Dominant Suppression of Repeat-Induced Point Mutation in Neurospora crassa by a Variant Catalytic Subunit of DNA Polymerase-ζ

Ranjan Tamuli and Durgadas P. Kasbekar

Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

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ABSTRACT

Crosses involving the Adiopodoumé strain of Neurospora crassa are defective for repeat-induced point mutation (RIP), a genome defense mechanism of fungi. We show here that the Adiopodoumé strain possesses an incompletely penetrant and variably expressible dominant suppressor of RIP (Srp) that maps to an ~34-kbp genome segment that is ~26 kbp proximal to mat on linkage group II. Gene disruption experiments revealed that Srp is the upr-1 allele of Adiopodoumé (upr-1Ad) that is contained within this segment. The upr-1 gene codes for the catalytic subunit of the translesion DNA polymerase-ζ (Pol-ζ) and it is unusually polymorphic in Neurospora. That the upr-1 gene contains upstream ORFs that overlap with the main ORF is potentially relevant to the incomplete penetrance and variable expressivity of the suppressor. Crosses between heterokaryons that contain upr-1Ad and strains that prevent mating events involving nuclei that contain upr-1Ad yielded no progeny in which RIP had occurred, consistent with the idea that the suppressor encoded by upr-1Ad is diffusible. The potential involvement of the Pol-ζ subunit in two functions, translesion DNA synthesis and RIP regulation, might account for the rapid evolution of its gene in Neurospora.

A

N unusual strain of Neurospora crassa was isolated in the 1950s from Adiopodoumé (a village and research station near Abidjan, Cote D’Ivoire, West Africa), in that it was the only wild-isolated Neurospora strain found harboring active copies of a LINE-like transposable element named Tad (Kinsey and Helber, 1989). All the other wild isolates of Neurospora examined (including those of species other than N. crassa) contained only mutation-inactivated relics of Tad (Kinsey, 1989; Kinsey et al., 1994). No other active transposable element has since been found in Neurospora. Subsequently, the Adiopodoumé strain was identified as one of 7 wild-isolated N. crassa strains (of ~450 screened) possessing the ability to dominantly suppress repeat-induced point mutation (RIP) (Noubissi et al., 2000, 2001; Bhat et al., 2003). RIP is a genome defense process of fungi that occurs during the premeiotic phase of a sexual cross and hypermutates and methylates duplicated DNA sequences, thereby protecting the genome against the proliferation of transposable elements and other parasitic DNA (Cambareri et al., 1989; Selker, 1990; Galagan et al., 2003; Galagan and Selker, 2004). Presumably the dominant RIP suppressor phenotype facilitated Tad’s survival in the Adiopodoumé strain. Screening for the dominant RIP suppressors was done by crossing the wild isolates with strains of the opposite mating type bearing the transgene Dp(erg-3), which duplicates a segment of the erg-3 gene. Crosses of the Dp(erg-3) strains with most wild isolates yielded RIP-induced erg-3 mutant progeny at frequencies in the 2–25% range, but in the crosses with the dominant RIP suppressors (and with a derivative of the Adiopodoumé A strain called T-430-Hyg a) this frequency was consistently <0.5%. The RIP suppressor phenotype also extended to a duplication of the downy gene (Vyas et al., 2006). Presumably it applies generally. Initial mapping studies suggested that the Adiopodoumé strain contains a dominant RIP suppressor (hereafter referred to as Srp for “Suppressor of RIP”) linked to the mating-type locus (mat) on linkage group (LG) II (Bhat et al., 2003). Here we show that Srp is the Adiopodoumé allele of the ultraviolet photoreactivation-1 (upr-1) gene. This gene encodes the catalytic subunit of the translesion DNA polymerase-ζ (Pol-ζ) (Sakai et al., 2002). This finding revealed that at least a subset of upr-1 alleles can act as potential regulators of RIP. A role for upr-1 in RIP regulation could have driven its rapid evolution, as evidenced by the unusually high polymorphism exhibited by this gene in N. crassa (Tamuli et al., 2006).

