
Translesion DNA polymerases Pol ζ , Pol η , Pol ι , Pol κ and Rev1 are not essential for repeat-induced point mutation in *Neurospora crassa*[†]

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Pol ζ , Pol η , Pol ι , Pol κ and Rev1 are specialized DNA polymerases that are able to synthesize DNA across a damaged template. DNA synthesis by such translesion polymerases can be mutagenic due to the miscoding nature of most damaged nucleotides. In fact, many mutational and hypermutational processes in systems ranging from yeast to mammals have been traced to the activity of such polymerases. We show however, that the translesion polymerases are dispensable for repeat-induced point mutation (RIP) in *Neurospora crassa*. Additionally, we demonstrate that the *upr-1* gene, which encodes the catalytic subunit of Pol ζ , is a highly polymorphic locus in *Neurospora*.

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1. Introduction

Specialized DNA polymerases called translesion polymerases are among the major determinants of spontaneous and DNA damage-induced mutation in both prokaryotes and eukaryotes (Livneh 2001). The classical replicative DNA polymerases can synthesize DNA with remarkable efficiency and fidelity in a template-dependent fashion but they are unable to replicate through a DNA lesion. DNA damage can be induced by a variety of intrinsic and extrinsic mutagens including oxygen free radicals, radiation and alkylating agents, and if a damaged nucleotide escapes repair mechanisms that ordinarily remove it and restore the original sequence, it can present a block to the replicative polymerase. In current models for DNA replication a sliding clamp of accessory proteins forms a ring around the replicating DNA, close to the primer-template junction, and the replicative and translesion DNA polymerases are tethered to this ring (Sabbioneda et al 2005). The replicating DNA passes through the central hole of the ring. The sliding clamp has been likened to a “tool belt”

with the polymerases as the attached ‘tools’. When a lesion at the replication fork causes the replicative polymerase to stall, the replication machinery switches to engage a translesion polymerase to replicate a few bases through the lesion and then switches back to re-engage the replicative polymerase and resume normal replication.

Lesion bypass by translesion polymerases can be mutagenic, due to the miscoding nature of most damaged nucleotides; or it can be non-mutagenic, because the polymerases are tailored to incorporate the ‘correct’ nucleotide opposite a damaged base. For example, inactivation of the DNA polymerase zeta (Pol ζ) in the yeast *Saccharomyces cerevisiae* causes a dramatic decrease in the frequency of both spontaneous and induced mutations (Lemontt 1979; Quah et al 1980; Datta and Jinks-Robertson 1995; Holbeck and Strathern 1997; Kozmin et al 2003; Gibbs et al 2005; Zhang et al 2006). This indicates that translesion replication by Pol ζ is mutagenic. Indeed the *S. cerevisiae* *REV3* and *REV7* genes, that encode the structural and accessory subunits of Pol ζ , were first defined by mutants obtained in screens for reduced

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Abbreviations used: NER, Nucleotide excision repair; ORF, open reading frame; RIP, repeat-induced point mutation; uORFs, upstream out-of-frame ORFs.

[†]Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession Nos DQ 231523, DQ 231524, DQ 235021, DQ 235525 - DQ 235541, DQ 240287, DQ 240288, DQ 354228, DQ 354235 - DQ 354237, DQ 386416 - DQ 386422, DQ 387872, DQ494492 - DQ494503 and DQ417211 - DQ417220.

UV-induced reversion of an *arg4-17* ochre allele. *In vitro*, Pol ζ functions as a “mispair extender”, that is, it can efficiently extend from primer-terminal mismatches containing template lesions and it likely functions in the mutagenic replication of damaged DNA by extending from nucleotides inserted opposite lesions by other polymerases (Johnson *et al* 2000; Haracska *et al* 2003). Down-regulation of Pol ζ in mouse fibroblasts decreased UV-induced mutations in the *hprt* gene (Diaz *et al* 2003) and in human B cells *in vitro* it decreased somatic hypermutation of immunoglobulin genes (Zan *et al* 2001). The yeast *REV1* gene, defined by a mutant with a phenotype similar to that of *rev3* and *rev7*, encodes a polymerase with dCMP transferase activity. Pol ζ belongs to the B-family of DNA polymerases whereas Rev1 polymerase belongs to the Y-family. For reviews on Pol ζ and Rev1 see Lawrence (2002) and Murakumo (2002).

