Genetic Analysis of Wild-Isolated *Neurospora crassa* Strains Identified as Dominant Suppressors of Repeat-Induced Point Mutation

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ABSTRACT

Repeat-induced point mutation (RIP) in Neurospora results in inactivation of duplicated DNA sequences. RIP is thought to provide protection against foreign elements such as retrotransposons, only one of which has been found in *N. crassa*. To examine the role of RIP in nature, we have examined seven *N. crassa* strains, identified among 446 wild isolates scored for dominant suppression of RIP. The test system involved a small duplication that targets RIP to the easily scorable gene *erg-3*. We previously showed that RIP in a small duplication is suppressed if another, larger duplication is present in the cross, as expected if the large duplication competes for the RIP machinery. In two of the strains, RIP suppression was associated with a barren phenotype—a characteristic of Neurospora duplications that is thought to result in part from a gene-silencing process called *m*eiotic *s*ilencing by *u*npaired *D*NA (MSUD). A suppressor of MSUD (*Sad-1*) was shown not to prevent known large duplications from impairing RIP. Single-gene duplications also can be barren but are too short to suppress RIP. RIP suppression in strains that were not barren showed inheritance that was either simple Mendelian or complex. Adding copies of the LINE-like retrotransposon *Tad* did not affect RIP efficiency.

A mutational process called repeat-induced point mutation (RIP) occurs during the premeiotic stage of the Neurospora sexual cycle and causes the hypermutation of any DNA sequences that are duplicated in an otherwise haploid genome (for reviews see Selker 1990; Irelan and Selker 1996). It had been suggested that RIP might serve to protect the genome against the proliferation of transposable elements. This hypothesis gained experimental support with the demonstration that the only wild-isolated Neurospora strain in which an active transposable element was found was also a dominant partial suppressor of RIP in the *erg-3* test system (Noubissi *et al.* 2000). This was the *Neurospora crassa* Adiopodoumé [Fungal Genetics Stock Center (FGSC) 430] strain that contains the LINE-like transposable element *Tad* (Kinsey and Helber 1989). All other Neurospora strains examined contained only RIP-inactivated relics of *Tad* (Kinsey 1990; Kinsey *et al.* 1994). Subsequently, we reported the identification of two more RIP suppressor strains: Adiopodoumé-7 (a *Tad*-free strain from the same Ivory Coast location as Adiopodoumé 430) and Sugartown (from Louisiana; Noubissi *et al.* 2001). In double-blind experiments all three suppressors could be distinguished from other wild strains solely on the basis of their dominant RIP suppressor phenotype. In this article the term "RIP suppressor" is used as an abbreviation for any factor that suppresses the RIP of *erg-3* in our test system (described below).

In addition to suppressing RIP, crosses with Sugartown as one parent also displayed a barren phenotype; that is, they produced perithecia that yielded only very few ascospores (Noubissi *et al.* 2001). Barrenness is a characteristic of strains that contain large duplications (Raju and Perkins 1978; Perkins 1997 and references therein). The duplications trigger a gene-silencing process called *m*eiotic *s*ilencing by *u*npaired *D*NA (MSUD) whereby any DNA sequence that is unpaired in meiosis is silenced itself and causes the silencing of all sequences that are homologous to it, including genes that may themselves be paired (Aramayo and Metzenberg 1996; Shiu *et al.* 2001; Shiu and Metzenberg 2002). Since large duplications can contain many genes, including some that may be required for meiosis or ascospore development, and one copy necessarily remains unpaired in meiosis of a heterozygous cross, gene silencing by MSUD renders the cross barren. The semidominant mutation *Sad-1* imposes a defect for MSUD and thereby suppresses the barren phenotype of duplication strains (Shiu *et al.* 2001; Shiu and Metzenberg 2002).

The present study follows the identification by Fehmer *et al.* (2001) of wild-isolated strains that suppress the barren phenotype of large duplications. It also makes use of the demonstration that presence of a large duplication suppresses RIP in a smaller duplication (Bhat and Kasbekar 2001; Fehmer *et al.* 2001). We

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have proposed that large duplications do so by titrating out limiting amounts of the RIP machinery.

The *ergosterol-3* (*erg-3*) gene is located in distal linkage group (LG) III and it encodes the ergosterol biosynthetic enzyme C-14 reductase (Ellis *et al.* 1991; Papavinasasundaram and Kasbekar 1994; Prakash and Kasbekar 2002). We have constructed strains in which a tagged duplicate copy of a 1.3-kb fragment of *erg-3*, designated *Dp 1.3^{ec} hph*, was introduced ectopically to target RIP to *erg-3* (Prakash *et al.* 1999). RIP-induced *erg-3* mutants are viable and have altered sensitivities to isoflavonoids and to the steroidal glycoside α-tomatine (Sengupta *et al.* 1995). Whereas the wild type is resistant to isoflavonoids and sensitive to tomatine, *erg-3* mutants are resistant to tomatine and sensitive to isoflavonoids. Most important for our objective, the colonies generated from *erg-3* mutant ascospores exhibit a characteristic growth morphology on Vogel's sorbose agar medium that allows them to be unambiguously identified by inspection under a dissection microscope (for a picture see Noubissi *et al.* 2000).

In our screens we had crossed the wild strains with strains bearing the *Dp 1.3^{ec} hph* transgene. Although both parents in these crosses were *erg-3^{+}*, the presence of the duplicated fragment resulted in the generation of *erg-3* mutant progeny. By determining the frequency of *erg-3* mutants produced in these crosses, we were able to identify the wild isolates that conferred a dominant RIP defect. We have now extended our screens to another 242 wild-isolated strains and identified four more dominant RIP suppressors: Golur-1 (P0334), Fred (P0833), Coon (P0881), and Bayan Lepas (P2663; see appendix). Crosses of *Dp 1.3^{ec} hph* strains with the standard Oak Ridge (OR) strains yield *erg-3* mutant progeny at frequencies typically in the range 8–13%, whereas in crosses with the RIP suppressor strains this frequency is generally ≤0.5%.

The primary objective of this work was to analyze the genetic basis of dominant RIP suppression in the seven strains. For this, each suppressor strain was crossed with the standard OR strains and the f_1 progeny from these crosses were scored for inheritance of the suppressor phenotype. Our studies reveal that natural populations of *N. crassa* harbor two classes of dominant RIP suppressor strains. One class, represented by the Sugartown strain, was barren in crosses, and this is attributed to the presence of a large duplication. The second class was not barren.

MATERIALS AND METHODS

Strains: All strains, unless otherwise indicated, were from the FGSC, University of Kansas Medical Center, Kansas City. These included the 242 wild-isolated *mat a* strains, the standard laboratory wild-type strains 74-OR23-1 A (FGSC 987) and OR8-1 a (FGSC 988), and the wild-isolated *mat A* strains Adiopodoumé (FGSC 430), Adiopodoumé-7 (P 4305), and Sugartown (P 0854). The T-430-a (Hyg^{r}; FGSC 8609) strain is derived from the Adiopodoumé strain by switching the mating type to *mat a* by transformation (Anderson *et al.* 2001).

The *helper-1* strain *a^{m1} ad-3B cyh-1* (FGSC 4564; see Perkins *et al.* 2001) was used to construct heterokaryons with OR-compatible mutant strains of either mating type. The *helper-1* component of such heterokaryons is a passive partner in a cross because the *mat a* allele *a^{m1}* is inactive.

The *Dp 1.3^{ec} hph a* and *Dp 1.3^{ec} hph A* strains were from our laboratory collection and have been described by Prakash *et al.* (1999). *Dp 1.3^{ec} hph* is essentially a tagged duplication of a 1.3-kb *Hin*dIII fragment from *erg-3* that is inserted as a single copy locus into an ectopic site unlinked to *erg-3*. The duplicated segment is marked by the bacterial *hph* gene for resistance to the antibiotic hygromycin B. It does not encode a functional sterol C-14 reductase but serves to target RIP to the *erg-3* gene. Double duplication strains were constructed that contain the *Dp 1.3^{ec} hph* transgene together with another larger duplication (Bhat and Kasbekar 2001; Fehmer *et al.* 2001). The large duplications are *Dp(AR17)* (in A40), *Dp(OY329)* (in C25-3 and C25-8), and *Dp(IBj5)* (in E30 and E42). The large duplications were obtained as segregants from crosses between translocation and normal sequence strains containing the *Dp 1.3^{ec} hph* transgene. The double duplication strains were barren in crosses with the wild type (Bhat and Kasbekar 2001; Fehmer *et al.* 2001). The strain designated 39 was obtained in this work as a segregant from an Adiopodoumé × *Dp 1.3^{ec} hph a*. It has an abnormal growth morphology and contains active *Tad* elements.

