The 3'→5' exonucleases of both DNA polymerases δ and ε participate in correcting errors of DNA replication in *Saccharomyces cerevisiae*

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**Abstract.** DNA polymerases II (δ) and III (ε) are the only nuclear DNA polymerases known to possess an intrinsic 3'→5' exonuclease in *Saccharomyces cerevisiae*. We have investigated the spontaneous mutator phenotypes of DNA polymerase δ and ε 3'→5' exonuclease-deficient mutants, *pol3-01* and *pol2-4*, respectively. *pol3-01* and *pol2-4* increased spontaneous mutation rates by factors of the order of 10² and 10¹, respectively, measured as *URA3* forward mutation and *hisT-2* reversion. Surprisingly, a double mutant *pol2-4* *pol3-01* haploid was inviable. This was probably due to accumulation of unedited errors, since a *pol2-4/pol2-4* *pol3-01/pol3-01* diploid was viable, with the spontaneous *hisT-2* reversion rate increased by about 2 x 10³-fold. Analysis of mutation rates of double mutants indicated that the 3'→5' exonucleases of DNA polymerases δ and ε can act competitively and that, like the 3'-5' exonuclease of DNA polymerase J, the 3'→5' exonuclease of DNA polymerase ε acts in series with the *PMS1* mismatch correction system. Mutation of conserved 3'→5' exonuclease active site residues of the *S. cerevisiae* DNA polymerases δ or ε reduces the ratio of exonuclease:polymerase activities of the partially purified enzymes by at least 100-fold, and causes a spontaneous mutator phenotype with no concomitant cell growth defect (Simon et al. 1991; Morrison et al. 1993). The DNA polymerases δ and ε therefore appear to be the only known nuclear DNA polymerases capable of performing 3'→5' exonucleolytic editing.

**Key words:** DNA polymerases ε and δ – 3'→5' Exonuclease – Replication errors – Spontaneous mutations – *Saccharomyces cerevisiae*

**Introduction**

Prokaryotic DNA polymerases possess a tightly associated 3'→5' exonuclease whose role is to edit out incorrectly inserted nucleotides and thus enhance accuracy of DNA replication (Echols and Goodman 1991). In eukaryotes, as exemplified by the yeast *Saccharomyces cerevisiae*, five nuclear DNA polymerases have been observed or predicted: DNA polymerases I (α), II (δ), III (ε), IV (β) and the putative *REV3* DNA polymerases (Wang 1991; Morrison and Sugino 1993). Current evidence suggests that only DNA polymerases α, δ and ε participate in DNA replication (Morrison and Sugino 1993). Purified DNA polymerases α and δ both possess a tightly associated 3'→5' exonuclease proofreading activity, while five active site residues of an evolutionarily conserved 3'→5' exonuclease domain have been identified in the N-terminal regions of their catalytic subunits (Morrison et al. 1991; Simon et al. 1991). The human DNA polymerase α catalytic subunit is devoid of 3'-5' exonuclease activity (Copeland and Wang 1991), as is the yeast DNA polymerase α holoenzyme (Kunkel et al. 1989). DNA polymerase β of higher eukaryotes lacks both the conserved 3'→5' exonuclease active site residues (Morrison et al. 1991; Morrison and Sugino 1993). The human DNA polymerase α catalytic subunit is devoid of 3'→5' exonuclease activity (Copeland and Wang 1991), as is the yeast DNA polymerase α holoenzyme (Kunkel et al. 1989). DNA polymerase β of higher eukaryotes lacks both the conserved 3'→5' exonuclease domain and an intrinsic 3'→5' exonuclease activity (Matsukage et al. 1987; Linn 1991); although a nuclease, exonuclease V, is sometimes associated with DNA polymerase β, it does not have the properties of an editing exonuclease (Linn 1991). DNA polymerases δ and ε therefore appear to be the only known nuclear DNA polymerases capable of performing 3'→5' exonucleolytic editing.

Mutation of conserved 3'→5' exonuclease active site residues of the *S. cerevisiae* DNA polymerases δ or ε reduces the ratio of exonuclease:polymerase activities of the partially purified enzymes by at least 100-fold, and causes a spontaneous mutator phenotype with no concomitant cell growth defect (Simon et al. 1991; Morrison et al. 1993, 1991). The mutation rate increase is about 130- to 400-fold in the case of *pol3* (Simon et al. 1991; Morrison et al. 1993). The DNA polymerase δ 3'→5' exonuclease corrects a broad spectrum of single-base mutations and acts in series with the *PMS1* mismatch correction system. Muta-
correction system so that their effects are multiplied (Morrison et al. 1993). Such data provide evidence for a proofreading role of the 3′→5′ exonuclease of DNA polymerase δ in vivo.

Based on an examination of epistatic relationships, we propose here that the DNA polymerase ε 3′→5′ exonuclease also participates in proofreading in vivo. The total contribution of both 3′→5′ exonucleases of DNA polymerases δ and ε to the reduction of spontaneous mutations appears to be of the order of 10^4-fold, and a haploid deficient in both activities is inviable. We also compare the mutational spectra of the 3′→5′ exonuclease-deficient DNA polymerases δ and ε mutants, and discuss the results in terms of the roles of the DNA polymerases in DNA replication.