Inactivation in yeast of another Y-family translesion polymerase, DNA polymerase eta (Pol η), leads to an increase in the frequency of UV-induced mutations. This indicates that the replication of UV-induced lesions by Pol η is not mutagenic. *In vitro*, Pol η has the ability to accurately replicate through a thymine dimer, a common UV-induced lesion, but it shows a relatively low fidelity when replicating undamaged DNA. In humans, defects in hRAD30A the structural gene for Pol η , are responsible for a cancer predisposition disorder, the variant form of xeroderma pigmentosum (XP-V). A paralogous gene, hRAD30B, encodes DNA polymerase iota (Pol ι), which is a very low fidelity polymerase. Pol ζ and Pol ι act sequentially to bypass DNA lesions: Pol ι incorporates correct or incorrect nucleotides opposite DNA lesions, and Pol ζ extends from the mispair (Johnson *et al* 2000). Interestingly, human Pol ι misincorporates dGTP when opposite a template base T about ten times more efficiently than dATP. Thus Pol ι may have evolved to correct a potential mutagenic T in a template, e.g. in a T:G mismatch, where the thymine residue was formed by deamination of 5-methylcytosine (Bebenek *et al* 2001). See McDonald *et al* (2001) for a review on Pol η and Pol ι . Human polymerase kappa (Pol κ) is encoded by the homologue of the *Escherichia coli* *DinB* gene for DNA pol IV. Pol κ also can extend from mismatched base pairs on undamaged and damaged DNAs, but does so with less efficiency than Pol ζ (Haracska *et al* 2002; Washington *et al* 2002). Pol κ -deficient mouse cells have substantially reduced levels of nucleotide excision repair (NER) of UV damage, thus providing evidence for an unexpected role for Pol κ in mammalian NER (Ogi and Lehmann 2006).

Given that translesion polymerases underlie mutational and hypermutational processes in systems as diverse as yeast and mammals we asked whether they have a role in repeat-induced point mutation (RIP), a mutational process of fungi. RIP induces multiple G:C to A:T mutations in duplicated DNA sequences during the premeiosis of a sexual cross

(Cambareri *et al* 1989; Selker 1990). In *Neurospora crassa* RIP serves as a genome defense process that protects the genome against the proliferation of transposable elements and other parasitic DNA (Galagan and Selker 2004). The *N. crassa* homologues of *S. cerevisiae* *REV1*, *REV3* and *REV7* are *mus-42*, *upr-1* and *mus-26*, respectively and the corresponding Pol ζ and Rev1 mutants have been shown to reduce UV-induced mutagenesis (Sakai *et al* 2002, 2003). Analysis of the *N. crassa* genome sequence has led to the identification of homologues for Pol η , Pol ι and Pol κ (Galagan *et al* 2003; Borkovich *et al* 2004). We report here the construction of *N. crassa* Pol η , Pol ι and Pol κ mutants by RIP and their use, together with the Pol ζ and Rev1 mutants, to explore whether the translesion polymerases are essential for RIP. We have also sequenced the *upr-1* locus of several *Neurospora* strains and found that it is highly polymorphic.

2. Materials and Methods

2.1 Strains

The following strains were obtained from the Fungal Genetics Stock Center (FGSC), University of Missouri, Kansas City, MO 64110, USA: The *N. tetrasperma* strain 85 *A* and 85 *a*. The *N. crassa* strains of the standard Oak Ridge background 74-OR23-1 *A* (FGSC 987) and OR8-1 *a* (FGSC 988); the *N. crassa* wild-isolated strains Adiopodoumé *A* (FGSC 430), Adiopodoumé -7 (P4305), Dagguluru (P3360), Makaba-2 (P3816), Golur (P0334), Colonia Paraiso (P4212), Franklin (P4467), Coon (P0881) and Fred (P0833). The *N. crassa* RIP5A strain, a RIP-induced mutant of *upr-1* (Sakai *et al* 2003), was kindly provided by Hirokazu Inoue (Saitama University, Japan).