MATERIALS AND METHODS

Gene and phenotype symbols: Gene symbols are italicized, while phenotype symbols are not. For example, the nonitalicized
symbol Srp\(^{+}\) signifies nonsuppressor phenotype, nonitalicized Srp\(^{-}\) signifies intermediate phenotype, and nonitalicized Srp\(^{±}\) signifies suppressor phenotype.

**General genetic manipulation of N. crassa and the assay for RIP:** Neurospora genetic analysis was essentially as described by Davis and De Serres (1970). The frequency of RIP-induced erg-3 mutant progeny among ascospores harvested at 31 days was used as a measure of RIP efficiency and determined as the proportion of colonies with the erg-3 mutant morphology, as seen under a dissection microscope.

**General strains:** Unless otherwise stated all N. crassa strains were obtained from the Fungal Genetics Stock Center (FGSC, University of Missouri, Kansas City). They included the standard Oak Ridge (OR) strains 74-OR23-1 A (FGSC 987) and OR8-1 a (FGSC 988) and the mutants erg-3 a (FGSC 2725) and erg-3 A (FGSC 3439). The erg-3 mutation confers a female-sterile phenotype. The Round spore (R) strains R A (FGSC 4022) and R a (FGSC 4023) contain an ascus-dominant 20- to 30-kbp deletion that when crossed with the wild type causes unpairing of the \( R^{a} \) allele and its consequent meiotic silencing; consequently, all eight ascospores become round rather than ellipsoid. R also confers a female-sterile phenotype. The auxotrophic strains are arg-1 A (FGSC 325), arg-3 A (FGSC 1068), leu-3 A (FGSC 4002), leu-3 a (FGSC 4003), his-3 A (FGSC 6103), his-3 a (FGSC 7088), his-1 A (FGSC 401), his-1 a (FGSC 402), trp-1 A (FGSC 2093), and trp-1 a (FGSC 2098) and the wild-isolated strain Adiopodoume\(^{A}\) (FGSC 430) and its derivative T-430-Hyg\(^{a}\) (FGSC 8699) that was derived from Adiopodoume\(^{A}\) by replacement of the \( mat^{a} \) idiomorph by transformation with DNA of the \( mat^{a} \) idiomorph (Anderson et al. 2001). Both Adiopodoume\(^{A}\) and T-430-Hyg\(^{a}\) display the dominant RIP suppressor phenotype linked to \( mat^{a} \) on LG II. (Bhat et al. 2003). The translocation strains are \( T(VII \rightarrow II)(H)B^{5} cpe-1 A \) (FGSC 4433) and \( T(VII \rightarrow II)(H)B^{5} cpe-1 a \) (FGSC 4434), in which a segment of VII, extending from \( cpe-1 \) through \( yoa-1 \), is translocated to IR. One-third of the viable progeny from a cross of the translocation with a normal sequence contain a duplication of the translocated segment, designated \( Dp(VII \rightarrow II)(H)B^{5} \). The duplication is stably barren in crosses. The duplication is ~400 kbp in size and serves as an effective dominant suppressor of RIP (Vyas et al. 2006).

The \( Dp(erg-3) \) A and a strains, described by Bhat et al. (2003) and P rankash et al. (1999), contain the transgene \( Dp(erg-3) \) that duplicates a fragment of the LG IIIR gene ergosterol-3 (erg-3) tagged with the bacterial hph gene for resistance to hygromycin. The ectopically duplicated segment serves to target RIP to erg-3. The resulting RIP-induced erg-3 mutant progeny can be scored under a dissection microscope by their distinct colony morphology on Vogel’s-sorbosce agar medium (Noubissi et al. 2000). The \( Dp(dow) \) A and a strains were previously referred to as \( Dp1.5\) dow\(^{a}\) A and a (Vyas et al. 2006). They contain the \( hph \) tagged transgene \( Dp(dow) \) that duplicates a fragment of the LG IIIR gene downy (dow) and targets RIP to dow. The mus-26 (NcRev7) mutant strains were constructed in the laboratory and have been described by Tamuli et al. (2006).

