Escape From Repeat-Induced Point Mutation of a Gene-Sized Duplication in *Neurospora crassa* Crosses That Are Heterozygous for a Larger Chromosome Segment Duplication

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ABSTRACT

In *Neurospora crassa* the ability of an ectopic gene-sized duplication to induce repeat-induced point mutation (RIP) in its target gene was suppressed in crosses that were heterozygous for another larger chromosome segment duplication. Specifically, the frequency of RIP in the *erg-3* gene due to a 1.3-kb duplication was reduced if the chromosome segment duplications *Dp(IIIR > [I;II]) AR17*, *Dp(VIR > IIIR) OY329*, or *Dp(IVR > VII) S1229* were present in either the same or the other parental nucleus of the premeiotic dikaryon. We suggest that the larger duplications act as sinks to titrate the RIP machinery away from the smaller duplication. In contrast, RIP efficiency was relatively unaffected in comparably unproductive interspecies crosses with *N. intermedia* and *N. tetrasperma*. These findings offer a novel explanation for the observed persistence of the transposable element *Tad* in only a subset of Neurospora strains.

REPEAT-INDUCED point mutation (RIP) is a unique mutational process that occurs in the sexual cycle of *Neurospora crassa* during the dikaryotic stage between fertilization and karyogamy. As a result of RIP, duplicated DNA sequences in the otherwise haploid nuclei suffer multiple G:C to A:T transition mutations and methylation of many of the remaining cytosine residues (for reviews see Selker 1990; Irelan and Selker 1996). RIP has been used to ascertain the null phenotype of cloned genes. In these studies, the duplications are produced by transformation of the cloned DNA and its insertion into ectopic chromosomal locations. Such duplications are typically only a few kilobases in size. But RIP can also occur in much larger duplications that are obtainable as segregants from crosses heterozygous for translocation chromosomes (Perkins *et al.* 1997). Crosses heterozygous for large chromosome segment duplications (segmental aneuploidy) are characteristically barren; *i.e.*, only a few exceptional asci produce a few viable ascospores. The efficiency of RIP appeared to be reduced in large duplications and when it did occur, the mutagenesis and methylation seemed milder than that typically induced by gene-sized duplications (Perkins *et al.* 1997). Since both large (*e.g.*, >100 kb) and small (*e.g.*, <10 kb) duplications can induce RIP and serve as its substrates, it was of interest to determine whether the ability of a small duplication to induce RIP in its target gene was affected by the presence of a larger chromosome segment duplication in the same cross. In this article we examine whether induction of RIP in the *erg-3* gene by an ectopically integrated 1.3-kb fragment of *erg-3*, designated *Dp 1.3*^{ec}, is affected in crosses that are also heterozygous for the much larger chromosome segment duplications *Dp(IIIR > [IR; IIR]) AR17*, *Dp(VIR > IIIR) OY329*, and *Dp(IVR > VII) S1229*.

The *erg-3* gene is located in LGIII and encodes the *erg*osterol biosynthetic enzyme sterol C-14 reductase (Ellis *et al.* 1991; Papavinasasundaram and Kasbekar 1994). RIP-induced null mutants of *erg-3* are viable but have altered sensitivities to isoflavonoids and to the steroidal glycoside α-tomatine (Sengupta *et al.* 1995; Prakash *et al.* 1999). Whereas the wild type is resistant to isoflavonoids and sensitive to tomatine, *erg-3* mutants are resistant to tomatine and sensitive to isoflavonoids. Additionally, the colonies generated from *erg-3* mutant ascospores exhibit a characteristic slow growth morphology on Vogel's-sorbose agar medium, thereby making them easy to score by mere inspection of plates under a dissection microscope (Noubissi *et al.* 2000).

MATERIALS AND METHODS

Strains: The wild-type *N. crassa* strains *74-OR23-1 matA* [Fungal Genetics Stock Center (FGSC) no. 987] and *OR8-1 mata* (FGSC no. 988); the mutant strains *erg-3 mata* (FGSC no. 2725), *dow erg-3 matA* (FGSC no. 7243), *col-18 matA* (FGSC no. 8283), and *col-18 mata* (FGSC no. 8284); the translocation strains *T(VIR > IIIR) OY329, matA* (FGSC no. 3670) and *T(VIR > IIIR) OY329, mata* (FGSC no. 3671); and the duplication strains *Dp(IVR > VII) S1229 matA* (FGSC no. 264) and *Dp(IVR > VII) S1229 mata* (FGSC no. 265) were obtained from the FGSC, University of Kansas Medical Center, Kansas City, KS 66103.

T(VIR > IIIR) OY329 [also referred to as *T(OY329)*] is an insertional translocation in which a segment of VIR is inserted

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into IIIR; the translocated segment includes the wild-type allele of the VIR marker *colonial-18* (*col-18*; Perkins *et al.* 1997). The viable progeny from crosses between a *T(OY329)* strain and a normal sequence strain include those that contain a duplication of the VIR segment, designated *Dp(VIR > IIIR) OY329*. *Dp(VIR > IIIR) OY329* strains are stably barren in crosses.

The translocation strain *T(IIIR>[I;II])AR17, mata* (Perkins lab stock no. xx-366 = FGSC no. 1463) was provided by David D. Perkins (Stanford University). This strain is a complex insertional translocation involving IR, IIR, and IIIR. One-third of the viable progeny from crosses of this strain with a normal sequence strain contain a duplication of a distal IIIR segment, designated *Dp(IIIR>[I;II])AR17*. The duplication covers the IIIR marker *downy* (*dow*) but not *erg-3* (Perkins 1997). (There is an error in the literature; Figure 2 of Perkins *et al.* 1997 mistakenly suggests that the duplication covers *erg-3*.) The *dow* and *erg-3* loci are separated by 10% crossover distance (Perkins *et al.* 1982). Although *Dp(IIIR>[I;II])AR17* strains are stably barren in crosses they do nevertheless produce a sufficient number of viable ascospores for meaningful analysis (Perkins *et al.* 1997).