The *Dp1.3^{ec} hph A* and *a* strains have been described (Prakash *et al* 1999; Bhat *et al* 2003). They contain the transgene *Dp1.3^{ec} hph*, tagged with the bacterial *hph* gene for hygromycin-resistance that duplicates a fragment of the *erg-3* gene and thus targets RIP to *erg-3* during a cross. RIP-induced *erg-3* mutant progeny are easily scored by their distinct colony morphology on Vogel's-sorbose agar medium (Noubissi *et al* 2000).

2.2 Growth, crosses, ascospore collection and scoring of *erg-3* mutants

Crossing and maintenance of *Neurospora* strains were essentially as described by Davis and De Serres (1970). Crosses were performed by confrontation between mycelia inoculated as plugs on synthetic crossing medium in petri dishes. Ascospores began to be shot within 16–18 days and were harvested by washing the lids with ~ 1 ml water. The

frequency of *erg-3* mutant progeny was determined as the proportion of colonies with the mutant morphology, as seen under a dissection microscope. In this article the frequency of *erg-3* mutant progeny is used as a measure of RIP efficiency. It is known that the frequency of RIP increases with the age of the cross (Singer *et al* 1995). Therefore the *erg-3* mutation frequencies were determined in ascospores harvested at 31 days, by which time RIP frequencies have plateaued.

2.3 PCR amplification, molecular markers, and transformation

PCRs were performed using custom oligonucleotide primers purchased from Bioserve (Hyderabad, India). The reaction conditions used, other molecular methods and transformation protocols were essentially the same as previously described (Bhat *et al* 2004).

2.4 RIP-induced mutants in *upr-1*, *mus-26*, or *mus-42*

RIP-induced mutants of *upr-1*, *mus-26* or *mus-42* were generated by Sakai *et al* (2002, 2003). We constructed additional mutants by the same approach. A 1531 bp fragment of *upr-1* was PCR amplified using the primers P3F 5' CCTGTACGGCTGTAAGTACC and P3R 5' GACGGGAAAGTTGATCGTAC. Fragments of 807 bp from *mus-26* and 1427 bp from *mus-42* were amplified using, respectively, the primers nrev7F and nrev7B; and nrev1F and nrev1B (Sakai *et al* 2003). The amplified fragments were cloned into the plasmid vector pCSN44 and transformed by electroporation into conidia of the strain *his-3 A* (FGSC 6103) by previously described methods (Bhat *et al* 2004). From crosses made with the transformants we obtained four *upr-1*, three *mus-26* and four *mus-42* mutant progeny. Partial sequencing of the endogenous loci confirmed that the mutant alleles contained multiple RIP mutations, including nonsense mutations (accession numbers: DQ 235525 - DQ 235541). Based on this we infer that the mutants were null. We also confirmed that the *upr-1*, *mus-26*, and *mus-42* mutants generated by us showed a UV-sensitive phenotype similar to one described previously by Sakai *et al* (2002, 2003). These mutants were used to construct the strains *upr-1*; *Dp1.3^{ec} hph*, *mus-26*; *Dp1.3^{ec} hph*; *mus-42*; *Dp1.3^{ec} hph*, and *upr-1^{Ad}*; *mus-26* used in the crosses summarized in table 1.

2.5 RIP-induced mutants in *polh*, *poli* and *polk*

In the sequenced *N. crassa* genome, the genes NCU 1936.1 (*polh*), NCU 6757.1 (*poli*) and NCU 2457.1 (*polk*) were identified as the homologues coding for Pol η , Pol ι and Pol κ (Borkovich *et al* 2004). The following pairs

Table 1. RIP in crosses mutant for *upr-1*, *mus-26*, *polh*, *poli*, *polk* and *mus-42*.