Strains Sad-2 (RIP32) A (RLM 30-12) and Sad-2 (RIP32) a (RLM33-12) (Shiu et al. 2006) were kindly provided by Robert L. Metzenberg (Department of Biology, California State University, Northridge, CA). They were used to construct the heterokaryons \([\text{leu-3 Srp}; \text{Sad-2 a}] + \) (erg-3; \text{his-1 a}) and \([\text{Dp}(H)B^{5})/\text{trp-1}; \text{Sad-2 a}] + \) (erg-3; \text{his-1 a}). These heterokaryons were crossed with the strain \text{Sad-2; Dp(dow) A}\) to construct RIP amplification, molecular markers, and transformation:

Oligonucleotide primers for PCRs were custom purchased from Bioserve (Hyderabad, India). The reaction conditions, other molecular methods, and transformation protocols were as described in Bhat et al. (2004). PCR-based RFLPs were identified to distinguish between the Adiopodoume and OR genomes in the mat-proximal region of LG II (Figure 1B). Supplemental Table 1 lists the primers used to amplify 11 genomic segments (P1–P11) from the mat-proximal sequence of super-contig 7.3, as well as the restriction enzymes that enable one to distinguish between the Adiopodoume and OR alleles of the amplified fragment.

**An OR A-specific DNA insert in molecular marker P7:** The molecular marker P7 is a 1658-bp PCR amplicon when OR A DNA was used as the template, but a smaller fragment was amplified when the template DNA was from the AdiopodoumeA strain.

Figure 1.—(A) Protrophic progeny produced by crossover if Srp\(^{+}\) is proximal to \( mat^{a} \). For class 1 prototrophs: (I) Srp\(^{+}\) A, (II) Srp A, and (III) Srp\(^{-}\) A. For class 2 prototrophs: (I) Srp\(^{+}\) A, (II) Srp\(^{-}\) a, and (III) Srp a. If, instead, Srp\(^{±}\) is distal to \( mat^{a} \), then crossovers in II yield Srp\(^{+}\) a in class 1 and Srp A in class 2 but the products of crossover in I and III remain unaffected. (B) Molecular markers in the \( mat^{a} \) proximal region. The distances (kilobase pairs) between consecutive pairs of markers from the left (\( mat^{a} \)) are 12.1, 12.8, 4.1, 2.7, 4.9, 7.0, 7.0, 5.9, 5.6, 8.5, 66.7, and >1042. (C) Localization of Srp\(^{±}\) to an ~34-kbp region proximal to \( mat^{a} \). Locations of the crossovers that produced the class 1 Srp A prototroph no. 117 and the class 2 Srp a prototroph no. 51 are shown. P2.1 and P2.2 are single-nucleotide polymorphisms (SNPs) in the interval P2–P3 and P8.1 and P8.2 are SNPs in the interval P8–P9.
TABLE 1

<table>
<thead>
<tr>
<th>Cross</th>
<th>erg-3 mutants/progeny examined</th>
<th>% erg-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 117-11 A × Dp(erg-3) a</td>
<td>0/560</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>2. 117-44 A × Dp(erg-3) a</td>
<td>0/200</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>3. 117-66 A × Dp(erg-3) a</td>
<td>1/332</td>
<td>0.3</td>
</tr>
<tr>
<td>4. 117-25 A × Dp(erg-3) a</td>
<td>50/400</td>
<td>12.5</td>
</tr>
<tr>
<td>5. 82-46 a × Dp(erg-3) A</td>
<td>0/262</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>6. 82-51 a × Dp(erg-3) A</td>
<td>0/512</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>7. 82-51-6 a × Dp(erg-3) A</td>
<td>8/900</td>
<td>0.9</td>
</tr>
</tbody>
</table>