The strain *ad-3B cyh-1 mata*^{m1} (FGSC no. 4564) was provided by Ramesh Maheshwari (Indian Institute of Science, Bangalore, India). It is an adenine auxotroph and contains a defective *mat* allele and is useful for constructing heterokaryons that can be maintained on adenine-less medium. The *col-18* nuclei were maintained in (*col-18* + *ad-3B cyh-1 mata*^{m1}) heterokaryons that were used in crosses.

The construction of the *Dp 1.3*^{ec} *hph mata* and *Dp 1.3*^{ec} *hph matA* strains has been described by Prakash *et al.* (1999). These strains contain the wild-type allele at the *erg-3* locus and also duplication of a 1.3-kb *Hin*dIII fragment from *erg-3* that is inserted as a single copy into LGI linked to *mata*. The duplicated segment does not encode a functional sterol C-14 reductase but serves to target RIP to the *erg-3* gene. The duplicated fragment is marked by the bacterial *hph* gene, which encodes the enzyme hygromycin phosphotransferase that when expressed confers resistance to the antibiotic hygromycin B. These strains have a tomatine-sensitive and hygromycin-resistant phenotype.

The *N. intermedia* standard reference strain *Shp-1 matA* (FGSC no. 3416) was provided by Ramesh Maheshwari and the *N. tetrasperma* reference strain *85 matA* (FGSC no. 1240) was obtained from the FGSC.

Growth conditions: Crossing and maintenance of the Neurospora strains was essentially as described by Davis and De Serres (1970). Antibiotic resistance was scored by streaking conidia onto 1.5% agar plates containing Vogel's N medium plus "sorbose" (0.05% fructose, 0.05% glucose, and 2% sorbose) and supplemented with the antibiotic. The antibiotics tested were α-tomatine (Sigma, St. Louis) at 90 μg/ml made from a 25 mg/ml stock solution in DMF and hygromycin B (Sigma) 200 μg/ml made from a 100 mg/ml aqueous stock solution. After an overnight incubation at 30° on tomatine-supplemented medium, growth can be observed of only the *erg-3* mutant strains (Sengupta *et al.* 1995). Only strains expressing the *hph* gene could grow on hygromycin medium. The *ad-3B cyh-1 mata*^{m1} strain was grown on Vogel's-sucrose medium supplemented with adenine (0.5 mg/ml).

Ascospore collection: Crosses were performed in petri dishes. Ascospores were collected by washing the lids with ~1 ml water. The frequency of RIP can differ greatly between early *vs.* late collected ascospores (Singer *et al.* 1995). Therefore we pooled the early and late ascospores before determining RIP frequencies. Typically ascospores began to be shot within 16–18 days in fertile crosses whereas in barren crosses the first ascospores were seen only after 21 days. For fertile crosses the spores were collected at regular intervals for up to 31 days, and the spores from all the collections of each cross were pooled. For barren crosses usually only one collection was feasible, after 31 days; thereafter the number of additional spores was negligible.

Scoring for *erg-3* mutant segregants: RIP-induced *erg-3* mutants can be distinguished from the wild type by the characteristic slow growth phenotype of their ascospore-derived colonies on Vogel's-sorbose agar (Prakash *et al.* 1999; Noubissi *et al.* 2000). Therefore, to score for *erg-3* mutants among the segregants from a cross we merely counted all colonies with the mutant growth morphology. In most cases we subsequently confirmed their mutant phenotype on tomatine medium. Likewise most of the wild-type colonies were scored merely on the basis of their normal growth phenotype. In each cross a significant number (~50) of "normal growers" was tested to confirm that they all indeed possessed the tomatine-sensitive wild-type phenotype.

RESULTS

Construction of *Dp 1.3*^{ec} *hph*; *Dp(AR17)* strains: A cross was performed between the strains *T(IIIR > [IR; IIR]) AR17 mata* [henceforth referred to as *T(AR17)*] and *Dp 1.3*^{ec} *hph matA* to produce progeny that should include *Dp (IIIR > [IR; IIR]) AR17* segregants [henceforth referred to as *Dp(AR17)*] that also contain the *Dp 1.3*^{ec} *hph* transgene (Figure 1). Although both parental strains are *erg-3*$^{+}$ and therefore have a tomatine-sensitive phenotype, *erg-3* mutants can be generated in this cross due to the induction of RIP in the *Dp 1.3*^{ec} *hph* nucleus. These mutants could be distinguished by the characteristic slow growth phenotype of the ascospore-derived colonies on Vogel's-sorbose agar medium (see Noubissi *et al.* 2000 for a figure) and also on the basis of their resistance to tomatine. Of 67 segregants examined, 17 (25.4%) were mutant in *erg-3*. This frequency is the same as that reported previously for the recovery of *erg-3* mutants from a cross between *Dp 1.3*^{ec} *hph mata* and the wild-type strain *74-OR23-1 matA* (Prakash *et al.* 1999). Of the remaining 50 tomatine-sensitive segregants, 30 (60%) were hygromycin resistant, indicating that they also contained the *Dp 1.3*^{ec} *hph* transgene. Each of the hygromycin-resistant, tomatine-sensitive segregants was crossed with the wild-type strains *74-OR23-1 matA* or *OR8-1 mata*. These crosses are referred to as series A. Twenty-five of the series A crosses were determined to be fertile and 5 (involving segregant nos. A13, A17, A30, A40, and A52) were barren. The proportion of barren crosses (5/30) is <1/3, presumably because the segregants were first screened for the hygromycin-resistance phenotype, so a crossover between the *Dp 1.3*^{ec} *hph* transgene and the *T(AR17)* breakpoint on LGI would be necessary for them to also possess *Dp(AR17)* (Figure 1). Ascospore production was estimated by eye to be 100- to 1000-fold lower in the barren crosses than in the fertile crosses. These results suggested that the segregants producing the barren crosses ("barren segregants") were *Dp 1.3*^{ec} *hph*; *Dp(AR17)* double duplication strains.