Cross	<i>erg-3</i> progeny (N) %
<i>upr-1</i> (94-26); <i>Dp1.3^{ec} hph a</i> x <i>OR A</i>	41.1 (548)
<i>upr-1</i> (52-34); <i>Dp1.3^{ec} hph A</i> x <i>OR a</i>	8.0 (500)
<i>upr-1</i> (94-26); <i>Dp1.3^{ec} hph a</i> x <i>upr-1</i> (34) <i>A</i>	24.9 (639)
<i>upr-1</i> (52-34); <i>Dp1.3^{ec} hph A</i> x <i>upr-1</i> (94) <i>a</i>	3.2 (526)
<i>mus-26</i> (41-7); <i>Dp1.3^{ec} hph</i> ; <i>a</i> x <i>OR A</i>	21.7 (600)
<i>mus-26</i> (3B-14); <i>Dp1.3^{ec} hph a</i> x <i>OR A</i>	18.5 (400)
<i>mus-26</i> (12B-27); <i>Dp1.3^{ec} hph</i> ; <i>A</i> x <i>OR a</i>	43.8 (290)
<i>mus-26</i> (41-7); <i>Dp1.3^{ec} hph</i> ; <i>a</i> x <i>mus-26</i> (12B) <i>A</i>	30.5 (180)
<i>mus-26</i> (3B-14); <i>Dp1.3^{ec} hph a</i> x <i>mus-26</i> (12B) <i>A</i>	17.7 (300)
<i>polh</i> (43-6); <i>Dp1.3^{ec} hph</i> ; <i>a</i> x <i>OR A</i>	30.0 (200)
<i>polh</i> (43-6); <i>Dp1.3^{ec} hph</i> ; <i>a</i> x <i>polh</i> (43-27) <i>A</i>	10.3 (379)
<i>poli</i> (94-6); <i>Dp1.3^{ec} hph</i> ; <i>A</i> x <i>OR a</i>	49.2 (560)
<i>poli</i> (94-6); <i>Dp1.3^{ec} hph</i> ; <i>A</i> x <i>poli</i> (94) <i>a</i>	36.1 (205)
<i>poli</i> <i>Dp1.3^{ec} hph</i> ; <i>a</i> (140-57) x <i>OR A</i>	40.9 (220)
<i>poli</i> <i>Dp1.3^{ec} hph</i> ; <i>a</i> (140-57) x <i>poli</i> (140) <i>A</i>	39.3 (393)
<i>polk</i> (120-10); <i>Dp1.3^{ec} hph</i> ; <i>A</i> x <i>OR a</i>	29.3 (290)
<i>polk</i> (120-10); <i>Dp1.3^{ec} hph</i> ; <i>A</i> x <i>polk</i> (120) <i>a</i>	25.9 (613)
<i>polk</i> (134-31); <i>Dp1.3^{ec} hph</i> ; <i>a</i> x <i>OR A</i>	38.0 (200)
<i>polk</i> (134-31); <i>Dp1.3^{ec} hph</i> ; <i>a</i> x <i>polk</i> (134) <i>A</i>	43.2 (220)
<i>mus-42</i> (51-6); <i>Dp1.3^{ec} hph A</i> x <i>OR a</i>	20.0 (500)
<i>mus-42</i> (51-45); <i>Dp1.3^{ec} hph A</i> x <i>OR a</i>	37.5 (500)
<i>mus-42</i> (51-45); <i>Dp1.3^{ec} hph A</i> x <i>mus-42</i> (31) <i>a</i>	30.0 (500)
<i>mus-42</i> ; <i>mus-26</i> (31); <i>Dp1.3^{ec} hph A</i> x <i>OR a</i>	14.7 (150)
<i>mus-42</i> ; <i>mus-26</i> (31); <i>Dp1.3^{ec} hph A</i> x <i>mus-42</i> ; <i>mus-26</i> (15) <i>a</i>	34.3 (300)
<i>upr-1</i> ; <i>Dp1.3^{ec} hph</i> ; <i>polk</i> (52-6) <i>A</i> x <i>OR a</i>	8.0 (200)
<i>upr-1</i> ; <i>Dp1.3^{ec} hph</i> ; <i>polk</i> (52-6) <i>A</i> x <i>upr-1</i> ; <i>Dp1.3^{ec} hph</i> ; <i>polk</i> (94-43) <i>a</i>	11.4 (220)
<i>mus-26</i> ; <i>polk</i> ; <i>Dp1.3^{ec} hph</i> (B-31-3) <i>a</i> x <i>OR A</i>	12.1 (214)
<i>mus-26</i> ; <i>polk</i> ; <i>Dp1.3^{ec} hph</i> (B-31-3) <i>a</i> x <i>mus-26</i> ; <i>polk</i> (A-31-25) <i>A</i>	13.6 (176)
<i>mus-42</i> ; <i>polk</i> ; <i>Dp1.3^{ec} hph</i> (B-31-10) <i>A</i> x <i>OR a</i>	16.3 (300)
<i>mus-42</i> ; <i>polk</i> ; <i>Dp1.3^{ec} hph</i> (B-31-10) <i>A</i> x <i>mus-42</i> ; <i>polk</i> (A-3-22) <i>a</i>	12.9 (240)