RIP Suppression by upr-1st

RESULTS AND DISCUSSION

**Srp shows incomplete penetrance and variable expressivity:** Segregation of the Srp phenotype was examined in 120 F1 progeny from the cross T-430-Hyg a × 74-OR23-1 A by crossing them to Dp(erg-3) strains of the opposite mating type and determining the frequency of erg-3 mutant progeny in the F2. All 65 mat A progeny were Srp+ (erg-3 mutation frequency >1.0%), whereas 51 of the 55 mat a progeny were Srp- (erg-3 mutation frequency <0.5%) and 4 had an intermediate (Srpra) phenotype (erg-3 mutation frequency 0.5–1.0%). These results confirmed the linkage of Srp to mat. That 4/55 mat a but 0/65 mat A progeny showed an intermediate phenotype alerted us to the possibility that occasionally (here, ~7%) Srp might show variable expressivity and confer an Srpra rather than an Srp− phenotype.

Of 733 F1 progeny examined from the cross T-430-Hyg a × leu-3 A, 673 were parental type with respect to leu-3 and mat (452 leu− a and 221 leu-3 A) and 60 (8.2%) were crossover types (32 leu− A and 28 leu-3 a). All the crossover progeny and 42 leu-3 A and 54 leu+ a progeny were tested for their Srp phenotype. The 74 mat A progeny were all Srp+, whereas 64 of the 82 mat a progeny were Srp−, 8 were Srpra, and 10 were Srp−.
Although the 10 Srp+ a progeny could, in principle, have arisen by crossover between Srp and mat, the absence of the complementary Srp- A progeny led us to postulate that they might not be products of cross-over but might reflect an extreme version of the Srp+/− phenotype. That is, Srp might also be incompletely penetrant.

To confirm the variable expressivity and incomplete penetrance of Srp, we tested Srp-homozygous crosses for the production of non-Srp- progeny. The cross T-430-Hyg+ a × Adiopodoumé A is infertile (Anderson et al. 2001; Bhat et al. 2003), but crosses of the Srp a strains T-430-Hyg+ a, 82-46 a, and 82-51 a with the Srp A strains 117-11 A, 117-44 A, and 117-66 A were fertile (see MATERIALS AND METHODS for strain descriptions). Five of 30 and 10 of 18 progeny examined, respectively, from crosses involving T-430-Hyg+ a, or crosses of 117-11 A with 82-46 a or 82-51 a, showed the Srp+/− or Srp+ phenotypes (Table 2). Confirmation of Srp’s incomplete penetrance signaled that the Srp- segregants from a Srp × Srp+ cross need not necessarily be of the Srp+ genotype and cautioned us to use only the Srp- segregants (whose genotype is necessarily Srp) for mapping Srp.

The non-Srp- phenotypes of Srp strains were reversible through crosses. Strains 117-25 A and 82-51-6 a are, respectively, Srp+ and Srp+/−, but derive their LG IL region around mat from the Adiopodoumé background (data not shown; see MATERIALS AND METHODS for strain descriptions). Two of the 20 progeny tested from 117-25 A × 82-51-6 a showed reversion to Srp− (Table 2). Seven other progeny that inherited the mat locus from the Srp+/− parent showed a further conversion to Srp−.