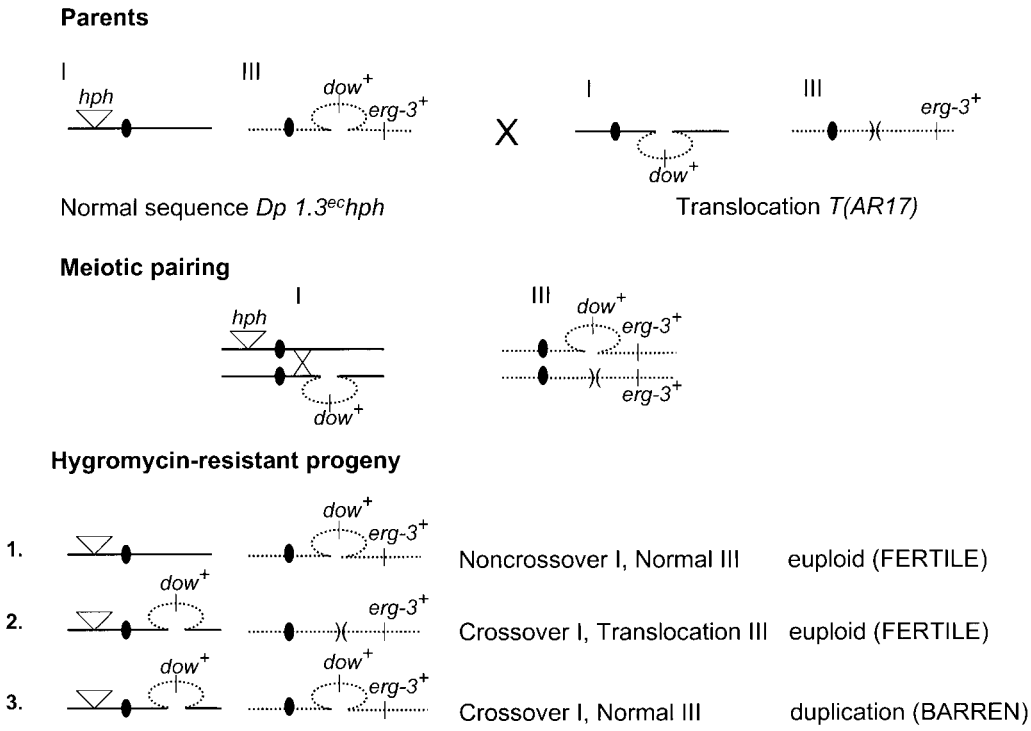

FIGURE 1.—Generation of *Dp(AR17)*; *Dp1.3*^{ec} double duplication strains by crossing over in the interval between the *hph* and *T(AR17)* insertions into linkage group I. All strains are *erg*$^{+}$ and, hence, tomatine sensitive. The drawing of *T(AR17)* is simplified by ignoring involvement of linkage group II, which is irrelevant to the present experiment. In this and the following figure, segments of linkage group I are shown as solid lines and those of linkage group III as dotted.

That four of the barren segregants were indeed *Dp 1.3*^{ec} *hph*; *Dp(AR17)* double duplication strains was confirmed by the recovery of *downy* (*dow*) mutants among their progeny (Table 1). The *dow* locus is covered by *Dp(AR17)* and these mutations are presumed to have resulted from RIP in the large duplication. The *dow* mutation frequencies in these four barren crosses were comparable with the 4.7% frequency reported by PERKINS *et al.* (1997). No *dow* mutants were found among the 35 progeny examined from the cross parented by the barren segregant A30 and we did not attempt to scale up this cross further to obtain additional progeny. No *dow* mutants were found among any of the segregants examined from the fertile crosses (data not shown).

From the barren cross between segregant A13, *mata* and *74-OR23-1 matA* we initially obtained 44 progeny (all tomatine-sensitive) of which 22 were hygromycin resistant. Twenty of the hygromycin-resistant progeny and their 22 hygromycin-sensitive sibs were crossed with the wild-type strains *74-OR23-1 matA* or *OR8-1 mata*. These crosses are referred to as series B. Twenty-eight crosses were fertile and 14 were barren. Of the fertile segregants, 10 were *mata* and 5 of the barren segregants (including B40 and B41, both hygromycin sensitive and the latter with a *dow* phenotype) were *matA*. These 15/42 segregants represented crossovers between the *mat* locus and a *T(AR17)* breakpoint on LGI. Such loose linkage is consistent with the assignment of the *T(AR17)* breakpoint to LGIR (PERKINS 1997).

The two hygromycin-sensitive and barren segregants, B40 and B41, represented *Dp(AR17) matA* strains. They were crossed with *T(AR17) mata*. Progeny from these crosses can be either *Dp(AR17)* or *T(AR17)* strains (see PERKINS *et al.* 1997 for an explanatory figure). One cross yielded 2 *dow* segregants out of 11 examined, and the other, 3 out of 20. One of these *dow* segregants was fertile and thus represented a *T(AR17), dow* strain.