The MSUD suppressor strains 96-01 (*Sad-1 A*) and 96-02 (*Sad-1 a*) were kindly provided by Robert L. Metzenberg (Stanford University). We generated *Sad-1; Dp 1.3^{ec} hph A* and *Sad-1; Dp 1.3^{ec} hph a* strains as segregants from the crosses *Dp 1.3^{ec} hph a* × *Sad-1 A* and *Dp 1.3^{ec} hph A* × *Sad-1 a*. The presence of *Sad-1* in these strains was confirmed by verifying their ability to suppress the barren phenotype in crosses with *Dp(IBj5)* strains, and the presence of *Dp 1.3^{ec} hph* was confirmed by verifying that RIP-induced *erg-3* mutant progeny were generated in crosses with 74-OR23-1 A or OR8-1 a. Sixty segregants were examined from each of the crosses *Dp 1.3^{ec} hph a* × *Sad-1 A* and *Dp 1.3^{ec} hph A* × *Sad-1 a*, and segregants 19 and 21, respectively, had a hygromycin-sensitive phenotype. Since both *Dp 1.3^{ec} hph* and *Sad-1* are marked by the *hph* gene, these results suggested that *Dp 1.3^{ec} hph* and *Sad-1* were unlinked. Since *Sad-1* is linked to *mat* (Shiu and Metzenberg 2002), these results cast doubt on earlier results suggesting linkage between *Dp 1.3^{ec} hph* and *mat* (Prakash *et al.* 1999). More extensive linkage experiments have now shown that the *Dp 1.3^{ec} hph* transgene is linked to *al-3* on LG VR (3/80 crossovers) and that it segregates independently of markers for all other linkage groups (data not shown).

Metzenberg also provided the strains FGSC 8752, FGSC 8754, FGSC 8756, FGSC 8758, and FGSC 8760. These are *pan-2 a* strains that contain, respectively, functional copies of the "meiosis-essential" genes *act^{+}* (actin), *hH3hH4-1^{+}* (histones H3 and H4-1), *Bml^{R}* (β-tubulin), *pma-1^{+}* (plasma membrane ATPase), and *mei-3^{+}* (the Neurospora *RecA/Rad51* ortholog *meiotic-3*) inserted into the *his-3* locus. In heterozygous crosses, the induction of MSUD silences these genes and thereby causes the cross to become barren (Shiu *et al.* 2001). The strain FGSC 8750 that contained an insertion of the *his-3* vector alone was used as the control. These strains were used to test whether gene-sized duplications that are capable of inducing barrenness can function as dominant RIP suppressors.

For linkage analysis of the barren phenotype of the Sugartown strain we used the single mutant strains *ad-8 a* (FGSC 453), *al-1 a* (FGSC 2085), *al-2 a* (FGSC 3448), *al-3 a* (FGSC 4073), *arg-5 a* (FGSC 4035), *col-18 a* (FGSC 8284), *cot-1 a*

(FGSC 4066), *cum a* (FGSC 3878), *cys-10 a* (FGSC 4054), *dow a* (FGSC 4052), *lys-1 a* (FGSC 4070), *met-9 a* (FGSC 3280), *pi a* (FGSC 4027), *pyr-1 a* (FGSC 8250), *slo-2 a* (FGSC 1533), *spco-4 a* (FGSC 1372), *thi-3 a* (FGSC 4084), and *ylo-1 a* (FGSC 4100). The mutations are described by Perkins *et al.* (2001).

The strains *In(H4250) aur R A* (FGSC 3156), *R A* (FGSC 4022), and *R a* (FGSC 4023) contain the dominant *Round spore* mutation that confers a female-sterile phenotype. These strains were used in experiments that revealed that a high proportion of the progeny of Adiopodoumé and T-430-a (Hyg^r) are female sterile.

The *rid A* (N1977) and *rid a* (N2148) strains were kindly provided by Eric U. Selker and Michael Freitag (University of Oregon) and are described by Freitag *et al.* (2002). The *rid A* strain was found to have a hygromycin-sensitive phenotype whereas the *rid a* strain was hygromycin resistant. We obtained *rid; Dp 1.3^{ec} hph A* and *rid; Dp 1.3^{ec} hph a* segregants (designated 10 and 2, respectively) from the crosses *Dp 1.3^{ec} hph a* × *rid A* and *Dp 1.3^{ec} hph A* × *rid a*. The genotypes of these segregants was confirmed by verifying that RIP-induced *erg-3* mutant progeny failed to be produced (frequency <0.5%) in crosses with *rid* strains of the opposite mating type but were produced (frequency ⩾0.5%) in crosses with 74-OR23-1 *A* or OR8-1 *a*. These strains were used in experiments to test linkage between *rid* and the Adiopodoumé RIP suppressor.

Growth and cross 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 dimethylformamide 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.

Crosses were performed by confrontation between mycelia inoculated as plugs on synthetic crossing medium in petri dishes. Generally ascospores began to be shot within 14–16 days. Ascospores were harvested by washing the lids with ~1 ml water. Unless indicated otherwise, a first harvest was made 31 days after the crosses were set up. Then the petri dish lids were replaced, and a second harvest was made after an additional 14 days.

Determination of RIP efficiencies: RIP was assayed in crosses with strains bearing the *Dp 1.3^{ec} hph* transgene. Progeny ascospores were germinated on Vogel's N-sorbose agar plates and the fraction of colonies with the *erg-3* mutant morphology was counted under a dissection microscope. Reliability of identifying the *erg-3* mutant phenotype in this way was established by confirming the ability of the conidia to germinate and grow on tomatine medium (Sengupta *et al.* 1995). This assay enabled us to score even very rare RIP events (*e.g.*, <0.04%) in large numbers of crosses. In this article we use the frequency of *erg-3* mutant progeny as a measure of RIP efficiency. In general, this frequency is one-half of the RIP frequency, the proportion of asci that have undergone RIP, since in crosses heterozygous for *Dp 1.3^{ec} hph* only one-half of the progeny ascospores inherit an *erg-3* gene that was exposed to RIP.

RESULTS

Barren RIP suppressors: Seven RIP suppressor strains have now been identified, including Golur-1 (P0334), Fred (P0833), Coon (P0881), and Bayan Lepas (P2663; see appendix), and Adiopodoumé (FGSC 430), Adiopodoumé-7 (P4305), and Sugartown (P0854) (from previous surveys). Of these suppressor strains, Sugartown and Golur-1 were barren in crosses, suggesting that suppression might be caused by a segmental duplication. In addition, the screens also identified three other barren strains: Georgetown-6 (P2622), Batu Ferringi-1 (P2681), and Brabadougou (P4296).

The Sugartown strain: A cross was performed between Sugartown and OR8-1 a, and 81 *mat A* f_1 progeny were crossed with *Dp 1.3^{ec} hph a*. Of these crosses, 40 were barren, 37 were fertile, and 4 had an intermediate productivity. This showed that the barren phenotype segregated 1:1 and was unlinked to *mat*. Among the barren crosses, 13 produced <100 ascospores so it was not possible to obtain a meaningful *erg-3* mutation frequency for them (however, none of the f_2 progeny examined from these 13 crosses was mutant in *erg-3*). In the 27 barren crosses that could be tested, the mutation frequencies were generally <0.5%, whereas in all the fertile crosses they were in the 2–24% range. Of the four crosses with intermediate productivity, one gave a mutation frequency <0.5% and the other three were in the 0.5–1% range. These results showed a tight linkage between RIP suppression and the barren phenotype. Since barrenness is a characteristic of strains bearing large duplications and large duplications suppress RIP in a smaller duplication, the linkage between the two phenotypes suggested that both were caused by the presence of one or more large duplications in the Sugartown strain.

The barren phenotype was tested for linkage with several markers (see materials and methods). It was unlinked to the following markers (numbers in parentheses indicate nonparental/total progeny): *mat* on IL (50/101), *pi* on IIL (43/85), *arg-5* on IIR (18/40), *cum* on IIIL (39/71), *dow* on IIIR (21/40), *cys-10* on IVL (43/82), *cot-1* on IVR (47/86), *pyr-1* on IVR (19/34), *lys-1* on VL (41/85), *al-3* on VR (45/84), *ade-8* on VIL (42/87), *ylo-1* on VIL (15/41), and *col-18* on VIR (34/68). The table provided by Perkins (1994) was used to decide whether these observed ratios indicated deviations from 1:1 segregation of parental:nonparental. Significant deviation from the 1:1 ratio was expected if there was no linkage in tests with the markers *al-1* on IR (23/68), *al-2* on IR (20/67), *spco-4* on VIIL (14/49), and *met-9* on VIIR (24/63). The tests for linkage with *al-1* and *al-2* were repeated and the results (11/38 and 12/38, respectively) once again showed significant deviation from the 1:1 ratio. Linkage of the barren factor was seen also with an additional LG VII marker, *slo-2* (11/38).