The only RIP-defective recessive mutant known so far is *rid-1*. Crosses homozygous mutant in *rid-1* produce no RIP-induced *erg-3* progeny (typically expressed as <0.5%, that is, no *erg-3* colonies out of > 200 examined). Thus none of the crosses in this table show a comparable RIP-defect.

of oligonucleotide primers were used to PCR amplify fragments of these genes: polh F 5' GCA TGA AGC GTT CAC GGT TC and polh R 5' CAG AGT CAA CCG TCG CTG TC; poli F 5' CGA TCG AGT CCA TGT TCG TC and poli R 5' GGT GCA AGA ACT GGT CTG AC; and polk F 5' CCT TAT TGC ACA GCT GGA GC and polk R 5' GGA TGT AGT AGC ATG CCG TC. The PCRs resulted in the amplification of a 1523 bp fragment of *polh*, a 1622 bp of *poli* and a 1561 bp fragment of *polk*. These fragments were cloned into the *EcoRV* site of pCSN44 and the resulting plasmids were transformed into *N. crassa* as described by Bhat *et al* (2004). From crosses homozygous for one or the other amplified fragment we obtained hygromycin-sensitive progeny, to enrich for progeny bearing a potentially RIP-induced mutant of the corresponding target gene. By sequencing the endogenous *polh*, *poli* or *polk* loci with primers flanking the duplicated segments we verified that all the mutants contain RIP-induced sequences alterations including at least some that introduced nonsense mutations. Based on this we infer that the mutants were null. Accession numbers for the partial sequences confirming the *polh* mutants are DQ494492-DQ494503 and for the *poli* and *polk* mutants DQ417211-20. The *polh*, *poli* or *polk* mutants were used to make the crosses described in table 1.

2.6 Strains deposited with the FGSC

The following strains constructed as described above have been deposited with the FGSC (numbers in parenthesis indicate the allele number and those in the box brackets indicate the FGSC strain number): *upr-1* (94) *his-3 a* [10139], *upr-1* (52) *his-3 A* [10140], *mus-26* (3B) *his-3 a* [10141], *mus-26* (12B) *his-3 A* [10142], *polh* (43) *a* [10143], *polh* (43-27) *A* [10144], *poli* (94) *a* [10145], *poli* (140) *A* [10146], *polk* (120) *a* [10147], *polk* (134) *A* [10148], *mus-42* (51) *his-3 A* [10149] and *mus-42* (31) *his-3 a* [10150].

3. Results and discussion

3.1 *Pol ζ, Pol η, Pol ι, Pol κ and Rev1 are dispensable for RIP*

We used the RIP-induced mutant alleles of *upr-1*, *mus-26* or *mus-42* generated by Sakai *et al* (2002, 2003), as well as additional mutants that were generated by us (see §2) to examine RIP frequencies in crosses homozygous for *upr-1*, *mus-26* or *mus-42* mutants. The results of these crosses are summarized in table 1. As can be seen in the table both the heterozygous and homozygous mutant crosses are about equally competent for RIP. Crosses doubly mutant for *mus-26* and *mus-42* also were not defective for RIP. These results

indicate that individually and collectively Pol ζ and Rev1 are dispensable for RIP.

We constructed RIP-induced mutants in the *polh*, *poli* and *polk* genes (see §2). As shown in the table 1, crosses homozygous mutant in these three genes also were not defective for RIP. These results allow us to conclude that Pol η, Pol ι and Pol κ also are not essential for RIP.