**GENETIC LOCALIZATION OF Srp:** We performed crosses between the strains arg-1 A or arg-3 A and the four leu-3 Srp a strains 398 a, 475 a, 514 a, and 603 a (see MATERIALS AND METHODS) and between leu-3 A and the four Srp arg-3 a strains 33 a, 82 a, 112 a, and 114 a (see MATERIALS AND METHODS). Most progeny from these crosses are auxotrophic for either leucine or arginine, but prototrophs (designated, respectively, as classes 1 and 2 from the two sets of crosses) are produced by crossover between leu-3 and arg-1 (or arg-3), which are, respectively, distal and proximal to mat. Since Srp and mat were closely linked, the auxotrophic markers also bracketed Srp, and the prototrophs were enriched for crossovers between Srp and mat. Depending on whether Srp is proximal or distal to mat, crossover between them yields class 1 and class 2 prototrophs that are, respectively, Srp− A and Srp+ a (Figure 1A) or Srp+ a and Srp− A. Of 81 class 2 prototrophs tested, 80 were parental types (42 Srp+ A + 38 Srp− a) and one, no. 87, was the crossover type, Srp− a (data not shown). This was consistent with Srp being proximal to mat, but also with the alternative model that the prototrophy of no. 87 resulted from a crossover outside mat-Srp and that its Srp− phenotype was due to the incomplete penetrance of Srp.

Of 177 class 1 prototrophs (83 mat a and 94 mat A), 2 (nos. 117 and 141) were Srp− A (data not shown), as would be expected only if Srp was proximal to mat. The
remaining 92 mat A class 1 prototrophs were Srp + and presumably resulted from crossovers proximal to Srp. Of the 83 mat a class 1 prototrophs, 40 were Srp + (data not shown) and were attributed to incomplete penetrance; thus penetrance can occasionally be as low as ~52%.

The genetic interval to which Srp maps is bounded distally by the most proximal of the two crossovers that produced the class 1 Srp - A prototroph no. 117 and 141 and proximally by the most distal of the 38 crossovers that produced the class 2 Srp - a prototrophs (i.e., crossovers in III). The crossovers were mapped with respect to molecular polymorphisms between Adiopodoumé and OR in the mat-proximal region of LG IL (Figure 1B and supplemental Table 1). Figure 1C summarizes this analysis for the crossovers that generated no. 117 and the class 2 Srp - a prototroph no. 51. The remaining 37 class 2 crossovers were proximal to that in no. 51 and the other class 1 crossover was distal to that in no. 117 (data not shown). Srp was localized to the ~34-kbp interval between the SNPs P2.1 and P8.2. Consistent with this localization, prototroph no. 87 was found to have resulted from a crossover from between mat and P1 (data not shown).

Srp is identical with upr-1Δ6: The ~34-kbp Srp interval contains 10 genes (or hypothetical genes) designated (proximal to distal) NCU01942.1–NCU01951.1 (http://www.broad.mit.edu/cgi-bin/annotation/fungi/neurospora_crasa_7). The sequence of the corresponding interval from the Adiopodoumé strain (accession no. DQ387872) revealed at least one nonsynonymous difference between the Adiopodoumé and the OR alleles of five genes (NCU01942.1, NCU01946.1, NCU01947.1, NCU01950.1, and NCU01951.1; supplemental Table 3). The largest candidate gene, NCU01951.1, also known as upr-1, had 118 nucleotide differences between its Adiopodoumé and OR alleles (upr-1Δ6 and upr-1ΔΔ6), including 66 nonsynonymous changes. Crosses homozygous for RIP-induced mutants of upr-1 are RIP competent (Tamuli et al. 2006), but this did not disqualify upr-1Δ6 from being Srp.

If Srp was identical with upr-1Δ6, disruption of upr-1Δ6 should abolish the Srp - phenotype. We used PCR to amplify an ~6-kbp DNA fragment that contained the bacterial hph gene for resistance to hygromycin flanked by 1410 and 1313 bp of sequences from, respectively, the 5' and 3' of upr-1Δ6 (Figure 2, also see MATERIALS AND METHODS). The amplified DNA was transformed into the Adiopodoumé strain by electroporation and transformants were selected on hygromycin medium. In N. crassa, transforming DNA can integrate either by non-homologous end joining into an ectopic site or, less frequently, by homologous integration. We identified three transformants (nos. 9, 21, and 24) in which integration had occurred by homologous recombination, thereby disrupting the upr-1Δ6 allele in the transformed nucleus (Figure 2). The transformants were heterokaryons; that is, they also contained additional untransformed nuclei. The heterokaryons were crossed with OR a, and hygromycin-resistant mat A progeny from these crosses represented homokaryons that contained the disrupted allele, whereas the hygromycin-sensitive mat A progeny were homokaryons with the intact upr-1Δ6 allele. All segregants that inherited the disrupted upr-1Δ6 allele were Srp -, whereas the Srp - or Srp a/Δ- phenotypes were seen only among segregants that inherited the intact upr-1Δ6 allele (Figure 3). Since disruption of upr-1Δ6 abolished the Srp - and Srp a/Δ- phenotypes, we could conclude that Srp is identical with upr-1Δ6.