The hypothesis that the barrenness and the RIP suppression phenotypes of the Sugartown strain might be due to one or more large duplications received indirect support with the recovery of a morphological mutant

among the progeny from a cross of the Sugartown strain with the auxotrophic strain *thi-3 a*. The mutant was prototrophic and it was barren in crosses with OR8-1 a. The mutation segregated in 10/36 progeny of this barren cross. RIP in the presumptive Sugartown duplication might have been responsible for generating this mutation. Alternatively, crosses with some duplications can also increase mutations in loci that are not covered by the duplication or even in unlinked loci, although it is not known whether such mutations have the molecular hallmarks of RIP (Perkins *et al.* 1997).

The Golur-1 strain: Although the barren phenotype of Golur-1 is stable, it appeared to become unstable in the f_1 progeny from Golur-1 × 74-OR23-1 A and Golur-1 × *Dp 1.3^{ec} hph A*. Of the exceptional f_1 progeny produced, 60 were tested from each cross. The crosses with these f_1 progeny appeared to be potentially barren or fertile until ~24 days, but by the 31st day all the crosses had comparable fertility and showed no evidence of suppression of RIP (data not shown). These results suggested that the presumptive duplication in Golur-1 is subject to a genotype-dependent instability. This instability foiled our attempts to test for linkage between the RIP suppressor and barren phenotypes of the Golur-1 strain.

Failure of the *Sad-1* suppressor of MSUD-induced barrenness to impair suppression of RIP by duplications in the *erg-3* test system: The semidominant *Sad-1* mutation imposes a defect for MSUD (Shiu *et al.* 2001; Shiu and Metzenberg 2002). The barren phenotype of the Sugartown, Golur-1, and Georgetown-6 strains, but not that of the Batu Ferringi-1 and Brabadougou strains, was suppressed in crosses with *Sad-1* strains (data not shown). These findings agreed with our model that the barren phenotype of the Sugartown and Golur-1 strains stems from MSUD-induced silencing of duplication-borne genes (see above).

We investigated whether large duplications retain their RIP suppression ability when their barren phenotype is suppressed by *Sad-1*. For this we used strains carrying *Dp 1.3^{ec} hph* together with one of the large duplications *Dp(AR17)*, *Dp(OY329)*, or *Dp(IBj5)*. The double duplication strains were crossed with *Sad-1* strains of the appropriate mating type. Control crosses were done between *Dp 1.3^{ec} hph* and *Sad-1* strains. As expected, the barren phenotype of all three large duplications was suppressed by *Sad-1* but as can be seen in the results summarized in Table 1A, the *erg-3* mutation frequencies continued to be very low. These results indicated that the defect for MSUD did not affect the ability of large duplications to suppress RIP in a smaller duplication.

Of 35 progeny examined from the A40 × *Sad-1* cross one had the *downy* (*dow*) phenotype. The *Dp(AR17)* duplication covers the *dow* locus; therefore, the *dow* mutant must have been generated by RIP. The *dow* mutation frequency (1/35 = 2.8%) was in agreement with results from previous crosses with *Dp(AR17)* (Perkins *et al.* 1997; Bhat and Kasbekar 2001). These results demonstrated that the *Sad-1* mutation does not impair RIP in either *Dp 1.3^{ec} hph* or *Dp(AR17)*.

Failure of barren gene-sized duplications to modify RIP in the *erg-3* test system: Even relatively small duplications can render a cross barren, for example, Dp (IVR>I)B362i, which covers only one known gene, *met-1* (see Perkins 1997). *Sad-1*-suppressible barrenness has also been demonstrated in crosses heterozygous for ectopic insertions of genes that code for functions required during meiosis (Shiu *et al.* 2001). These include β-tubulin (*Bml^R*), actin (*act$^+$*), histones H3 and H4-1 (*hH3hH4-1$^+$*), plasma membrane ATPase (*pma-1$^+$*), and the Neurospora *RecA/Rad51* ortholog *meiotic-3* (*mei-3$^+$*). The titration model, however, predicts that such gene-sized duplications might not be large enough to titrate out the RIP machinery and therefore would be incapable of functioning as dominant RIP suppressors. To test this we performed crosses between the strains bearing duplications for the "meiosis-essential" genes and *Sad-1 Dp 1.3^{ec} hph A* and scored the frequency of *erg-3* mutant progeny. The results summarized in Table 1B show that the gene-sized duplications did not suppress RIP in *erg-3*. This is consistent with the idea that gene-sized duplications are too small to act as dominant RIP suppressors.

A barren strain that is not a RIP suppressor: Although the Georgetown-6 (P2622) strain showed a *Sad-1*-suppressible barrenness, it did not display a RIP suppressor phenotype (see appendix). The frequency of *erg-3* mutant progeny from the cross of Georgetown-6 with *Sad-1 Dp 1.3^{ec} hph A* was 1.8% (n = 709). It is conceivable that this strain contains a gene-sized duplication of a "meiosis-essential" gene that is capable of inducing barrenness but is not large enough to titrate the RIP machinery.

Nonbarren RIP suppressor strains: The Adiopodoumé, Adiopodoumé-7, Bayan Lepas, Coon, and Fred RIP suppressor strains did not have an associated barren phenotype. It was conceivable that these strains contained a large duplication as well as a "*Sad-1*-like" mutation. To test such possibilities, each of these strains was crossed with 74-OR23-1 A or OR8-1 a and the f_1 progeny were examined for segregation of the suppressor and any cryptic barren phenotype.

The Adiopodoumé (FGSC 430) strain: The T-430-a (Hyg^r) strain is a derivative of the Adiopodoumé 430 strain in which the mating type has been switched to *mat a* by transformation (Anderson *et al.* 2001). A total of 116 f_1 progeny from T-430-a (Hyg^r) × 74-OR23-1 A were crossed with *Dp 1.3^{ec} hph* strains of the appropriate mating type. None of the crosses were barren, indicating that T-430-a (Hyg^r) does not contain any conventional duplication. We determined the frequency of RIP-induced *erg-3* mutant progeny from these crosses. In the crosses with the 59 *mat a* progeny, 52 (88.1%) gave *erg-3* mutation frequencies <0.5%, and in the crosses with the 57 *mat A* progeny, 50 (87.7%) gave *erg-3* mutation

TABLE 1

***erg-3* mutation frequencies in *Sad-1* heterozygous crosses**

Sad-1$^+$ parent	Segregants examined	*erg-3* mutants	Frequency (%)
A.[a]			
A40 a	569	3	0.5
C25-3 A	271	0	<0.4
C25-8 A	289	1	0.5
E30 a	474	0	<0.2
E42 a	415	0	<0.2
Dp 1.3^{ec}hph A	163	35	21.0
Dp 1.3^{ec}hph a	226	42	18.5
B.[b]			
his-3::his-3$^+$; pan-2 a	74	17	23.0
his-3::his-3$^+$ act$^+$; pan-2 a	72	13	18.0
his-3::his-3$^+$ hH3hH4$^+$; pan-2 a	80	18	22.5
his-3::his-3$^+$ Bml^R; pan-2 a	127	24	19.0
his-3::his-3$^+$ pma-1$^+$; pan-2 a	54	5	9.3
his-3::his-3$^+$ mei-3$^+$; pan-2 a	59	12	20.3

[a] The genotype of strain A40 is *Dp(AR17); Dp 1.3^{ec} hph*; of strains C25-3 and C25-8, *Dp(OY329); Dp1.3^{ec} hph*; and of strains E30 and E42, *Dp(IBj5); Dp1.3^{ec} hph*. They are all equally effective in suppressing RIP in the *erg-3* test system. These strains were crossed with *Sad-1 A* or *Sad-1 a*.

[b] The other parent was *Dp 1.3^{ec} hph; Sad-1 A*.

frequencies >1%. These results suggested that the suppressor was linked to the *mat* locus on LG IL and that the 7 *mat a* progeny with the nonsuppressor phenotype (*i.e.*, *erg-3* mutation frequencies >0.5%) and the 7 *mat A* progeny with the suppressor phenotype (*i.e.*, *erg-3* mutation frequencies <1%) represent the crossover types. From this we can infer that the suppressor is 14/116 (12.1%) units away from the *mat* locus.