3.2 Polymorphisms at *upr-1*

The Adiopodoumé A strain has a dominant RIP suppressor phenotype that is linked to *mat* on LG IL (Bhat *et al* 2003). In other studies we mapped the dominant RIP suppressor locus (*Srp*) to a ~34 kb genomic segment that is ~26 kb proximal to *mat* (Ranjan Tamuli and D P Kasbekar, unpublished results). This segment contains the *upr-1* gene that encodes the 1926 amino acid residues Pol ζ catalytic subunit. This finding prompted us to compare the sequence of the *upr-1* allele of the Adiopodoumé strain (*upr-1^{Ad}*, accession number DQ 23502.1) with that of the standard laboratory Oak Ridge (OR) allele (*upr-1^{ORA}*), and also to test for a RIP-defect in *upr-1* homozygous crosses. The two alleles share identical introns of 101 bases but their coding sequences differ at 118 positions, including 66 non-synonymous changes. Both the *upr-1^{Ad}* and *upr-1^{ORA}* alleles possess out-of-frame ATG codons that initiate open reading frames (ORFs) upstream of the main ORF and that terminate within the main ORF (figure 2). The ATG codon of the main ORF (designated In3) has 8/10 matches with the consensus Kozak sequence for translation initiation in *Neurospora* (Bruchez *et al* 1993). Another ATG codon (In2), located 29 bases upstream of In3, initiates an ORF of 14 amino acid residues and shares 6/10 matches with the consensus. In2 and In3 are present in both the OR and Adiopodoumé *upr-1* alleles. In addition, *upr-1^{Ad}* possesses a third upstream ATG codon (In1) that is in frame with In2 and initiates an ORF of 33 amino acid residues and has 4/10 matches with the consensus sequence. The ORF initiated by In1 is absent from the OR allele because of a single nucleotide polymorphism that produces a TAA stop codon upstream of In2. Similar upstream out-of-frame ORFs (uORFs) are conserved in the yeast and human homologues of *upr-1* (Gibbs *et al* 1998). Since uORFs reduce the translational efficiency of the main ORF (Morris and Geballe 2000), it has been suggested that the cellular levels of Pol ζ may in general be very low (Lawrence 2002). Experiments to determine whether or not *Srp* is identical with the *upr-1^{Ad}* allele are currently underway in our laboratory.

We also determined the sequence of the *upr-1* allele of the *N. tetrasperma* wild-type strain 85A (*upr-1^{85A}*, accession number DQ 231524). The *upr-1^{85A}* and *upr-1^{ORA}* alleles differed at three bases in the intron and at 118 positions in the coding sequences, including 50 non-synonymous

changes. Thus, the divergence of the coding sequences of the Adiopodoumé and OR alleles in *N. crassa* was comparable with the interspecies divergence between *N. crassa* OR and *N. tetrasperma* (figure 1). We also determined the *upr-1* gene

sequences of eight other *N. crassa* wild-isolates [accession numbers: Adiopodoumé-7 (DQ 354228), Makaba-2 (DQ 386417), Coon (DQ 386420), Dagguluru (DQ 386416), Franklin (DQ 386419), Fred (DQ 386421), Golur-1

