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Abstract

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.

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

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The duplication *Dp(D305)* is shown to cover the *erg-3* locus (which encodes the *erg*osterol 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(IIIR -> X; IIIR; VIL) D305* (also referred to as *T(D305)*) translocates a LG IIIR 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 IIIR segment. The duplication progeny are referred to as *Dp(D305)* (also as *Dp(IIIR)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 *erg*osterol 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^+ $Erg\text{-}3^+$, 44 Dow^- $Erg\text{-}3^-$, 7 Dow^+ $Erg\text{-}3^-$, and 2 Dow^- $Erg\text{-}3^+$. It should be noted that one-fourth of the progeny are expected to be inviable because of partial deletion of LG IIIR. If *Dp(IIIR)D305* covers *erg-3*, the duplication progeny should have the Dow^+ $Erg\text{-}3^+$ phenotype, otherwise they should be Dow^+ $Erg\text{-}3^-$. Furthermore, crossing the duplication segregants with the wild type should yield Dow^- $Erg\text{-}3^-$ progeny. Thirty Dow^+ $Erg\text{-}3^+$ 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^- $Erg\text{-}3^-$ segregants. These results indicate that *Dp(IIIR)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 *TipIIIR*. 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^+ $Erg\text{-}3^+$ 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(IIIR) 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 $Erg\text{-}3^+$ and 107 $Erg\text{-}3^-$ 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, #12 and #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 (Meenal 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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