Linkage between the RIP suppressor and the *mat* locus was also evident in the f_1 progeny from Adiopodoumé × OR8-1 a. Of the 54 *mat A* progeny tested, 32 (59.3%) gave a low *erg-3* mutation frequency (<0.5%), 7 (12.9%) gave an intermediate frequency (0.5–1%), and 15 (27.8%) gave a high frequency (>1%). And of the 30 *mat a* progeny tested, the mutation frequencies were high for 25 (83.3%), intermediate for 2 (6.7%), and low for 3 (10%). Thus, most of the *mat A* progeny had the suppressor phenotype whereas most of the *mat a* progeny had the nonsuppressor phenotype. The proportion of putative crossovers in this experiment was 27/84 (32.1%).

A newly identified locus on LG IL, designated *rid* (*RIP defective*), codes for a putative DNA methyltransferase and homozygous *rid* mutant crosses are defective for RIP (Freitag *et al.* 2002). We tested the Adiopodoumé RIP suppressor for linkage with *rid*. A total of 100 f_1 progeny from the cross T-430-a (Hyg^r) × *rid A* were crossed with *rid; Dp 1.3^{ec} hph* strains of the opposite mating types and the f_2 ascospores from these crosses were examined for *erg-3* mutants. Only two crosses produced any *erg-3* mutant progeny in the f_2. These two f_1 strains presumably represent crossover segregants that are wild type at both *rid* and the dominant RIP suppressor locus. Control crosses were done in which the f_1 progeny from OR8-1 a × *rid A* were crossed with the *rid; Dp 1.3^{ec} hph* strains and the *rid* and *rid*$^+$ markers showed ~1:1 segregation in the f_1 progeny (data not shown). Taken together, these results allow us to conclude that the Adiopodoumé RIP suppressor is distinct from, but linked to, *rid* (~4/100).

Infertility factors in the Adiopodoumé strain: The Adiopodoumé × T-430-a (Hyg^r) cross is infertile (Anderson *et al.* 2001). For all practical purposes this is a self-cross; therefore, it follows that the Adiopodoumé strain contains one (or more) recessive fertility defect(s). The 116 f_1 progeny from T-430-a (Hyg^r) × 74-OR23-1 A were backcrossed to Adiopodoumé or T-430-a (Hyg^r). Of the 59 *mat a* segregants, 56 (95%) were infertile in the backcrosses with Adiopodoumé and 3 (5%) were fertile, and of the 56 *mat A* segregants, 33 (59%) were fertile in the backcross with T-430-a (Hyg^r) and 23 (41%) were infertile. We interpret these results to suggest that the T-430-a (Hyg^r) strain (and therefore also the Adiopodoumé strain) contains two recessive infertility mutations: one with 95% linkage to *mat* and another unlinked to *mat*. Only 1 of the 3 *mat a* crossover types for the infertility factor was also a crossover type for the RIP suppressor. Since the linkage between the LG I infertility factor and *mat* was greater than that between the RIP suppressor and *mat*, the infertility locus and the dominant RIP suppressor are likely to be on opposite sides of the *mat* locus.

Crosses parented by the Adiopodoumé strain produce many female-sterile progeny: A high proportion of progeny from the crosses parented by the Adiopodoumé and T-430-a (Hyg^r) strains were found to have a female-sterile pheno-

type and could participate in crosses only as males. This was discovered in crosses of the progeny with a strain bearing the *Round spore* (R) mutation, which confers a female-sterile phenotype. Since the *erg-3* mutation also confers a female-sterile phenotype, in subsequent studies the female sterility of such progeny was tested in crosses with *erg-3* strains. Of 40 progeny examined from the cross Adiopodoumé × OR8-1 a, 14 (35%) were female sterile and of 113 examined from T-430-a (Hyg^r) × 74-OR23-1 A, 20 (17.7%) were female sterile. Since all four parental strains are fertile as both male and female, these results suggest that one or more mutations that cause female sterility occur at a high frequency in crosses parented by the Adiopodoumé strains. In control crosses none of the 50 progeny tested from 74-OR23-1 A × OR8-1 a were female sterile. Crosses of the Adiopodoumé and T-430-a (Hyg^r) strains with non-Oak Ridge partners also yielded high frequencies of female-sterile progeny. The Adiopodoumé strain was crossed with the *mat a* strains Venkatavarum (FGSC 4722), Madurai (FGSC 4718), Iowa (P0529), and Bichpuri-1 (P0748) and the frequencies of female-sterile progeny were, respectively, 37.5% (40), 35% (20), 40% (40), and 55% (20), (numbers in parentheses indicate the number of progeny examined). T-430-a (Hyg^r) was crossed with the *mat A* strains Esterillo Este Rd-3 (P4000) and Rondon (P4214) and the frequencies of female-sterile progeny from these crosses were, respectively, 12.5% (40) and 12.5% (40).

Adiopodoumé-7: Sixty *mat A* f_1 progeny from a cross between Adiopodoumé-7 (P4305) and OR8-1 a were crossed with *Dp 1.3^{ec} hph a.* Only six crosses gave a very low frequency of *erg-3* mutants in progeny ascospores harvested at 31 days and of these only three continued to exhibit this low frequency even in ascospores harvested at 45 days (data not shown). Additionally, 37 *mat a* f_1 progeny were crossed with *Dp 1.3^{ec} hph A*, but none of them appeared to have inherited the dominant RIP suppressor phenotype (data not shown). Thus the dominant RIP suppressor phenotype of Adiopodoumé-7 was inherited by only 1/10–1/20 of the *mat A* progeny and by an even lower fraction (<1/37) of the *mat a* progeny. These results are consistent with models in which RIP suppression by Adiopodoumé-7 requires the inheritance of four or five unlinked loci of which one might be located on LG IL. At any rate, the RIP suppressor of Adiopodoumé-7 does not appear to segregate as a single locus linked to *mat* and therefore the genetic basis of suppression appears to be different from that in the Adiopodoumé (FGSC 430) strain. It is noteworthy that two strains isolated from the same geographical region contain nonidentical RIP suppressors.

Bayan Lepas: We examined 59 f_1 progeny from Bayan Lepas × 74-OR23-1 A. Of these, 14 gave low *erg-3* mutation frequencies (<0.5%), 10 were intermediate (0.5–1.0%), and 35 were high (>1.0%). If the low and intermediate mutation frequencies indicate the presence of the suppressor, then the segregation of suppressor and wild-type phenotypes is 24:35, which is not significantly different from 1:1 (Perkins 1994). However, when 70 f_1 progeny from Bayan Lepas × *Dp 1.3^{ec} hph A* were examined, only 9 (~1/8) appeared to have inherited the RIP suppressor phenotype. This result suggests that the suppressor phenotype might require the inheritance of as many as three unlinked loci from the Bayan Lepas parent. Thus dominant RIP suppression in Bayan Lepas appears to have a complex genetic basis.

Coon and Fred: The Coon and Fred RIP suppressor strains were analyzed in a similar manner. Again only a minority of the f_1 progeny were found to inherit their RIP suppressor phenotype. Among the f_1 progeny parented by the Coon strain, 6 gave low *erg-3* mutation frequencies (<0.5%), 4 were intermediate (0.5–1.0%), and 54 were high (>1.0%), which indicated that the suppressor was inherited only by 10/64 progeny. The corresponding frequencies for the f_1 progeny parented by the Fred strain were 5 low, 7 intermediate, and 43 high, which indicated that the suppressor phenotype was inherited by only 12/55 progeny. These deviations from 1:1 segregation suggest that the RIP suppressor phenotype of these strains might require the inheritance of more than one locus.