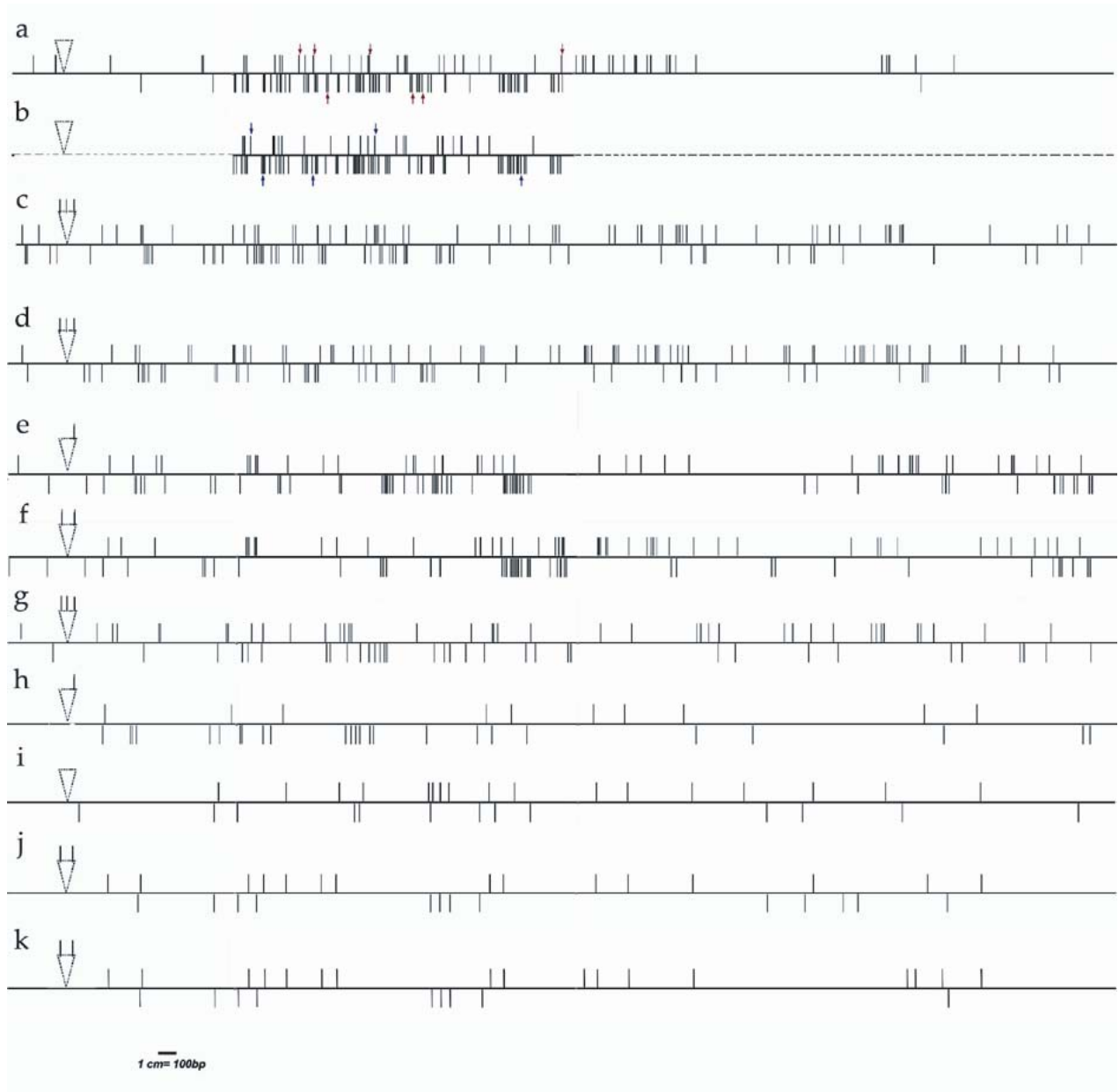


Figure 1. Sequence differences in *upr-1* alleles of different strains relative to the OR sequence. Synonymous and non-synonymous are shown as tick marks, respectively, above and below the corresponding lines. The triangles represent the 101 base intron sequences. **(a)** Adiopodoumé -1 (FGSC 430), **(b)** Adiopodoumé -7 (P4305), **(c)** *N. tetrasperma* 85 a, **(d)** *N. tetrasperma* 85 A, **(e)** Makaba-2 (P3816), **(f)** Dagguluru (P3360), **(g)** Golur (P0334), **(h)** Colonia Paraiso (P4212), **(i)** Franklin (P4467), **(j)** Fred (P0833) and **(k)** Coon (P0881). For the *upr-1* allele of Adiopodoumé -7 only the intron and a 1830 bp segment was sequenced. The unsequenced portion of the ORF is indicated by the dotted line. The arrows in **(a)** and **(b)** identify sequence changes that are specific to the Adiopodoumé -1 and Adiopodoumé -7 strains, respectively. The two strains were identical at all the other sites in the 1830 bp segment.