RIP in *erg-3* is reduced in crosses involving *Dp 1.3*^{ec} *hph*; *Dp(AR17)* strains: The *erg-3* mutation frequencies were determined for all 5 barren crosses and 18 of the 25 fertile crosses of series A and the results are summarized in Table 2. The crosses involving the barren

TABLE 1

Recovery of *dow* mutations from barren crosses of series A

Cross	Segregants examined	*downy* mutants	% *dow*
A13 *mata* × OR *matA*	184	4	2.17
A17 *mata* × OR *matA*	133	2	1.50
A30 *matA* × OR *mata*	35	0	<2.86
A40 *mata* × OR *matA*	50	2	4.00
A52 *mata* × OR *matA*	86	4	4.65

TABLE 2

***erg-3* mutation frequencies in series A crosses**

Segregant	Nature of cross	Ascospores examined	*erg-3* mutants	RIP (%)
A1	Fertile	520	8	1.54
A4	Fertile	301	16	5.32
A5	Fertile	446	27	6.05
A7	Fertile	479	10	2.08
A8	Fertile	1061	38	3.58
A9	Fertile	990	31	3.13
A14	Fertile	453	9	1.99
A15	Fertile	268	7	2.61
A16	Fertile	607	72	11.86
A19	Fertile	900	66	7.33
A32	Fertile	266	74	27.82
A35	Fertile	103	10	9.71
A42	Fertile	63	6	9.52
A46	Fertile	171	26	15.20
A48	Fertile	374	11	2.94
A51	Fertile	70	9	12.85
A62	Fertile	133	13	9.77
A66	Fertile	264	19	7.20
A13	Barren	1343	0	<0.07
A17	Barren	1050	2	0.19
A30	Barren	60	0	<1.60
A40	Barren	177	0	<0.56
A52	Barren	213	0	<0.47

segregants A13 and A17 were scaled up to obtain larger numbers of progeny. In the fertile crosses the *erg-3* mutation frequencies ranged between 1.5 and 27.8%. For the barren crosses parented by the four confirmed double duplication strains the range was between <0.07 and <0.56%. The clean separation of the two ranges allows us to conclude that the ability of *Dp 1.3^{ec}* to induce RIP in *erg-3* is suppressed in nuclei that contain *Dp(AR17)*. In fact, the frequency of RIP in *erg-3* was even lower than in *dow* (compare entries for segregants A13, A17, A40, and A52 in Tables 1 and 2).

Control crosses were made between *Dp 1.3^{ec} hph* and the *N. intermedia* standard reference strain *Shp-1 matA* and the *N. tetrasperma* standard reference strain *85 matA*. Interspecies crosses in Neurospora are usually quite unproductive; in fact these crosses were even less productive than the barren crosses described above and yielded ~1000-fold fewer ascospores than the fertile crosses of series A. Of 90 segregants examined from *Dp 1.3^{ec} hph* × *Shp-1 matA*, three (3.3%) were mutant in *erg-3*, and in *Dp 1.3^{ec} hph* × *85 matA* the *erg-3* mutation frequency was 15 out of 80 (18.8%). Thus, even though the productivity of the interspecies crosses was poor, their RIP efficiencies were within the range of the fertile crosses. This argues against the possibility that suppression of RIP in the crosses involving segmental aneuploidy is a trivial consequence of their poor productivity and instead implicates a role for *Dp(AR17)* in this effect. On the basis of these results we can conclude that the relatively smaller gene-sized duplication *Dp 1.3^{ec}* tends to be ignored by the RIP machinery in a nucleus that also contains the large chromosome segment duplication *Dp(AR17)*.

RIP efficiency is restored in the fertile segregants from a barren cross: Of the hygromycin-resistant segregants examined in series B (see above), 8 were fertile and 12 were barren. We examined the progeny of 7 fertile and 10 barren segregants and the results are summarized in Table 3. It can be seen that the *Dp 1.3^{ec} hph* transgene regains the ability to induce *erg-3* mutations in the fertile crosses and continues to be ignored by the RIP machinery in the barren crosses. These results allow us to conclude that the ability of *Dp 1.3^{ec} hph* to engage in RIP is restored subsequent to its segregation from *Dp(AR17)*.

***Dp(AR17)* suppresses induction of RIP by *Dp 1.3^{ec}* even in the *trans* configuration:** The *Dp(AR17)* and *Dp 1.3^{ec} hph*; *Dp(AR17)* segregants identified in the series A and B crosses were used to examine whether *Dp(AR17)* affected the induction of RIP by *Dp 1.3^{ec}* when the two duplications were in different parental nuclei of the premeiotic dikaryon (*i.e.*, *in trans*). The results of these crosses (Table 4) suggest that *Dp(AR17)* can suppress the induction of RIP by *Dp 1.3^{ec}* even *in trans*. However, this suppression was not always as severe as when the duplications were in the same nucleus.

We also performed crosses that were homozygous for the large duplication and either homozygous or heterozygous for the small duplication. Interestingly the productivity of the *Dp(AR17)* homozygous crosses was com-

TABLE 3

***erg-3* mutation frequencies in series B crosses**

Segregant	Nature of cross	Ascospores examined	*erg-3* mutants	RIP (%)
B4	Fertile	734	154	20.98
B15	Fertile	737	67	9.09
B20	Fertile	587	94	16.01
B30	Fertile	319	23	7.21
B31	Fertile	161	23	14.29
B37	Fertile	244	42	17.21
B43	Fertile	430	40	9.30
B7	Barren	137	0	<0.73
B9	Barren	533	0	<0.19
B18	Barren	255	0	<0.39
B19	Barren	69	0	<1.45
B23	Barren	383	0	<0.26
B28	Barren	180	2	1.1
B33	Barren	378	0	<0.26
B36	Barren	220	3	1.36
B42	Barren	66	0	<1.52
B44	Barren	254	0	<0.39

parable to that of the heterozygous crosses. Of a total of 2778 segregants examined from such crosses none was mutant in *erg-3*; therefore, the frequency of RIP in *erg-3* was <0.04%.

