⁻ progeny from this cross but none were Dow⁻. Thus the frequency of RIP-induced dow mutants generated in a Dp(D305) heterozygous cross was less than 1/207 (<0.5%), which is lower than the 1.5%-4.7% frequency with which dow segregants were recovered from crosses heterozygous for another large duplication, Dp(AR17), that covers dow (Bhat and Kasbekar 2001; Perkins et al. 1997). These results suggested that Dp(D305) may
be “invisible” to the RIP machinery, possibly because of duplication instability.

**Testing Dp(IIIr)D305 for instability:** We asked whether Dp(D305) was unstable during vegetative growth. If the duplication broke down during vegetative growth, then a subset of conidia from a Dp(D305), erg-3‘/erg-3 strain would be expected to display the tomatine-resistance phenotype of the uncovered erg-3 mutation (Sengupta et al. 1995 Fungal Genet. Newslett. 42: 71-72). We streaked conidia of three Dp(D305), dow‘ erg-3‘/dow erg-3 strains (4#4, 12#12 and 14#14) onto Vogel’s-FGS plates supplemented with tomatine but did not observe any conidia with the tomatine-resistance phenotype. This suggested that Dp(D305) was not vegetatively unstable. In contrast, a subset of conidia from a control heterokaryon made between a dow erg-3 strain and the helper-1 strain (FGSC No. 4564) were tomatine-resistant.

To determine whether Dp(D305) was unstable during the sexual phase we performed a cross between two confirmed Dp(D305), dow‘/dow strains of opposite mating types. Of 30 progeny examined, 26 were dow‘. These results indicated that Dp(D305) can indeed be lost in a cross.

However for it to be “invisible” to the RIP machinery Dp(D305) would have to be lost premeiotically rather than in meiosis. To test whether such is the case, we performed crosses between three different Dp(D305), dow‘ erg-3‘/dow erg-3 strains and an erg-3 laboratory strain. It has been previously reported that erg-3 mutants have a female sterile phenotype (Perkins 2001 The Neurospora Compendium Chromosomal Loci. Academic Press, San Diego, USA). Crosses homozygous for erg-3 produce only a few protoperithecia that fail to mature into perithecia. The female sterility of erg-3 is not rescued in heterokaryons with the helper-1 strain (Meean Vyas and D. P. Kasbekar, unpublished results). If Dp(D305) was indeed lost premeiotically from the Dp(D305), dow‘ erg-3‘/dow erg-3 parent then the affected mycelium should become female sterile. Thus ascospores can be produced only if the duplication is retained. We found that the three Dp(D305), dow‘ erg-3‘/dow erg-3 x erg-3 crosses were as sterile as erg-3 homozygous crosses whereas the control crosses Dp(D305), dow‘ erg-3‘/dow erg-3 x 74-OR23-1 were fertile. These crosses were performed by confrontation between mycelia inoculated as plugs on synthetic crossing medium in petri dishes. Our results allow us to conclude that although Dp(D305) is stable during vegetative growth it is highly unstable in the premeiosis of a sexual cross. Premeiotic instability might explain the low frequency with which duplication progeny are recovered from crosses. Dp(D305)‘s instability might also reflect its constitution from a terminal, rather than an interstitial, translocation.

Dp(D305)‘s premeiotic instability adds to the list of unusual genetic instabilities that occur in the premeiotic phase such as intrachromosomal recombination (reviewed by Selker 1990 Annu. Rev. Genet. 24: 597-613) and changes in rRNA gene copy-number in the nucleolus organizer (Butler and Metzenberg 1989 Genetics 122: 783-791). The fact that we were able to recover one RIP-induced erg-3 mutant suggests that RIP and the premeiotic instability may not be mutually exclusive.

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