Failure of the *Tad* retrotransposon to alter the efficiency of RIP: A *Dp 1.3^{ec} hph; col-18 A* strain that does not contain any *Tad* sequences was used as the naive strain into which *Tad* sequences were introduced by infection from a donor strain. Another strain, no. 39 (see MATERIALS AND METHODS), which was obtained as a segregant from Adiopodoumé × *Dp 1.3^{ec} hph a*, was found to contain active *Tad* elements and used as the donor strain. Strain 39 also has an abnormal growth phenotype (different from the col-18 phenotype). Heterokaryons were formed between these two morphologically abnormal strains and isolated on the basis of their wild-type morphology. The heterokaryons were passaged through 30 vegetative transfers during which time the naive nuclear component became infected with *Tad.* The newly infected nuclei were isolated in homokaryotic conidia, now designated as "infected derivatives," and these were maintained as heterokaryons with the *helper-1* strain. Figure 1 presents the results of the Southern analysis done to confirm the acquisition of *Tad* sequences by the infected derivative strains. When the infected derivatives were crossed with the wild type, there was no difference in the *erg-3* mutation frequencies relative to control crosses with the naive strain (Table 2). These results lead us to conclude that the RIP efficiency of a naive strain is not affected following its infection by *Tad.*

DISCUSSION

Of the 446 *N. crassa* wild isolates that we screened, 7 dominant RIP suppressor strains were identified. Each

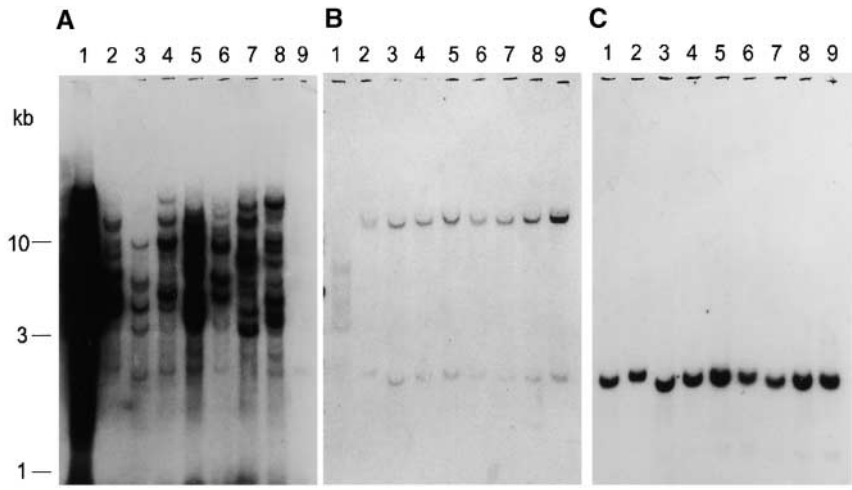

FIGURE 1.—Acquisition of *Tad* sequences by "infected derivatives" of the naive strain. Genomic DNA of the Adiopodoumé strain (lane 1), the *Dp 1.3*^{ec} *hph; col-18 A* naive strain (lane 9), and seven infected derivative strains (lanes 2–8) was digested with *Eco*RI and subjected to Southern analysis. The blot was probed for *Tad* (A), *hph* (B), and *erg-3* (C). Note that *Tad* sequences are present in the Adiopodoumé strain and in all the infected derivatives but are absent from the naive strain. The *Dp 1.3*^{ec} *hph* transgene is present in the infected derivatives and the naive strain but not in the Adiopodoumé strain. The relatively constant hybridization intensities in B and C indicate that the proportion of infected derivative nuclei in the heterokaryons with *helper-1* are roughly equal (see text for details).

of these strains was crossed with the standard OR background and the inheritance of the suppressor phenotype was examined in the f_1 progeny. The RIP suppressors from the Adiopodoumé and Sugartown strains showed 1:1 segregation in the f_1 progeny, whereas the suppressors of the Adiopodoumé-7, Bayan Lepas, Coon, and Fred strains appeared to show a more complex inheritance pattern. We could not establish the segregation pattern of the Golur-1 suppressor. Additionally, our studies reveal that the dominant RIP suppressors fall into two classes. One class, represented by the Sugartown strain and possibly by the Golur-1 strain, appears to use large duplications to suppress RIP. To our knowledge, this is the first report identifying duplications in natural populations of Neurospora. The second class, which included the Adiopodoumé, Adiopodoumé-7, Bayan Lepas, Coon, and Fred strains, did not appear to involve duplications. However, we cannot rigorously exclude the possibility that a subset of these strains might in fact contain duplications that do not cover any "meiosis-essential" genes whose silencing by MSUD would evoke barrenness.

TABLE 2

***erg-3* mutation frequencies in crosses between "infected derivative" strains and the wild type**

Infected derivative	Segregants examined	*erg-3* mutants (%)
1	346	2.6
2	173	1.7
3	241	2.5
4	72	4.2
5	151	4.6
6	382	1.6
7	251	2.4
8	154	3.9
9	322	3
Naive strain	31	5.1

The naive strain is *Dp 1.3*^{ec} *hph; col-18 A*. The infected derivative strains have an identical genotype plus the introduced *Tad* elements.

Duplications can affect Neurospora crosses in two ways. During the premeiotic stage they serve as substrates for RIP and in this way they can lead to the generation of novel mutations among the progeny. Witness the morphological and *dow* mutants produced, respectively, in the crosses with the Sugartown and *Dp(AR17)* duplications (also see PERKINS *et al.* 1997). By acting as substrates for RIP, large duplications might also titrate out the RIP machinery and thereby act as suppressors of RIP in a smaller duplication. Subsequently during meiosis, duplications can cause the silencing of unpaired genes by MSUD and thereby render the cross barren. RIP and MSUD are independent processes. It was shown previously that the barren phenotype of a duplication strain was not alleviated in crosses with the Adiopodoumé-dominant RIP suppressor strain (NOUBISSI *et al.* 2000). Now we have shown that suppression of the barren phenotype by *Sad-1* does not reduce the RIP suppressive effect of large duplications. The picture emerging from these studies is that the size of the duplication ("quantity") determines whether it will act as a RIP suppressor, and its coverage of meiosis-essential genes ("quality") determines whether or not it causes barrenness.

It is not known whether rearrangements other than duplications can trigger MSUD when they are heterozygous. Pairing would be disrupted in the vicinity of breakpoints or with short inversions. Such rearrangements should look like dominant-barren mutations that are suppressible by *Sad-1* but do not suppress RIP. The Georgetown-6 strain is a candidate for such a rearrangement. The inability of *Sad-1* to suppress the barren phenotype of the Batu Ferringi-1 and Brabadougou strains suggested that these two strains might not contain dupli-

cations. Although Brabadougou was not tested for RIP suppression, the Batu Ferringi-1 strain happened to be tested *en passant* in a double-blind experiment (see APPENDIX) and found not to behave as a dominant RIP suppressor. This result also argues against the presence of a duplication in this strain.

Infection of a naive strain by *Tad* did not affect RIP. But we were somewhat surprised to find that *Tad* sequences made no contribution at all to the RIP suppression phenotype of the Adiopodoumé strain. This strain contains ~40 copies of *Tad* dispersed throughout the genome. A complete *Tad* element is ~7 kbp in size; therefore, the Adiopodoumé strain could harbor as much as 280 kb of duplicated DNA, which is comparable in size with some large duplications (SMITH *et al.* 1996). But its RIP suppressor phenotype appeared to show quite straightforward linkage to the *rid* and *mat* loci on LG IL rather than to multiple dispersed loci that would be expected to represent the *Tad* elements. Although some copies of *Tad* might have already suffered enough sequence alterations to preclude their involvement in further rounds of RIP, Southern analysis of suppressor and nonsuppressor f_1 progeny did not reveal any consistent difference in *Tad* sequences (data not shown). Thus our results suggest that duplicated DNA might be effective in titrating out the RIP machinery only if it is present in one contiguous stretch, as in a segmental aneuploid strain, and not if it is composed of relatively shorter segments dispersed in the genome.

Of the 116 progeny tested from T-430-a (Hyg^r) × 74-OR23-1 A, 12.1% represented products of crossovers between the RIP suppressor and *mat* loci, whereas the crossover frequency was considerably higher (32.1%) among the 84 progeny tested from Adiopodoumé × OR8-1 a. One explanation for this difference could be that the T-430-a (Hyg^r) strain, which represents a transformed nucleus that was essentially "cloned" out of the Adiopodoumé strain, is homokaryotic for an inversion for which the parental Adiopodoumé strain is heterokaryotic. Homokaryosis for an inversion could have reduced the crossover frequency. Chromosome rearrangements have been noted to be frequent in the Adiopodoumé strain (by KINSEY and HELBER 1989, as a personal communication from David Perkins). Alternatively, the putative inversion might have been generated during the transformation done to switch the mating type. PERKINS *et al.* (1993) have reported that chromosome rearrangements can accompany transformation at surprisingly high frequencies. However, we do not have any explanation for our observation that the frequencies of female-sterile progeny were consistently lower (12.5–17.5%) in crosses parented by the T-430-a (Hyg^r) strain than in crosses parented by Adiopodoumé (35–55%).