Crossover between the *T(AR17)* breakpoint in LGIII and *erg-3*: A *T(AR17) mata* × *dow erg-3 matA* cross can produce progeny with either the *dow* phenotype or with a tomatine-sensitive and barren phenotype if there has been a crossover in LGIII between the translocation breakpoint and *erg-3* and such segregants represent one-third of the viable crossover products (Figure 2). Of 81 segregants examined from this cross, one was *dow*; thus, the crossover frequency was (1 × 3)/ 81, *i.e.*, 3.7%. In a second experiment 309 segregants were examined from the cross *T(AR17) mata* × *erg-3 matA* and 5 were tomatine sensitive and barren. In this experiment the crossover frequency was (5 × 3)/309, *i.e.*, 4.8%. The latter determination may be more accurate because it was based on the examination of more segregants.

Construction of *Dp 1.3^{ec} hph*; *Dp(OY329)* strains: To determine if other chromosome segment duplications, besides *Dp(AR17)*, also suppress RIP in *Dp 1.3^{ec}*, we examined crosses that were heterozygous for the duplication *Dp(VIR > IIIR) OY329* [also referred to as *Dp(OY329)*]. We constructed *Dp(OY329) Dp 1.3^{ec} hph* double duplication strains and determined the frequency of *erg-3* mutants among progeny parented by these strains.

A cross was performed between the *Dp 1.3^{ec} hph mata* and *col-18 matA* strains. The frequency of RIP-induced *erg-3* mutants in this cross was 11/165 (6.7%). Thirty-two of 78 progeny (41%) had the colonial mutant phenotype (col$^-$) and, of 27 col$^-$ segregants examined, 12 (44.4%) were hygromycin resistant. The col$^-$, hygromycin-resistant phenotype represented the *Dp 1.3^{ec} hph*; *col-18* progeny.

Another set of crosses was performed between the *col-18* and *T(OY329)* strains. *Dp(OY329)* has been reported to cover *col-18* (Perkins 1997), so the ratio of col$^-$ to col$^+$ progeny was expected to be 1:2. The col$^+$ segregants should include both the *T(OY329)* and *Dp(OY329)*, *col-18$^+$*/*col-18* progeny, and the latter should be distinguishable by their barrenness and ability to yield *col-18* progeny in crosses with the wild type. Surprisingly, the observed ratio of col$^-$ to col$^+$ segregants from *col-18 matA* × *T(OY329) mata* was 54:25 and from *col-18 mata* × *T(OY329) matA* it was 56:21. We do not have a

TABLE 4

Effect of *Dp (AR17)* on the induction of RIP in *Dp 1.3^{ec} hph in trans*

Cross	Segregants examined	*erg-3* mutants	% *erg-3*
A17 *mata* × *Dp 1.3^{ec} hph matA*	173	1	0.56
A30 *matA* × *Dp 1.3^{ec} hph mata*	126	0	<0.79
A40 *mata* × *Dp 1.3^{ec} hph matA*	301	4	1.33
A52 *mata* × *Dp 1.3^{ec} hph matA*	218	0	<0.46
B18 *mata* × *Dp 1.3^{ec} hph matA*	274	4	1.46
B40 *matA* × *Dp 1.3^{ec} hph mata*	782	8	1.02