The mus-26 gene encodes a regulatory subunit of Pol-ζ (Sakai et al. 2003). Homozygous mus-26 mutant crosses are not ordinarily RIP defective (Tamuli et al. 2006). We found that RIP was suppressed in the cross Srp / mus-26 (84) a × mus-26 (27); Dp(erg-3) A (frequency of erg-3 progeny <0.5). Therefore suppression of RIP by upr-1Δ6 is independent of the regulatory subunit.

A difference in dominant suppression of RIP by Srp and that by large chromosome segment duplications: The heterokaryon [(1:3 Srp; Sad-2 a) + (erg-3; his-1 a)] was isolated on the basis of the complementation between its auxotrophic components and used to make
crosses with the strain R; Sad-2; Dp(dow) A. Mating of R; Sad-2; Dp(dow) A with the *leu-3* *Sr*p; Sad-2 a component is nonproductive due to homozygosity for Sad-2 and crosses of R; Sad-2; Dp(dow) A with adventitiously formed erg-3; his-1 a homokaryons also are nonproductive because both *R* and erg-3 confer female sterility. However, the heterokaryon is female fertile and therefore progeny can be obtained from the mating between its erg-3; his-1 a component and R; Sad-2; Dp(dow) A. We examined 120 erg-3 progeny from this cross but none were RIP-induced *dow* mutants. In contrast, the control Sad-2; Dp(dow) A × erg-3 a crosses yielded RIP-induced *dow* mutants among the erg-3 progeny at frequencies typically >8%. These results suggested that the *Sr*p-phenotype of *Sr*p is able to spread to the *Sr*p × *Sr*p ascus of the mosaic perithecium that form in [*Sr*p × *Sr*p] × *Sr*p crosses. (Strictly speaking, since RIP actually occurs in the premeiotic dikaryon that precedes karyogamy, it is more accurate to say the *Sr*p- phenotype can spread into the premeiotic dikaryons that go on to generate productive karyogamies. The term “asci” is used here merely as a matter of convenience.) This result is consistent with the idea that RIP suppression is due to the synthesis of a diffusible, *upr-1* *dow* -encoded, suppressor Pol-ζ subunit.

The ∼400-kbp duplication *Dp(1Bj5)* is an effective dominant suppressor of RIP (Vyas et al. 2006). We constructed the heterokaryon [(*Dp(1Bj5)*); *trp-1; Sad-2 a] + (erg-3; his-1 a)] and crossed it with the R; Sad-2; Dp(dow) A strain. Again, progeny are generated only from the mating of R; Sad-2; Dp(dow) A with the erg-3; his-1 a component of the heterokaryon. Seven of the 70 erg-3 progeny examined were RIP-induced *dow* mutants. Thus, although the RIP suppressor phenotype of *Dp(1Bj5)* is dominant within an ascus, in a mosaic perithecium it did not affect asci that do not contain the *Dp*. That is, the presumed titration of the RIP machinery requires the presence of the *Dp* in the ascus.