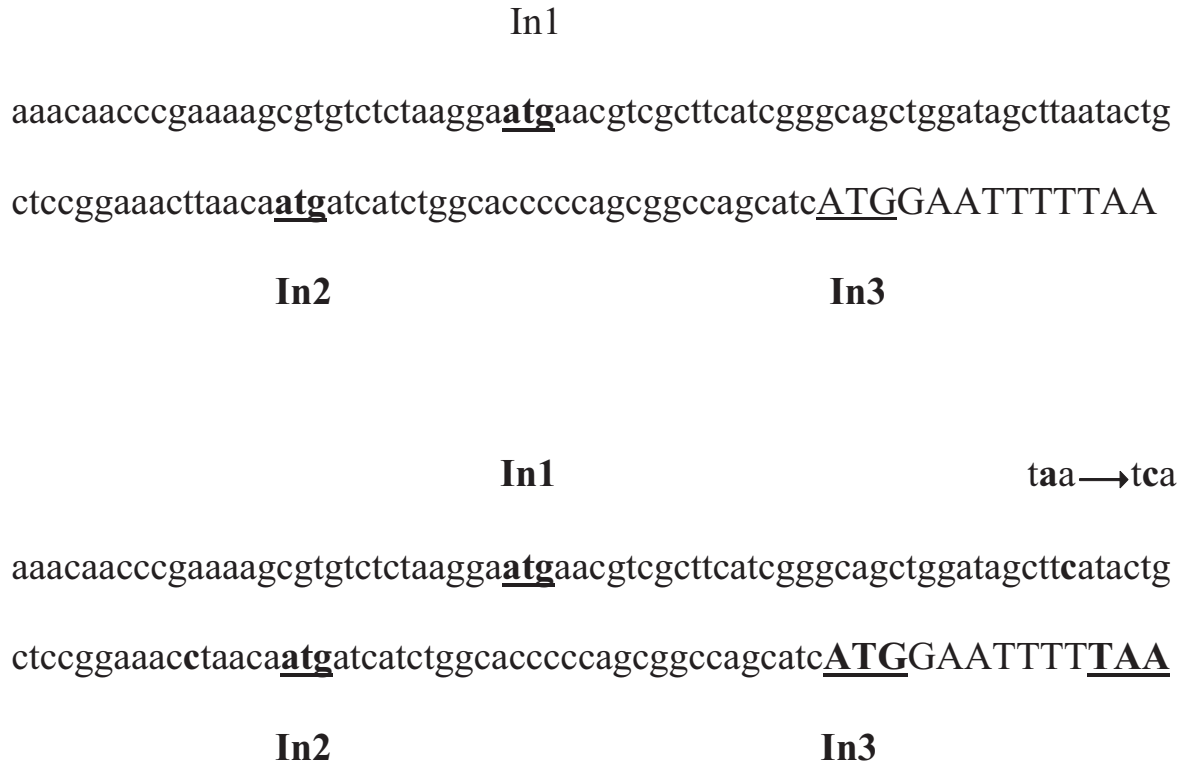


Figure 2. The upstream ORFs of the *upr-1* alleles of OR A (top) and Adiopodoumé -1 (bottom). The main ORF is shown in upper case and the upstream ORFs are in lower case. A stop codon (TAA) in the OR A allele is altered to a TCA in Adiopodoumé -1 allele (affected base shown in bold) that codes for serine. Another synonymous alteration (T → C) also is shown.

(DQ 386422) and Colonia Paraiso (DQ 386418)] and of the *N. tetrasperma* strain 85a (DQ 231523). All showed substantial divergence from the *upr-1^{ORA}* allele sequence. In fact, no two strains examined shared the same *upr-1* allele (figure 1).

For comparison, we also sequenced the *mus-26* alleles of *N. tetrasperma* 85A and *N. crassa* Adiopodoumé (accession numbers: DQ 240287, DQ 240288) and the *mus-42* alleles of *N. tetrasperma* 85A and 85 a, and *N. crassa* Adiopodoumé (accession numbers: DQ 354236, DQ 354237, DQ 354235), but did not find any evidence for an anomalously high intraspecies sequence divergence in them (data not shown). These results demonstrate that the Pol ζ catalytic subunit is unusually polymorphic in *Neurospora*.

4. Conclusions

We have shown that *N. crassa* strains null for any of the translesion polymerases Pol ζ, Pol η, Pol ι, Pol κ and Rev1 are viable, fertile and can support RIP in homozygous

mutant crosses. Additionally, we found evidence for an unusually high polymorphism at the *upr-1* locus.

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