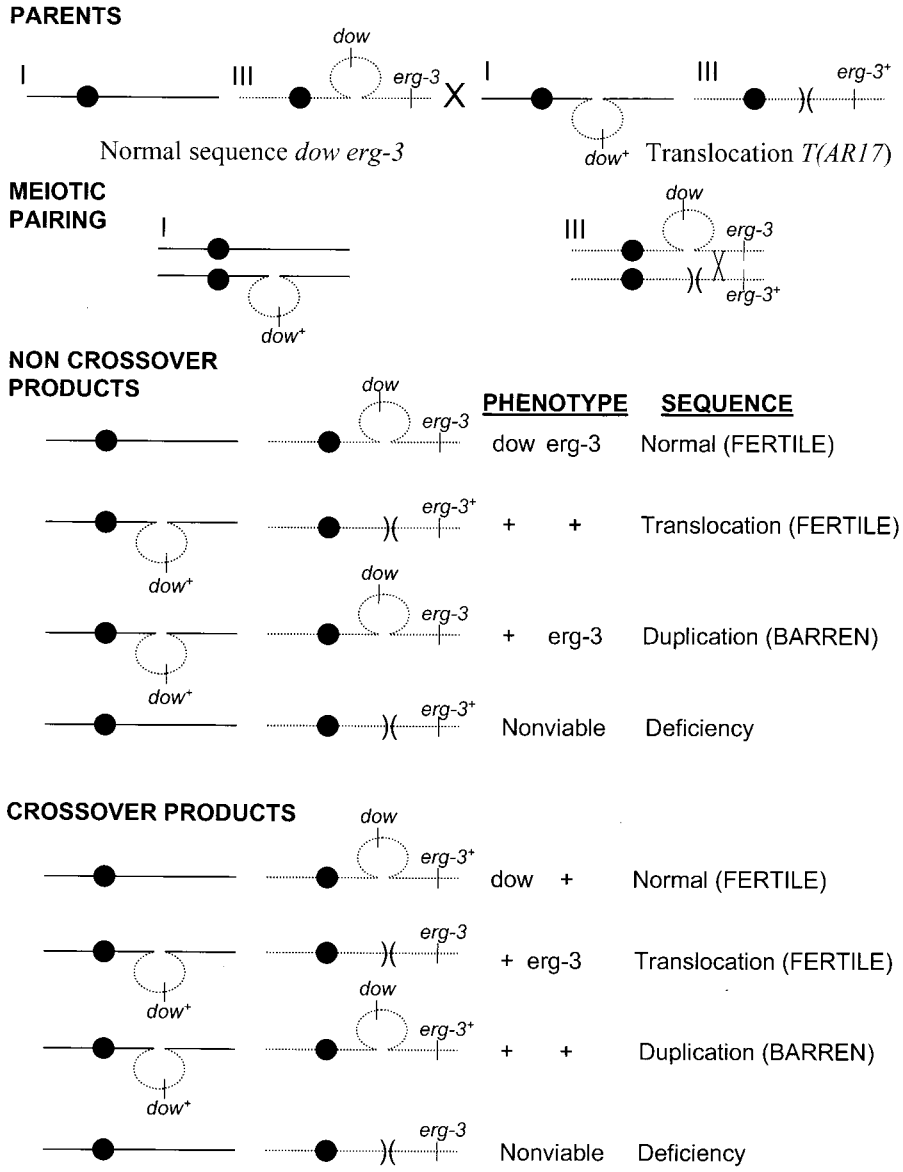

FIGURE 2.—Progeny produced in a cross between *T(AR17)* and a normal-sequence *dow erg-3* strain. Note that progeny with the *dow* phenotype represent one-third of all viable crossover products and that progeny that are phenotypically tomatine sensitive and barren are produced only if there has been a crossover in linkage group III between the translocation breakpoint and *erg-3*. Conventions as in Figure 2.

simple explanation for this discrepancy between the expected and observed segregation frequencies (but we consider a potentially interesting and testable hypothesis in the DISCUSSION). We did, however, identify one col^+ segregant that was barren in a cross with *Dp 1.3^{ec} mata* and this cross yielded 20 col^- segregants out of 52 progeny examined. Thus this col^+ segregant represented a *bona fide Dp(OY329), col-18$^+$/col-18 matA* strain. The *erg-3* RIP frequency in the cross between this strain and *Dp 1.3^{ec} mata* was 3/172 (1.7%), which was comparable with the frequencies in crosses where *Dp(AR17)* was present *in trans* (Table 4).

The confirmed *Dp(OY329), col-18$^+$/col-18 matA* strain was crossed with two *Dp 1.3^{ec} hph*; *col-18 mata* strains (designated 19 and 25). Both crosses were barren and, as expected, they produced col^+ and col^- segregants in approximately 1:1 ratios (20:27 and 10:11). Of the col^+ segregants, 9 from the cross parented by 19 and 5 from the one parented by 25 were also resistant to hygromycin. These 14 hygromycin-resistant col^+ strains were presumably the *Dp 1.3^{ec} hph*; *Dp(OY329), col-18$^+$/col-18.* double duplication progeny. And the segregants with the hygromycin-resistant col^- phenotype were their *Dp 1.3^{ec} hph*; *col-18* euploid siblings (Figure 3).

RIP in *erg-3* is reduced in crosses involving *Dp 1.3^{ec} hph*; *Dp(OY329)* strains: Nine col^+ putative double duplication segregants from the cross parented by strain 19 and four from the cross parented by 25 (see above) were crossed with the wild-type strains *74-OR23-1 matA* or *OR8-1 mata*. And seven col^- segregants (two and five from the crosses parented by 19 and 25, respectively) were also crossed with the wild type as controls. These crosses are referred to as series C.

Surprisingly, six of the col^+ segregants (all derived from 19) were fertile and only seven were barren (Table 5). The six fertile col^+ segregants did not yield any col^-

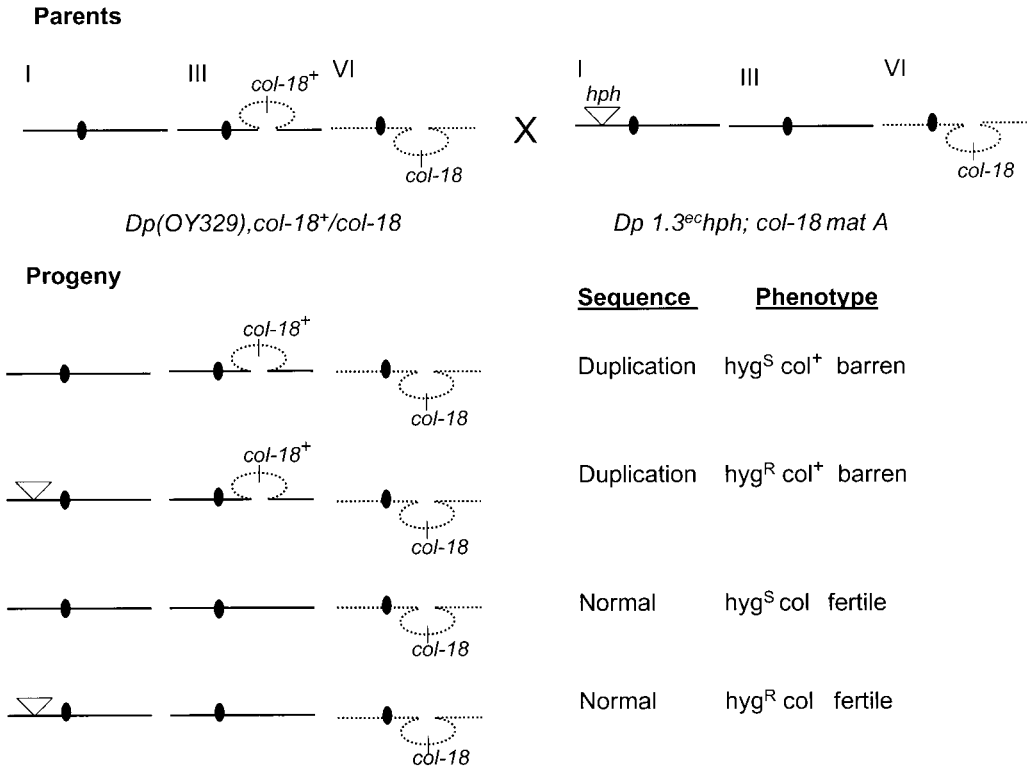

FIGURE 3.—Generation of *Dp(OY329)*; *Dp1.3*^{ec} double duplication strains. All strains are *erg*$^{+}$ and, hence, tomatine sensitive. See text for details of *Dp(OY329)*, *col-18*$^{+}$*/col-18* and *Dp1.3*^{ec} *hph*; *col-18* strain construction. Segments of linkage group VI are shown as dotted lines and those of linkage groups I and III as solid.

progeny (20 progeny were examined for each cross), which confirmed that the fertile col^{+} segregants were not *Dp(OY329)* strains, but they did segregate the *Dp 1.3*^{ec} *hph* transgene. We discuss later the possible origin of these col^{+} nonduplication strains. The remaining seven barren col^{+} segregants all segregated col^{-} progeny (data not shown), which confirmed that they were indeed *Dp(OY329)* strains. The col^{-} segregants were all fertile, which confirmed that they were nonduplication strains. Table 5 summarizes the *erg-3* RIP frequencies in the barren and fertile crosses of series C.