Conclusions: We have shown that *Sr*p, the dominant RIP suppressor of the wild-isolated Adiopodoumé strain, is identical with its *upr-1* allele (*upr-1*/*dow*). Although Pol-ζ and the other translesion polymerases (Pol-η, Pol-κ, and Rev1) are dispensable for RIP (Tamuli et al. 2006), a RIP-suppressive Pol-ζ catalytic subunit encoded by *upr-1*/*dow* might be well placed to interfere with the assembly of other factors essential for RIP in Neurospora. RIP suppression by *upr-1*/*dow* did not require the Pol-ζ regulatory subunit encoded by *mus-26*. Recently, Acharya et al. (2006) established that the *Saccharomyces cerevisiae* Rev1 protein binds separately to the catalytic and regulatory subunits of Pol-ζ (i.e., Rev3 and Rev7, respectively), suggesting the possibility of separate Rev1–Rev3 and Rev1–Rev7 complexes in *viv o*. Since Rev1 has been suggested to play a role in assembling Pol-η, Pol-κ, and Pol-ζ at the replication fork in mouse and human cells (Prakash et al. 2005), it is possible that Rev1 could play a role in assembling the Pol-ζ catalytic subunit at replication forks separately from the regulatory subunit encoded by *mus-26*. Presumably, the association of a complex without the *mus-26*-encoded regulatory subunit would not be capable of polymerization but may be capable of inhibiting the association of other factors essential for RIP. The Neurospora Rev1 homolog, encoded by the *mus-42* gene, is located 358 kbp distal to *upr-1* on LG II, which is why we have not yet obtained the rare *mus-42* *upr-1* *dow* crossover type that would enable us to put this idea to a genetic test.

Both *upr-1* *mus-26* and *upr-1* *dow* possess out-of-frame ATG codons that initiate short open reading frames upstream of the main *upr-1* ORF (Tamuli et al. 2006). Similar upstream out-of-frame ORFs (uORFs) are conserved in the yeast and human homologs of *upr-1* (Gibbs et al. 1998). Since uORFs reduce the translational efficiency of the main ORF (Morris and Geralle 2000), it has been suggested that they serve to keep cellular levels of Pol-ζ very low (Lawrence 2002). It is
tempting to speculate that the uORFs might contribute to the variable expressivity and incomplete penetrance of Srp. That is, Srp strains that are otherwise genetically identical might differ in uORF expression and this might generate differences in the amount of the suppressor Pol-Δ translated from the main ORF, thus giving rise to the variable expressivity and incomplete penetrance. That is, the variable expressivity and incomplete penetrance might reflect consequences of differences in uORF activity. If the effective cellular levels of the suppressor subunit decrease to below the threshold required to suppress RIP, then a \textit{upr-1}\textsuperscript{med} strain would mimic the \textit{upr-1} null mutant. Incomplete penetrance of the Srp phenotype allows us to reconcile our observations of RIP suppression with the observations of RIP in other Srp-homozygous crosses (Anderson et al. 2001 and this work). Temporary loss of suppressor function and its partial or complete recovery in subsequent generations might also involve an epigenetic change reminiscent of paramutation in maize or metastable epialleles in other systems (Hollick et al. 1997; Poirier et al. 2002; Rakyan et al. 2002; Chandler and Stam 2004; Della Vedova and Cone 2004) or it can be due to stochastic events during development (Lalucque and Silar 2004). Although we have no experimental evidence as yet, it is conceivable that Srp strains are effectively heterokaryotic and express \textit{upr-1}\textsuperscript{med} in only a subset of nuclei and epigenetically switch it off in other nuclei. Among the progeny from crosses parented by such a heterokaryon, those inheriting the epigenetically silenced \textit{upr-1}\textsuperscript{med} gene would be phenotypically Srp\textsuperscript{+}. The range in \textit{Srps} penetrance seen in this work (52–100%) might reflect the range in the proportion of nuclei in which \textit{upr-1}\textsuperscript{med} is silenced. In sum, the rapidly evolving \textit{upr-1} gene (Tamuli et al. 2006) might represent the key to solve the riddle of \textit{Tads} survival in the Adiopodoumé strain, wrapped in the mystery of incompletely penetrant dominant RIP suppressors, inside the enigma of RIP regulation.

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**LITERATURE CITED**


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