We have shown here that the Adiopodoumé suppressor was distinct from, but closely linked to, *rid*. Another LG I candidate gene that should probably be tested for allelism with the Adiopodoumé suppressor is *eth-1* (*ethionine resistant-1*), the structural gene for *S*-adenosylmethionine synthetase (see PERKINS *et al.* 2001). SELKER (1990) had proposed an attractive model in which limiting cellular levels of S-adenosylmethionine could increase the efficiency of RIP. The model was considered in further detail by MAUTINO and ROSA (1998). In this model a putative DNA-(5-cytosine) methyltransferase (possibly the enzyme encoded by *rid*) interacts with the C6 of cytosine to form an unstable intermediate 5,6-dihydrocytosine complex with the enzyme. The intermediate either can receive the methyl group from *S*-adenosylmethionine to generate 5-methylcytosine or, alternatively, in *S*-adenosylmethionine-limiting conditions, can tautomerize to an imino group, which can be easily hydrolyzed to generate uracil. Conceivably, if an overactive *S*-adenosylmethionine synthetase of the Adiopodoumé strain caused overaccumulation of *S*-adenosylmethionine, the efficiency of RIP might become depressed. In parallel with the testing of candidate genes we are continuing our efforts to obtain a more accurate localization of the suppressor locus on IL as a prelude to its molecular characterization.

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Note added in proof: The Adiopodoumé suppressor was found not to be allelic with *eth-1* (6/80) (Ranjan TAMULI, unpublished results).

LITERATURE CITED

ANDERSON, C., T. TANG and J. A. KINSEY, 2001 Elimination of active *Tad* elements during the sexual phase of the *Neurospora crassa* life cycle. Fungal Genet. Biol. **33:** 49–57.

ARAMAYO, R., and R. L. METZENBERG, 1996 Meiotic transvection in fungi. Cell **86:** 103–113.

BHAT, A., and D. P. KASBEKAR, 2001 Escape from repeat-induced point mutation of a gene-sized duplication in *Neurospora crassa* crosses that are heterozygous for a larger chromosome segment duplication. Genetics **157:** 1581–1590.

DAVIS, R. H., and J. F. DE SERRES, 1970 Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **A17:** 79–143.

ELLIS, S. W., M. E. ROSE and M. GRINDLE, 1991 Identification of a sterol mutant of Neurospora deficient in $\Delta^{14,15}$-reductase activity. J. Gen. Microbiol. **137:** 2627–2630.

FEHMER, M., A. BHAT, F. K. NOUBISSI and D. P. KASBEKAR, 2001 Wild-isolated *Neurospora crassa* strains that increase the fertility of crosses with segmental aneuploids used to establish that a larger duplication suppresses RIP in a smaller duplication. Fungal Genet. Newsl. **48:** 13–14.

FREITAG, M., R. L. WILLIAMS, G. O. KOTHE and E. U. SELKER, 2002

A cytosine methyltransferase homologue is essential for repeat-induced point mutation in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **99:** 8802–8807.

Irelan, J. T., and E. U. Selker, 1996 Gene silencing in filamentous fungi: RIP, MIP and quelling. J. Genet. **75:** 313–324.

Kinsey, J. A., 1990 Restricted distribution of the *Tad* transposon in strains of Neurospora. Curr. Genet. **15:** 271–275.

Kinsey, J. A., and J. H. Helber, 1989 Isolation of a transposable element from *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **86:** 1929–1933.

Kinsey, J. A., P. W. Garrett-Engele, E. B. Cambareri and E. U. Selker, 1994 The Neurospora transposon *Tad* is sensitive to repeat-induced point mutation (RIP). Genetics **138:** 657–664.

Mautino, M. R., and A. L. Rosa, 1998 Analysis of models involving enzymatic activities for the occurrence of C → T transition mutations during repeat-induced point mutation (RIP) in *Neurospora crassa*. J. Theor. Biol. **192:** 61–71.

Noubissi, F. K., K. McCluskey and D. P. Kasbekar, 2000 Repeat-induced point mutation (RIP) in crosses with wild-isolated strains of *Neurospora crassa*: evidence for dominant reduction of RIP. Fungal Genet. Biol. **31:** 91–97.

Noubissi, F. K., K. Aparna, K. McCluskey and D. P. Kasbekar, 2001 Evidence for dominant suppression of repeat-induced point mutation (RIP) in crosses with the wild-isolated *Neurospora crassa* strains Sugartown and Adiopodoumé-7. J. Genet. **80:** 55–61.

Papavinasasundaram, K. G., and D. P. Kasbekar, 1994 The *Neurospora crassa erg-3* gene encodes a protein with sequence homology to both yeast sterol C-14 reductase and chicken lamin B receptor. J. Genet. **73:** 33–41.

Perkins, D. D., 1994 Deviations from 1:1 and numbers of progeny necessary for establishing linkage. Fungal Genet. Newsl. **41:** 69–70.

Perkins, D. D., 1997 Chromosome rearrangements in Neurospora and other filamentous fungi. Adv. Genet. **36:** 239–398.

Perkins, D. D., J. A. Kinsey, D. K. Asch and G. D. Frederick, 1993 Chromosome rearrangements recovered following transformation of *Neurospora crassa*. Genetics **114:** 729–736.

Perkins, D. D., B. S. Margolin, E. U. Selker and S. D. Haedo, 1997 Occurrence of repeat induced point mutation in long segmental duplications of Neurospora. Genetics **147:** 125–136.

Perkins, D. D., A. Radford and M. S. Sachs, 2001 *The Neurospora Compendium. Chromosomal loci.* Academic Press, San Diego.

Prakash, A., and D. P. Kasbekar, 2002 The sterol C-14 reductase encoded by the *Neurospora crassa erg-3* gene: essential charged and polar residues identified by site-specific mutagenesis. Mol. Genet. Genomics **266:** 787–795.

Prakash, A., S. Sengupta, K. Aparna and D. P. Kasbekar, 1999 The *erg-3* (sterol Δ14, 15 reductase) gene of *Neurospora crassa*: generation of null mutants by repeat-induced point mutation and complementation by proteins chimeric for human lamin B receptor sequences. Microbiology **145:** 1443–1451.

Raju, N. B., and D. D. Perkins, 1978 Barren perithecia in *Neurospora crassa*. Can. J. Genet. Cytol. **20:** 41–59.

Selker, E. U., 1990 Premeiotic instability of repeated sequences in *Neurospora crassa*. Annu. Rev. Genet. **24:** 597–613.

Sengupta, S., T. Bhavani Prasanna and D. P. Kasbekar, 1995 Sterol 14, 15 reductase (*erg-3*) mutants switch the phenotype of *Neurospora crassa* from sensitivity to the tomato saponin α-tomatine to sensitivity to the pea phytoalexin pisatin. Fungal Genet. Newsl. **42:** 71–72.

Shiu, P. K. T., and R. L. Metzenberg, 2002 Meiotic silencing by unpaired DNA: properties, regulation and suppression. Genetics **161:** 1483–1495.

Shiu, P. K. T., N. B. Raju, D. Zickler and R. L. Metzenberg, 2001 Meiotic silencing by unpaired DNA. Cell **107:** 905–916.

Smith, M. L., C. J. Yang, R. L. Metzenberg and N. L. Glass, 1996 Escape from *het-6* incompatibility in *Neurospora crassa* partial diploids involves preferential deletion within the ectopic segment. Genetics **144:** 523–531.

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APPENDIX

Screening of additional wild *N. crassa* strains for dominant suppression of RIP: A total of 242 wild-isolated *mat a* strains were each crossed with *Dp 1.3^{ec} hph A* and the frequency of RIP-induced *erg-3* mutants was determined in the f_1 progeny of these crosses. For crosses that gave a low frequency (<1%) of *erg-3* mutants in ascospores harvested at 31 days, the mutation frequency was determined again in a second harvest taken at 45 days. Crosses with the three strains Fred (P0833), Coon (P0881), and Bayan Lepas (P2663) gave low *erg-3* mutation frequencies in both harvests (Table A1).

Crosses with four other strains, Golur-1 (P0334), Georgetown-6 (P2622), Batu Ferringi-1 (P2681), and Brabadougou (P4296), were barren. This is relevant because segmental duplications make crosses barren, and long segmental duplications are also known to suppress RIP in our test system. Of the barren crosses only the one with Golur-1 produced sufficient numbers of ascospores after 45 days to allow an estimation of the *erg-3* mutation frequency. This frequency was low. The other three barren crosses together produced <100 progeny. To confirm that the low frequency of *erg-3* mutants in the crosses with the Coon, Fred, Bayan Lepas, and Golur-1 strains was reproducible and not merely a sampling artifact, we repeated these crosses and again the frequency of *erg-3* mutant progeny was very low (data not shown). Once again the cross with the Golur-1 strain was barren and took 45 days to produce sufficient numbers of ascospores. Thus the Coon, Fred, and Bayan Lepas strains resembled the previously tested Adiopodoumé 430 strain in that the RIP suppression by these strains was not associated with a barren phenotype, whereas the Golur-1 strain was like the Sugartown strain in that its RIP suppression phenotype was associated with barrenness. It was not possible to determine from these results whether the barrenness of the Georgetown-6, Batu Ferringi-1, and Brabadougou strains was associated with a dominant RIP suppression phenotype.

