A Role for *REV3* in Mutagenesis During Double-Strand Break Repair in *Saccharomyces cerevisiae*

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ABSTRACT

Recombinational repair of double-strand breaks (DSBs), traditionally believed to be an error-free DNA repair pathway, was recently shown to increase the frequency of mutations in a nearby interval. The reversion rate of *trp1* alleles (either nonsense or frameshift mutations) near an HO-endonuclease cleavage site is increased at least 100-fold among cells that have experienced an HO-mediated DSB. We report here that in strains deleted for *rev3* this DSB-associated reversion of a nonsense mutation was greatly decreased. Thus *REV3*, which encodes a subunit of the translesion DNA polymerase ζ, was responsible for the majority of these base substitution errors near a DSB. However, *rev3* strains showed no decrease in HO-stimulated recombination, implying that another DNA polymerase also functioned in recombinational repair of a DSB. Reversion of *trp1* frameshift alleles near a DSB was not reduced in *rev3* strains, indicating that another polymerase could act during DSB repair to make these frameshift errors. Analysis of spontaneous reversion in haploid strains suggested that Rev3p had a greater role in making point mutations than in frameshift mutations.

THE ability to repair damaged or broken chromosomes is common to all organisms. DNA synthesis is an important component of most DNA repair and recombination processes. In yeast, three pathways of DNA repair have been identified (reviewed in FRIEDBERG 1988). The genes of the *RAD3* pathway are needed for ultraviolet (UV) excision repair, in which a short section of the damaged DNA strand is removed and replaced by DNA synthesis (WANG *et al.* 1993) using the undamaged strand as template. Genes in the *RAD6* group (which includes *REV3*) are used in the translesion error-prone repair pathway. In this type of DNA repair the damaged (adducted) DNA is used as template for DNA synthesis. The genes in the *RAD52* group are needed to repair double-strand breaks (DSBs) by recombination with an undamaged homologue.

Many models of recombination, including the DSB repair model of SZOSTAK *et al.* (1983), propose that the ends of a DSB are resected to expose single-stranded ends that are used to search for homology. Repair DNA synthesis is then required to fill in the remaining gaps. STRATHERN *et al.* (1995) asked whether this DNA synthesis event has as high a fidelity as S-phase replication, *i.e.*, whether recombination can induce mutations. They found that reversion of an allele near an HO-endonuclease recognition site was elevated at least 100-fold among cells that had undergone (and repaired) an HO-induced DSB. This was true for both a nonsense allele and two frameshift mutations. This demonstrates that misincorporation occurring during DNA synthesis accompanying recombinational repair of a DSB results in a localized elevation of the mutation rate.

We have begun to search for genes that affect the fidelity of DNA synthesis during DSB repair. As a first step we analyzed the role of *REV3* in this process. *REV3* has been proposed to encode the error-prone translesion polymerase, since *REV3* is required for UV-induced mutagenesis (LEMONTT 1971). DNA polymerase ζ (zeta), comprised of Rev3p and Rev7p was recently shown to synthesize across a template containing a thymine-thymine dimer (NELSON *et al.* 1996). We reasoned that a translesion DNA polymerase might be expected to have a lower fidelity than other DNA polymerases, since it must be able to recognize the nonstandard bases in a damaged DNA template. We report here that the increased reversion of a nonsense allele observed near a DSB was dependent on *REV3*, demonstrating that Pol ζ has access to this (presumably) nondamaged template. However, reversion of frameshift alleles near a DSB was largely *REV3*-independent. This specificity of *REV3* for point mutations was also seen for spontaneous mutation.

MATERIALS AND METHODS

Strains and plasmids: The genotypes of the strains used are listed in Table 1. Strains carrying the *trp1-his3* recombination substrate have been previously described (MCGILL *et al.* 1990; STRATHERN *et al.* 1995). To make gene disruptions, haploid strains were transformed with plasmids using a rapid LiAc protocol (GIETZ *et al.* 1992). Plasmid pAM56 (kindly provided