Materials and methods

Yeast strains. Yeast strains were: CG379 (MATa ade5-1 his7-2 leu2-3,-112 trp1-289 ura3-52), CG378 (MATα ade5-1 can1-leu2-3,-112 trp1-289 ura3-52), AMY360-8D (MATα pol3-01 his7-2 ade5-1 leu2-3,-112 trp1-289 ura3-52) (Morrison et al. 1993) and AMY360-11A (MATα leu2-3,-112 trp1-289 ura3-52 ade5-1 his7-2 pol3-01).

Plasmids. pBL304 (11.05 kb), containing the 3.7 kb MluI-HindIII interval of POL3 DNA cloned as a Sall-HindIII fragment into the Sall-HindIII sites of YCp50, was constructed by P.J.M. Burgers. pBLAM1 was derived from pBL304 by replacement of POL3 by pol3-01 (Morrison et al. 1993). PAM140 was derived from pBLAM1 by replacement of a 1.5 kb NruI-SmaI interval containing URA3 with a 2 kb HpaI fragment from YEp13 containing LEU2 in the orientation opposite to that of pol3-01. PAM150, a pol2-4 TRP1 CENIV plasmid, was derived from YCPOL2 (Araki et al. 1992) by replacement of the POL2 MluI-BamHI fragment with the corresponding pol2-4 fragment from YIpJB1 (Morrison et al. 1991).

Construction of yeast strains. Construction of the pol2-4 and pol3-01 derivatives of CG379 and insertion of URA3 (in the LR orientation, i.e. the URA3 coding sequence is the lagging strand relative to the ARS306 replication origin) about 4.4 kb distal to the ARS306 replication origin (Reynolds et al. 1989) were described previously (Morrison et al. 1991, 1993). Insertion of URA3 in the opposite RL orientation followed the same procedure; transplacement of the 1.1 kb URA3 BamHI fragment into the BglII interval of ARS306 DNA of PAM81 (Morrison et al. 1993) naturally generated both orientations. Activity of the ARS306 origin was confirmed by 2-D gel electrophoresis of replicating DNA (Brewer and Fangman 1987) from several different strains (unpublished observations). A pms1 mutation was introduced into CG379 and its pol2-4 derivative by transplacement of the coding region of the chromosomal PMS1 gene by LEU2 using plasmid PAM58 (Morrison et al. 1993). Diploids used in Table 2 were derived from crosses of CG379 and CG378 or their pol2-4, pol3-01 or pms1 derivatives. PMS1 segregants from a cross of CG379 and CG378 pms1 were crossed with CG379 to make the control +/+ +/+ diploid. AMY360-8D, a pol3-01 segregant obtained from a cross of CG379 pol3-01 and CG378, was crossed with CG379 pol2-4 to make diploid AMY410. AMY410 was transformed with the POL3 URA3 plasmid pBL304, sporulated, and segregants were crossed to make the other diploids listed in Table 2. These diploids were plated on 5-fluoroorotic acid (FOA) to remove pBL304 before the his7-2 reversion measurements (Boeke et al. 1984).

Yeast methods. Measurement of spontaneous mutation rates and genotyping of pol3-01 using the polymerase chain reaction (PCR) were as described (Morrison et al. 1993). To determine pol2-4 genotypes, a 0.28 kb segment of POL2 DNA was amplified from yeast genomic DNA by PCR with 30 cycles of 94°C, 42°C, 72°C using an oligonucleotide primer matching the complement of bases 1705-1730 (Morrison et al. 1990) and either oligonucleotide 5'-AATGGCATTTGCTATAGC-3', matching nucleotides 1449-1466 of POL2+ (Morrison et al. 1990), or 5'-AATGGCATTTGATATAGA-3', matching the equivalent pol2-4 sequence. The latter two primers each generated the expected 0.28 kb DNA fragment only with their cognate template DNA. A PCR test, using 30 cycles of 94°C, 52°C, 72°C, was employed to check the orientation of URA3 integrated near to ARS306. Reactions contained an oligonucleotide primer corresponding to nucleotides 69539-69560 of chromosome III (Oliver et al. 1992) and either primer P4 representing nucleotides 785-805 of URA3 (Rose et al. 1984) or primer P8 representing the complement of URA3 nucleotides 475-455. With URA3 in the LR orientation (see Fig. 1 of Morrison et al. 1993), primer P4 generated an approximately 430 bp PCR DNA fragment; with URA3 in the RL orientation, primer P8 generated an approximately 520 bp PCR DNA fragment.

Nucleotide sequence of ura3 mutations. The URA3 gene of 5-fluoroorotic acid-resistant (FOA+) mutant yeast cells was amplified by PCR and sequenced as described (Morrison et al. 1993). For URA3 in the LR orientation, 20/31 pol3-01 and 20/33 pol2-4 ura3 mutations were determined for strains cultured in YPDA medium. Similarly, 20/36 pol3-01 and 13/23 pol2-4 ura3 mutations with URA3 in the RL orientation were determined for strains cultured in YPDA medium. The remainder were cultured in synthetic complete medium, since it was found that the spontaneous FOA+ mutation rate of the control strain was lower in this medium (our unpublished results). From the relative (to the control strain) ura3 mutation rate of about 130 for pol3-01 in YPDA medium, we expect that none of the sequenced pol3-01 mutations in Fig. 2 is a background mutation (Lee et al. 1988). Since the relative mutation rate for pol2-4 is about 12 in YPDA medium and about 86 in synthetic complete medium, it is likely that about 2/33 sequenced mutations are due to background for URA3 in the LR orientation, and about 1/23 in the RL orientation. For the first 20 pol3-01 and