Double-blind tests of the RIP suppressor phenotype: We tested whether the dominant RIP suppression phenotype could be used as the defining character to identify the Coon, Fred, and Bayan Lepas strains in double-blind experiments. The T-430-a (Hyg^r) (FGSC 8609) strain is derived from the Adiopodoumé strain by switching of the mating type to *mat a* by transformation (Anderson *et al.* 2001). We also tested this strain to verify that it retained the ability of the Adiopodoumé strain to dominantly suppress RIP in *Dp 1.3^{ec} hph*. In each of these experiments 10 wild strains were coded and crossed with *Dp 1.3^{ec} hph A* and the frequency of RIP-induced *erg-3* mutants was determined in ascospores harvested after 31 days. The challenge was to identify which of the coded cultures represented the suppressors. The experimental protocol was essentially the same as the one used by Noubissi *et al.* (2000). The results of these experiments are summarized in Table A2 and they showed that in all cases the RIP suppressor strains could be correctly distinguished from the other wild isolates. In the double-blind test of the Bayan Lepas strain, we happened to also perform a cross with the

Batu Ferringi-1 strain. This cross was barren as noted previously (see above), but nevertheless we could obtain 246 ascospores and thus determine the *erg-3* mutation frequency. Despite its barren phenotype, the Batu Ferringi-1 strain did not display a RIP suppressive effect.

In the experiment to test the Coon strain, the cultures coded B, D, and E were the Coon strain. Those coded A, C, F, G, H, I, and J were the non-Coon strains, respectively, Makaba-1 (P3812), Aarey-1 (P0677), Sungai Ara (P2672), Bichpuri-1 (P0742), Homestead-3 (P1470), Fred-2 (P0828), and Klong Rangsit No. 7 (P4217). In the test of the Fred strain the cultures coded C, G, and H were the Fred strain and those coded A, B, D, E, I, and J were, respectively, Aarey-1 (P0678), Georgetown-2 (P2592), Colonia Paraiso (P1291), Welsh-1d (P0507), Georgetown-4 (P2608), and Groveland-1 (P0438). The strain coded F, Florida City (P1448), was found to behave as *mat A*. In the test of Bayan Lepas, the cultures coded D, H, and I were Bayan Lepas and those coded A, B, C, E, and G were Batu Ferringi-1 (P2681), Franklin (P4493), Kabah (P4126), Kabah (P4125), and Jaco-2 (P4017). The strains coded F and J, respectively, Maripasoula (P4088) and Madurai (P4360), were found to behave as *mat A*. In the test for T-430-a (Hyg^r) (FGSC 8609), the cultures A, E, F, G, and J were T-430-a (Hyg^r) whereas B, C, D, H, and I were OR8-1 a.

TABLE A1

erg-3* mutation frequencies in crosses between *N. crassa* wild isolates and *Dp 1.3*^{ec} *hph A

Strain name	Strain number[a]	Segregants examined	*erg-3* mutation frequency (%)
Brazil			
Rondon	P4038	109	8.2
Rondon	P4207	306	15.3
Rondon	P4215	434	2.7
Tucamanduba	P4592	436	3.2
Tucamanduba	P4593	166	9.6
Tucamanduba	P4602	435	7.6
Tucamanduba	P4603	262	8.4
Tucamanduba	P4609	150	11.3
British West Indies			
Old Man Bay-1	8175	209	16.2
Old Man Bay-1	P4782	350	6.8
Old Man Bay-1	P4783	322	8.1
Congo			
Bouanza	4819	321	21.8
Bouanza	P3855	18	11.1
Loubom	4820	495	17.4
Makaba-1	P3812	387	3.6
Makaba-2	4821	372	7.5
Continental United States			
Coon-1	3200	360	9.7
Coon	P880	359	11.4
Coon	P881	988 (927)	0.5 (0.4)
Elizabeth-8	P868	180	4.4
Elizabeth	P870	184	9.2
Elizabeth	P1140	365	11.5
Florida City[b]	3974	460	4.6
Franklin	7834	334 (430)	0.3 (6)
Franklin	P4450	284	6
Franklin	P4451	174	19.5
Franklin	P4452	240	2.5
Franklin	P4454	557	6.3
Franklin	P4455	554	13.9
Franklin	P4456	187	8.5
Franklin	P4457	249	1.2
Franklin	P4459	196	19.9
Franklin	P4465	329	10.9
Franklin	P4469	486	12.7
Franklin	P4470	200	12

(*continued*)

TABLE A1

(Continued)

Strain name	Strain number[a]	Segregants examined	*erg-3* mutation frequency (%)
Franklin	P4471	373	8
Franklin	P4472	456	13.6
Franklin	P4476	329	5.2
Franklin	P4479	380	4.2
Franklin	P4481	464 (682)	0.8 (1.7)
Franklin	P4483	214	3.7
Franklin	P4489	258	5.8
Franklin	P4491	142	5.6
Franklin	P4493	266	3.7
Franklin	P4496	150	4.6
Franklin	P4497	297	3
Franklin	P4498	323	4
Franklin	P4499	309	1.6
Franklin	P4500	458	8.9
Franklin	P4501	721	8.3
Fred-2	3225	362	9.6
Fred	P829	176	9.1
Fred	P830	208	9.6
Fred	P832	173	10.4
Fred	P833	482 (486)	<0.2 (<0.2)
Fred	P834	308 (508)	1 (0.4)
Fred	P1138	326	11
Georgia Plantation	8104	183	1.6
Georgia Plantation	P4507	163	3.7
Georgia Plantation	P4508	221	10.4
Georgia Plantation	P4509	129	1.5
Groveland-1	P438	150	10
Groveland-1	P441	234	4.7
Groveland-1	P445	177	8.5
Homestead-2	3971	74	10.8
Homestead-2	P1408	170	10
Homestead-3	P1460	184	2.7
Homestead-3	P1470	218	15.1
Morrero-1	P474	307	5.2
Houma	P548	121	1.6
Houma	P491	429	1.2
Houma	P492	667	4.6
Houma	P493	377	14.6
Houma	P496	234	3.4
Houma	P499	71	6.6
Houma-1	3943	191	5.2
Houma-1	2221	429	1.2
Iowa	P528	206	1.9
Iowa	P529	154	2
Iowa	P530	393	3.5
Iowa	P531	211	8.1
Iowa	P532	416	2.6
Iowa	P534	221	7.2
Iowa-1f	2223	416	2.6
Marrero-1d	2224	307	5.2
Mauriceville-1d	2226	139	9.3
Northside Planting	8141	126	11.9
Roanoke	P517	303	11.5
Roanoke	P520	128	2.3
Roanoke	P522	308	12.9
Roanoke	P525	463	1.1

(*continued*)

TABLE A1

(Continued)

Strain name	Strain number[a]	Segregants examined	*erg-3* mutation frequency (%)
Spurger	P840	114	9.6
Spurger-7	3202	155	7.1
Sugartown-7	3211	278	2.5
Sweetwater-2	3975	212	9.4
Welsh-1d	2230	496	4.6
Welsh	P509	256	5.1
Welsh	P511	140	6.4
Welsh	P512	561 (240)	0.8 (1.2)
Welsh	P513	421 (370)	0.7 (1.1)
Costa Rica			
Agudas Rd-1	6204	287	3.5
Agudas Rd-1	P3979	203	6.9
Agudas Rd-2	6205	406	7.4
Esterillo Este Rd-2	P3989	209	7.2
Esterillo Este Rd-2	P3992	290	12.4
Esterillo Este Rd-2	P3993	199	23.1
Esterillo Este Rd-2	P3995	333	24.9
Esterillo Este Rd-2	P3996	318	11.6
Esterillo Este Rd-2	P3998	152 (270)	0.6 (2.6)
Esterillo Este	6209	365	16.4
Esterillo Este	P4004	261	24.9
Esterillo Este	P4005	143	16.1
Esterillo Este	P4008	203	7.4
Esterillo Este	P4009	116	11.2
Jaco-1	6201	292	6.8
Jaco-1	P3965	306	8.8
Jaco-1	P3966	126	7.9
Jaco-1[b]	P3968	216	11.6
Jaco-2	6210	301	9.3
Jaco-2	P4015	171	9.3
Jaco-2	P4017	706	8.5
Jaco-2	P4018	205	18.0
Jaco-2	P4019	129	15.5
Jaco-2	P4037	448	4.7
Jaco-2[b]	P4013	359	9.7
Haiti			
Bas Quartier	4707	740	11.5
Berard	4709	448	13.2
Carrefour Dufort	P3427	359	10.3
Leogane	4712	165	7.9
Pescail	4715	329	4.9
India			
Aarey-1	P676	280	3.6
Aarey-1	P677	213	17
Aarey-1	P678	240	4.2
Aarey-1	P679	359	6.4
Aarey-1	P681	526 (74)	0.6 (4.0)
Aarey-1	P685	639	2.3
Aarey-1	2500	280	3.6
Aarey-1g	2712	561 (496)	0.9 (1.4)
Bichpuri-1	P742	128	16.4
Bichpuri-1	P745	212	8
Bichpuri-1	P748	320	5.3
Bichpuri-1	P749	595	3.5
Bichpuri-1	P750	355	2
Bichpuri-1	P754	319	3.4
Dagguluru	P1121	280	2.8