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TABLE 1

Yeast strains

		Reference
Haploids		
GRY1079	*MATa-inc::[trp1-488 HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ1 ura3-52*	McGill *et al.* (1993)
GRY1197	*MATα-inc::[trp1-488 his3-621] leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52*	Strathern *et al.* (1995)
GRY1279	*MATa-inc::[trp1-fsA HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52*	Strathern *et al.* (1995)
GRY1281	*MATa-inc::[trp1-fsT HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52*	Strathern *et al.* (1995)
GRY1282	*MATα-inc::[trp1-fsA his3-621] leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52*	Strathern *et al.* (1995)
GRY1283	*MATα-inc::[trp1-fsT his3-621] leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52*	Strathern *et al.* (1995)
SLH59	*MATα-inc::[trp1-488 his3-621] leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH86	*MATa-inc::[trp1-488 HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH113	*MATα-inc::[trp1-488 his3-621] leu2-Δ1 tyr7-1 trp1-Δ::hisG his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH114	*MATa-inc::[trp1-488 HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ::hisG his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH146	*MATa-inc::[trp1-488 HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ::hisG his3-Δ1 ura3-52*	This study
SLH147	*MATα-inc::[trp1-488 his3-621] leu2-Δ1 tyr7-1 trp1-Δ::hisG his3-Δ200 ura3-52*	This study
SLH310	*MATα-inc::[trp1-fsA his3-621] leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH391	*MATa-inc::[trp1-fsA HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH403	*MATα-inc::[trp1-fsT his3-621] leu2-Δ1 tyr7-1 trp1-Δ1 his3-Δ200 ura3-52 rev3::LEU2*	This study
SLH404	*MATa-inc::[trp1-fsT HO*-site *his3-192] cry1 leu2-Δ1 ade2-101 lys2-801 trp1-Δ1 his3-Δ200 ura3-52 rev3::LEU2*	This study
Diploids		
GRY1198	GRY1079 × GRY1197 [pGAL*HO*]	Strathern *et al.* (1995)
GRY1292	GRY1279 × GRY1282 [pGAL*HO*]	Strathern *et al.* (1995)
GRY1296	GRY1281 × GRY1283 [pGAL*HO*]	Strathern *et al.* (1995)
SLH89	SLH59 × SLH86 [pGAL*HO*]	This study
SLH139	SLH113 × SLH114 [pGAL*HO*]	This study
SLH148	SLH146 × SLH147 [pGAL*HO*]	This study
SLH394	SLH391 × SLH310 [pGAL*HO*]	This study
SLH406	SLH403 × SLH404 [pGAL*HO*]	This study

by Alan Morrison) carries a 7-kb *Bgl*II fragment of *REV3*, with a 1.75-kb *Sna*BI fragment deleted and replaced with a 2.1-kb *Hpa*I fragment carrying the *LEU2* gene. pAM56 was cleaved with *Xba*I before transformation. The presence of the *rev3*::*LEU2* null allele in Leu$^+$ transformants was confirmed by screening for loss of UV-induced mutation to canavanine resistance and by Southern blotting. The *trp1*Δ::*hisG* mutation, which in contrast to *trp1-Δ1* retains the upstream activation sequence (UAS) elements upstream of the neighboring *GAL3* gene, was constructed by PCR amplification of *TRP1* fragments representing the 5′ (oligo 1: 5′-GCGGCCGCGGAT CCAGATATTCCTTATGGCATGT and oligo 2: 5′-GCCGGC CCCGGGGGCGCCAGATCTGTACGTAAGGTGACAAG CTA) and 3′ (oligo 3: 5′-GGCGCCCCCGGGGCCGGCGTG CACTTGCCTGCAGGCCTT and oligo 4: 5′-GCGGCCGCT CTAGAATCTCTGACCGGAGAACTGAA) ends of the gene. One microliter of each product was added to 100 μl of water and subjected together to a second round of PCR with oligos 1 and 4. The resulting product was cloned into the pCRII vector (Invitrogen) following the manufacturer's protocol. The plasmid was cleaved with *Bgl*II and ligated to a *Bam*HI-*Bgl*II fragment containing *Salmonella typhimurium hisG* sequences flanking the *Saccharomyces cerevisiae URA3* gene (Alani *et al.* 1987) to generate pSH129 carrying the *trp1*Δ::*hisG-URA3-hisG* allele. pSH129 was cleaved with *Xba*I and *Bam*HI before transformation of yeast strains. Ura$^+$ transformants were analyzed for the presence of the allele by PCR and Southern blot analysis. Ura$^-$ derivatives, which had recombined between the *hisG* repeats to pop out *URA3*, were selected on 5-fluoro-orotic acid (Boeke *et al.* 1984).

Media: Yeast media were prepared as described in Sherman *et al.* (1986) and McGill *et al.* (1990).

Galactose induction fluctuation tests: Single yeast colonies from synthetic complete media-lacking uracil (SC −ura) plates were patched to SC −ura plates. These patches were used to inoculate 10-ml cultures of SC −ura + glucose media.