As can be seen in the table, in most of the barren crosses the *erg-3* mutation frequency was very low. In contrast, we could recover *erg-3* mutants from all the fertile crosses, although there was considerable variation in their RIP frequencies. These results allow us to conclude that, like *Dp(AR17)*, *Dp(OY329)* also can suppress the induction of RIP by *Dp 1.3*^{ec}.

RIP is reduced in crosses involving *Dp(IVR > VII) S1229*: The duplication *Dp(IVR > VII) S1229* [henceforth designated as *Dp(S1229)*] has been described by PERKINS (1997) and strains bearing this duplication were available from the FGSC. This duplication is stable in crosses and segregates 1:1 in progeny from *Dp* × *N*. Crosses were performed between the strains *Dp(S1229) matA* and *Dp 1.3*^{ec} *mata* and between *Dp(S1229) mata* and *Dp 1.3*^{ec} *matA*. These crosses were barren and ascospores could be harvested only after 2 months. No *erg-3* mutants were found among the 88 segregants examined from the first cross and the 137 segregants from the latter cross. Thus the *erg-3* RIP frequencies were, respectively, <1.13% and <0.73%. These frequencies are comparable with the frequencies where *Dp(AR17)* was present *in trans* (Table 4).

The hygromycin-resistant segregants from these crosses must contain the transgene, and they could be either *Dp(S1229) Dp 1.3*^{ec} double duplication strains (barren), or *Dp 1.3*^{ec} normal sequence strains (fertile). Ten hygromycin-resistant segregants were crossed with the wild-type strains *74-OR23-1 matA* or *OR8-1 mata*. After 43 days two crosses could unambiguously be scored as fertile, six as barren, and two were intermediate in ascospore productivity. Of the six unambiguously barren crosses, only one (designated D9) produced a sufficient number of ascospores to permit a meaningful estimate of RIP frequencies; one had to be discarded due to contamination; and the other four together yielded only 22 progeny (none were mutant in *erg-3*). The RIP frequencies in the five crosses that could be analyzed (series D) are summarized in Table 6. As can be seen in the table the barren cross showed a very low *erg-3* mutation frequency relative to the two fertile crosses. This was consistent with the idea that *Dp(S1229)* suppresses the induction of RIP by *Dp 1.3*^{ec} in *erg-3*.

The *erg-3* mutation frequency in the two crosses producing an intermediate number of ascospores was much lower than in the fertile crosses.

DISCUSSION

We have examined whether the ability of a small duplication to induce RIP in its target gene is affected by

TABLE 5

***erg-3* mutation frequencies in series C crosses**

Segregant	Phenotype	Nature of cross	Ascospores examined	*erg-3* mutants	RIP (%)
C19-7	col	Fertile	282	6	2.13
C19-22	col	Fertile	243	26	10.70
C25-1	col	Fertile	145	1	0.69
C25-4	col	Fertile	270	4	1.48
C25-6	col	Fertile	369	5	1.36
C25-9	col	Fertile	201	2	1.00
C25-10	col	Fertile	306	9	2.94
C19-2	+	Fertile	398	55	13.80
C19-5	+	Fertile	397	24	6.04
C19-6	+	Fertile	301	23	7.64
C19-8	+	Fertile	476	45	9.45
C19-15	+	Fertile	303	16	5.28
C19-20	+	Fertile	193	58	30.05
C19-3	+	Barren	186	0	<0.54
C19-4	+	Barren	128	0	<0.78
C19-19	+	Barren	220	0	<0.45
C25-3	+	Barren	265	0	<0.38
C25-7	+	Barren	112	0	<0.89
C25-8	+	Barren	220	0	<0.45
C25-11	+	Barren	72	2	2.78

the presence of a larger chromosome segment duplication in the same cross. For this we first constructed strains that contained both the large chromosome segment duplication, *Dp(AR17)*, and a smaller gene-sized duplication, *Dp 1.3^{ec}*. Assuming that *dow* and *erg-3* represent comparable targets for RIP, the frequency of *dow* and *erg-3* mutants among the progeny from crosses made with such double duplication strains provides estimates, respectively, of RIP efficiency in the large and small duplications. We found that the presence of the *Dp(AR17)* duplication suppressed the ability of the smaller gene-sized duplication to induce RIP in its target gene. In fact, the induction of RIP in *erg-3* was even lower than in *dow*. Suppression was evident even when the two duplications were in different nuclei of the premeiotic dikaryon. *Dp(AR17)* was initially chosen because we imagined that its linkage to *erg-3* would increase the sensitivity of our tests. But in view of the observation that *Dp(AR17)* suppresses RIP even *in trans*, linkage does not appear to be germane to this effect.