(*continued*)

TABLE A1

(Continued)

Strain name	Strain number[a]	Segregants examined	*erg-3* mutation frequency (%)
Dagguluru	P1122	209	1.4
Dagguluru	P1123	210 (364)	0.9 (2.1)
Dagguluru-1	3361	280	2.8
Golur-1[c]	P334	— (320)	— (0.6)
Golur-1	P335	141 (112)	0.7 (0.9)
Lankala Koderu-1	P1110	690	3.6
Lankala Koderu-1	P1111	231	2.5
Lankala Koderu-1	P1113	307 (168)	1 (3.0)
Lankala Koderu-2	P4329	156	1.3
Madurai	4718	372	5.1
Madurai	P2534	243	4.1
Madurai[b]	P4360	910	1.3
Mallilinatham	4719	74	2.7
Mallilinatham	P2566	93	10.7
Mallilinatham	P2568	171	8.1
Mallilinatham	P2571	197	4.6
Mallilinatham	P4358	107	5.6
Mughalsarai-1	P728	362 (129)	0.3 (0.8)
Mughalsarai-1	P730	358	2.2
Mughalsarai-1	P733	770 (177)	0.5 (1.1)
Mughalsarai-2	P734	418	2.4
Mughalsarai-2	P735	256	2.7
Mughalsarai-2	P739	114	1.7
Mughalsarai-2	P740	264	8.3
Rameshwaram[b]	P4362	362	2.2
Vallancheri	4721	411 (67)	0.5 (3)
Vallancheri	P2562	55	5.4
Vallancheri	P2563	499 (65)	0.6 (1.5)
Vehar-1	P667	271	5.5
Vehar-1	P669	309	3.5
Vehar-1	P672	324	6.5
Venkatavarum	4722	299	1.7
Venkatavarum	P2577	285	2.1
Ivory Coast			
Asikro	4828	242	10.3
Brabadougou	P4291	193	10.9
Brabadougou	P4294	397	4
Brabadougou	P4296	Barren	—
Eremankono	4832	226	7.1
Foro-Foro	4829	693	9.6
Grabiokoko	4831	332	5.7
Grabiokoko	P3593	287	13.2
N'Douci	4836	203	8.4
N'Douci	P3698	120	32.5
N'Douci	P3699	264	10.6
Tiassale	4826	286	4.8
Tiassale	4827	520	2.7
Tiassale	P680	390	9.7
Tiassale	P3682	594	7.1
Tiassale	P3685	193	5.2
Malaya			
Batu Ferringi-1	5359	Barren	—
Bayan Lepas	P2663	191 (361)	0.5 (0.3)
Georgetown-2	P2588	259	13.1
Georgetown-2	P2592	248	1.6
Georgetown-2	P2595	228	3.5

(*continued*)

TABLE A1

(Continued)

Strain name	Strain number[a]	Segregants examined	*erg-3* mutation frequency (%)
Georgetown-4	4724	120	1.7
Georgetown-4	P2607	177	2.8
Georgetown-4	P2608	252	6.3
Georgetown-5	P2610	118	3.4
Georgetown-5	P2611	246	2.8
Georgetown-6	4727	144 (49)	0.7 (2)
Georgetown-6	P2619	176	5.7
Georgetown-6	P2622	Barren	—
Georgetown-6	4728	226	9.2
Sungai Ara	P2668	70	4.3
Sungai Ara	P2672	257	17.1
Sunshine Beach	P2692	163	3.3
Tanjung Asam	P2647	92	4.3
Tanjung Asam	P2649	164	6.7
Tanjung Asam	P2650	449	1.5
Tanjong Tokong	4729	134	8.2
Telok Kumbar	P2640	38	2.6
Telok Kumbar	P2641	448	7.1
Telok Kumbar	P2723	292	2
Mexico			
Chemax	P4109	519	5.2
Chemax	6636	267	12
Chemax	P4120	339	2.9
Chemax	P4122	300	3
Chemax	P4180	71	35.2
Chichen Itza	P4160	258	9.3
Chichen Itza	P4163	324	12.9
Kabah	P4124	282	14.5
Kabah	6637	412	6.8
Kabah	P4126	254	7.9
Kabah	P4130	164	7.3
Sayil	P4150	136	5.9
Pakistan			
Lahore-1b	1825	131	4.6
Lahore	P356	370	1.3
Lahore	P4220	377	5.8
Puerto Rico			
Colonia Paraiso	3694	242	6.6
Colonia Paraiso	P4209	218	12.4
Colonia Paraiso	P4211	187	18.7
South America			
Ile St. Joseph	7553	191	8.9
Maripasoula[b]	6241	659	2.6
Puerto Ayachucho	6233	379	5.5
Rondon	4706	291	9
Thailand			
Khao Eto	6797	244	7
Klong Rangsit no.7	P4217	215	6.5
Venezuela			
Puerto Ayachucho	P4031	319	5.9
Puerto Ayachucho	P4253	260	5.4

Numbers in parentheses refer to ascospores harvested after 45 days. All other values refer to ascospores harvested after 31 days.

[a] Strain numbers preceded by a "P" are from the Perkins collection and those without a prefix are all FGSC numbers.

[b] These strains behave as *mat A*. The *erg-3* mutation frequencies were determined in crosses with *Dp1.3*^{rc} *hph a*.

[c] No ascospores could be harvested at 31 days.

TABLE A2

Double-blind experiments

Strain code	Coon (P0881)		Fred (P0833)		Bayan Lepas (P2663)		T-430-a (Hyg^r) (8609)	
	erg-3 progeny (%)	Strain	*erg-3* progeny (%)	Strain	*erg-3* progeny (%)	Strain	*erg-3* progeny (%)	Strain
A	3.23	Makaba-1 (P3812)	5.45	Aarey-1 (P0678)	2.43	Batu Ferringi-1[b] (P2681)	<0.26	T-430-a
B	0.25	Coon	1.77	Georgetown-2 (P2592)	2.42	Franklin (P4493)	8.17	OR8-1a
C	3.96	Aarey-1 (P0677)	0.36	Fred	6.59	Kabah (P4126)	8.33	OR8-1a
D	0.28	Coon	3.2	Colonia Paraiso (P1291)	0.43	Bayan Lepas	6.92	OR8-1a
E	0.32	Coon	4.38	Welsh-1d (P0507)	7.65	Kabah (P4125)	<0.28	T-430-a
F	7.05	Sungai Ara (P2672)	No perithecia	Florida City[a] (P1448)	No perithecia	Maripasoula[a] (P4088)	<0.24	T-430-a
G	2.86	Bichpuri-1 (P0742)	0.3	Fred	9.58	Jaco-2 (P4017)	<0.5	T-430-a
H	3.53	Homestead-3 (P1470)	0.39	Fred	0.55	Bayan Lepas	9.67	OR8-1a
I	6.39	Fred-2 (P0828)	1.19	Georgetown-4 (P2608)	0.42	Bayan Lepas	6.02	OR8-1a
J	2.10	Klong Rangsit-7 (P4217)	9.36	Groveland-1 (P0438)	No perithecia	Madurai[a] (P4360)	<0.27	T-430-a

Underline indicates crosses with low *erg-3* mutation frequencies (<0.6%) that were predicted to represent the RIP suppressor strains. All other crosses were predicted to represent nonsuppressor strains. See text for additional details.

[a] These strains behaved as *mat A*.

[b] Barren cross.