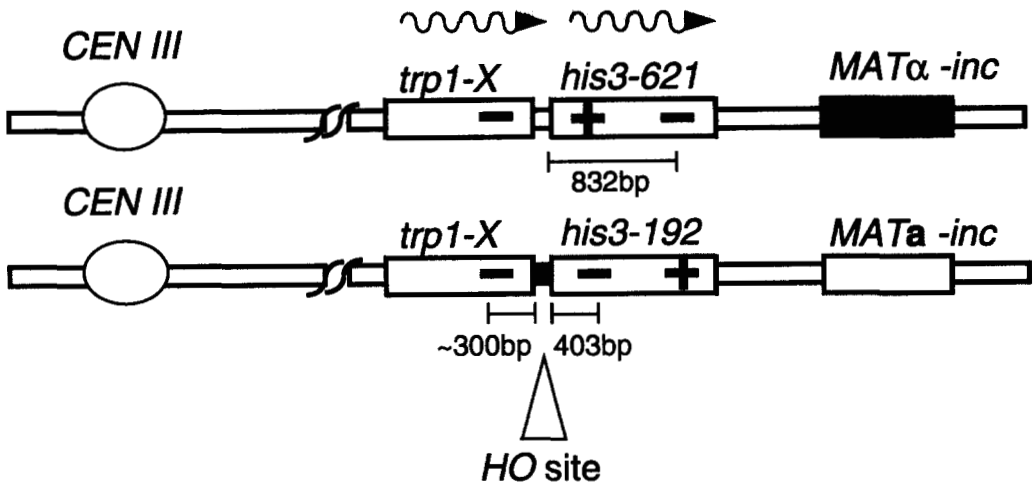

FIGURE 1.—Physical description of the *trp1-his3* interval. Construction of this genetic interval has been previously described (MCGILL *et al.* 1990; STRATHERN *et al.* 1995). Both chromosomes carry the same *trp1* mutation (*trp1-488, trp1-fsT* or *trp1-fsA*). The *trp1-488* allele was made by site-directed mutagenesis to change two bases at codon 163, creating a stop codon 314 bp from the *HO* cleavage site. The *trp1* frameshift alleles were made by insertion of 20-base oligonucleotides at the *Hin*dIII site (*trp1-fsA*, AGCTGGCCAAAAAATGGCCG; *trp1-fsT*, AGCTCGGCCATTTTTTGGCC), 280 bp from the *HO* cleavage site.

Each experiment used 11 independent cultures of each strain. Cultures were grown at 30° to ~2 × 10^7 cells/ml, then used to inoculate 200 ml cultures of SC −ura + glucose media. Cells were again grown to a density of ~2 × 10^7 cells/ml, washed twice with water and resuspended in 6 ml of water. Three milliliters were plated on YEPD, SC −histidine or SC −tryptophan plates at appropriate dilutions to determine the spontaneous frequency of His^+ recombinants and Trp^+ revertants. The remaining 3 ml of each culture were added to 100 ml of SC −ura + galactose media and grown for 48 hr (*trp1Δ1* strains) or 24 hr (*trp1Δ::hisG* strains) at 30° to induce HO expression. Total cell number increased two- to threefold during growth in galactose. Cells were washed once in water, resuspended in 3 ml of water and plated as described above. The median value from each set of 11 cultures was used to determine the frequency of His^+ recombinants and Trp^+ revertants. The percentage HO induction was determined by measuring what fraction of unselected colonies from cells plated after growth in galactose no longer had the starting configuration of alleles: **a**/α cells capable of galactose induction of recombination to His^+ (STRATHERN *et al.* 1995). This was done by measuring the fraction of Ura^+ cells that had recombined to become His^+ or homoallelic for one of the *his3* mutations, or that no longer retained the *HO* site as scored by the ability to be induced to recombine to His^+ by growth on galactose. The fraction of cells retaining the pGALHO plasmid was similar (80–90%) before and after growth in galactose.

Fluctuation tests to determine spontaneous mutation frequencies: Single yeast colonies on YEPD plates were inoculated into 10 ml YEPD broth and shaken at 30° for 14 hr. Nine independent cultures were used in each experiment. The 10-ml cultures were used to inoculate 100-ml cultures in YEPD. These were incubated for ~6 hr at 30°, then washed once with water and resuspended in 9 ml of water. Appropriate dilutions were plated on YEPD, SC −histidine, SC −tryptophan, SC −adenine, and SC −arginine + canavanine plates. The median value from each set of nine cultures was used to determine the frequency of reversion to His^+, Trp^+, Ade^+ and the frequency of forward mutation to canavanine resistance.

RESULTS

The experimental design for the HO-induced DSB experiments is shown in Figure 1. One copy of chromosome III had an insertion near *MAT* carrying an HO-endonuclease recognition site flanked by defective *trp1* and *his3* alleles. The other copy of chromosome III lacked the *HO* site, had the identical *trp1* allele, but a different *his3* allele. His^+ cells could arise by recombination between the *his3* heteroalleles. However, since the *trp1* alleles were identical, Trp^+ cells must have resulted from reversion (*i.e.*, mutation to restore a functional *trp1* gene). The *trp1* mutations are located in a region of the gene that is tolerant of many types of alterations, allowing the detection of many types of reversion events (STRATHERN *et al.* 1995). The strains also contained a plasmid with the *HO* gene under the control of a galactose-inducible promoter (JENSEN and HERSKOWITZ 1984). The normal HO recognition sites at *MAT* were mutated so that they were not cleavable by HO endonuclease. The frequency of spontaneous Trp^+ reversion and His^+ recombinants was determined by plating aliquots of glucose-grown cells. The remaining cells were washed, then incubated in galactose-containing medium to induce expression of the HO endonuclease. HO-induced frequencies of Trp^+ reversion and His^+ recombinants were then determined, as well as the total fraction of cells that had experienced an HO-induced repair event.