Like *Dp(AR17)*, the duplications *Dp(OY329)* and *Dp(S1229)* also suppressed the induction of RIP by *Dp 1.3^{ec}*, both *in cis* and *in trans*. Two crosses in the experiments with *Dp(S1229)* produced an intermediate number of ascospores (Table 6). These two segregants could not be unambiguously designated as either duplication or euploid strains because this experiment lacked a marker for *Dp(S1229)* like the *dow* and *col-18* markers for *Dp(AR17)* and *Dp(OY329)*, respectively. But even if we assume the devil's advocate position that the "intermediate" segregants represent the *Dp(S1229) Dp 1.3^{ec}* double duplication strains, the *erg-3* mutation frequency in the two crosses was much lower than that in the fertile crosses. Therefore these results do not negate the conclusion that *Dp(S1229)* reduces RIP in *Dp 1.3^{ec}*. Overall our results suggest that *any* large duplication can suppress a smaller duplication's ability to induce RIP in its target. It is conceivable that large duplications act as sinks to titrate the RIP machinery from the dikaryotic cell. Since a considerable proportion of nuclei in standard laboratory strains harbor rearranged chromosomes (Perkins and Kinsey 1993), studies of RIP in

TABLE 6

***erg-3* mutation frequencies in series D crosses**

Segregant	Nature of cross	Ascospores examined	*erg-3* mutants	RIP (%)
D6	Fertile	155	45	29.03
D10	Fertile	77	12	15.58
D4	Intermediate	312	10	3.21
D7	Intermediate	355	5	1.4
D9	Barren	318	3	0.94

such genetic backgrounds can potentially be confounded by the generation of cryptic duplications in a subset of the progeny.

The frequency of col^+ to col^- progeny from the *col-18* × *T(OY329)* crosses was expected to be 2:1 but the observed frequency was 1:2. This result would normally have led one to question whether *col-18* is covered by *Dp(OY329)*. But the recovery of one *bona fide Dp(OY329)*, *col-18*$^+$ / *col-18 matA* segregant with the col^+ phenotype confirmed that *Dp(OY329)* does in fact cover *col-18*. So how might one explain the discrepant segregation ratios? One possibility is that the *col-18* locus may be deleted from a subset of nuclei of the *T(OY329)* parents. Thus these strains may effectively be heterokaryons, in which nuclei with the active *col-18*$^+$ allele ensure their col^+ phenotype. It is well known that chromosome segment duplications often break down during vegetative growth by loss of one copy of the duplicated segment, and this loss occurs more frequently from the translocated position than from the normal position (see Perkins 1997 for a review). Similar processes might have led to the loss of the *col-18*$^+$ allele (as well as that of a nearby essential locus) from a subset of *T(OY329)* nuclei. In this case some of the col^- progeny from *col-18* × *T(OY329)* might represent the *Dp(OY329)*, *col-18*/ *col-18* genotype. Moreover, progeny inheriting the modified translocation chromosomes might be inviable. Both these effects could contribute to the discrepant phenotypic ratios. This hypothesis predicts that a cross between the *T(OY329)* and *Dp(OY329)*, *col-18*$^+$ / *col-18* strains will yield col^- segregants that are not products of RIP or gene conversion events.

Another unexpected finding was that six of nine col^+ segregants examined from *Dp(OY329)*, *col-18*$^+$ / *col-18 matA* (#19) × *Dp 1.3*^{ec} *hph*; *col-18 mata* were non-*Dp(OY329)* strains. This was surprising because all the col^+ segregants were expected to be genotypically *Dp(OY329)*, *col-18*$^+$ / *col-18* (Figure 3). The generation of non-*Dp(OY329)* col^+ progeny suggests that the *col-18* allele on the nontranslocation LGVI was gene converted to *col-18*$^+$ using the duplication-borne allele as template. Since only one of the two crosses examined exhibited such gene conversion, this event may represent a "jackpot." Thus the conversion event possibly occurred either during the vegetative growth of *Dp(OY329)*, *col-18*$^+$ / *col-18* (#19) or in the premeiotic dikaryon stage between fertilization and karyogamy.

Breakdown of chromosome segment duplications during vegetative growth restores euploidy (Perkins 1997). Only 5 of the 23 barren crosses examined in series A, B, C, and D yielded any *erg-3* mutants. If the mutations did not depend on a prior breakdown of the large duplications we would expect some of these mutants to display a barren phenotype. Of seven *erg-3* mutants examined, six were clearly fertile. One appeared to be barren, but none of its progeny were *dow* (A. Bhat, unpublished results). Thus it will be necessary to examine additional *erg-3* mutants before we can assert that *erg-3* can be RIPed in the presence of the larger duplication.

RIP frequencies in the fertile crosses of series A, C, and possibly D were more variable than in those of series B. Such unexplained variability in RIP frequencies is not without precedent (*e.g.*, Table 2 in Kinsey *et al.* 1994) and merits further investigation.

Might chromosome rearrangements have sheltered active copies of *Tad* from RIP? It has been suggested that RIP might serve to protect the genome against the proliferation of transposable elements (Selker 1990). Transposable elements can be regarded as gene-sized duplications, but they also have the potential to generate segmental aneuploidy via homologous recombination between unlinked copies followed by segregation of the resulting translocation chromosome with normal chromosomes in meiosis. Our results suggest that duplications generated in this way would protect the transposable elements from destruction by RIP and the residual fertility of the duplication-bearing strains might provide a virtually ineradicable source of active elements through successive generations. *Tad*, an active LINE-like Neurospora transposon was discovered in the Adiopodoume strain of *N. crassa*; it is noteworthy that translocations were observed to be unusually frequent in crosses involving this strain (reported by Kinsey and Helber 1989 as a personal communication from David Perkins). Kinsey *et al.* (1994) had suggested several factors that might account for *Tad*'s survival in Adiopodoume, and recent results from our laboratory (Noubissi *et al.* 2000) indicate that this strain even possesses dominant RIP suppressors. The results presented here suggest that segmental duplications also may have contributed to *Tad*'s RIP-free passage in the preceding generations. The translocations in the Adiopodoume strain might represent elements of those ancestral duplications (Kasbekar 1999).

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