HO-induced reversion of the nonsense mutant *trp1-488* is *REV3*-dependent: The *REV3* strains GRY1198 and SLH148 showed a large increase in reversion frequency of the *trp1-488* nonsense allele after receiving an HO-cut (Table 2). To determine the frequency of Trp^+ among the cells that had undergone repair of a DSB, the spontaneous frequency was subtracted from the galactose-induced frequency and the resulting frequency was divided by the fraction of cells in the culture that had repaired a DSB (as defined in MATERIALS AND METHODS). This correction yielded HO-induced reversion frequencies of 91 × 10^{-8} for GRY1198 and 62 × 10^{-8}

TABLE 2

Reversion frequency

	Before induction	After induction
Reversion of *trp1-488*		
GRY1198 (*REV3*)		35% *HO* Induction
Trp^+ (revertants)	1.2×10^{-8}	33×10^{-8}
His^+ (recombinants)	1.2×10^{-4}	310×10^{-4}
SLH148 · (*REV3*)		16% *HO* Induction
Trp^+ (revertants)	1.1×10^{-8}	11×10^{-8}
His^+ (recombinants)	11×10^{-4}	160×10^{-4}
SLH89 (*rev3*)		16% *HO* Induction
Trp^+ (revertants)	0.13×10^{-8}	0.33×10^{-8}
His^+ (recombinants)	0.52×10^{-4}	120×10^{-4}
SLH139 (*rev3*)		26% *HO* Induction
Trp^+ (revertants)	0.46×10^{-8}	0.56×10^{-8}
His^+ (recombinants)	8.4×10^{-4}	210×10^{-4}
Reversion of *trp1-fsT*		
GRY1296 (*REV3*)		9% *HO* Induction
Trp^+ (revertants)	0.05×10^{-8}	19×10^{-8}
His^+ (recombinants)	0.07×10^{-4}	58×10^{-4}
SLH406 (*rev3*)		7% *HO* Induction
Trp^+ (revertants)	0.04×10^{-8}	9.1×10^{-8}
His^+ (recombinants)	0.17×10^{-4}	33×10^{-4}
Reversion of *trp1-fsA*		
GRY1292 (*REV3*)		2.4% *HO* Induction
Trp^+ (revertants)	0.29×10^{-8}	1.5×10^{-8}
His^+ (recombinants)	0.09×10^{-4}	14×10^{-4}
SLH394 (*rev3*)		11% *HO* Induction
Trp^+ (revertants)	0.16×10^{-8}	5.5×10^{-8}
His^+ (recombinants)	0.06×10^{-4}	74×10^{-4}

for SLH148. Thus, the *REV3* strains showed a 56- to 76-fold increase in Trp^+ revertants among cells that had experienced an HO cut. The *rev3* strains SLH89 and SLH139 gave lower spontaneous Trp^+ reversions (nine- and twofold lower than the isogenic *REV3* strains); frequencies consistent with Rev3p having a role in spontaneous mutagenesis. After galactose induction of HO, Trp^+ reversion frequencies in these *rev3* strains increased only slightly. Correcting for HO activity yielded HO-induced reversion frequencies of 1.2×10^{-8} and 0.4×10^{-8}, respectively. Thus, among cells that had received an HO-induced DSB, the frequency of reversion of *trp1-488* in the *rev3* strains was only 1 to 2% of that found in the isogenic *REV3* strains. This result demonstrated that *REV3* function was responsible for most of the mutagenesis leading to reversion of this point mutation during recombination. The level of His^+ recombinants (when corrected for the level of HO-induced DSBs) was approximately the same in *rev3* strains as in *REV3* cells. This indicated that *REV3* was not needed for recombination, and that HO cleavage and access to recombination machinery were not diminished in the *rev3* strains.

HO-induced reversion of frameshift mutants *trp1-fsT* and *trp1-fsA* is *REV3*-independent: Similar analysis of reversion of two frameshift alleles of *trp1* (see legend Figure 1) gave a very different result (Table 2). These frameshift alleles can revert either by polymerase slippage or by template switching in quasipalindromic sequences (STRATHERN *et al.* 1995). Reversion of both *trp1-fsT* and *trp1-fsA* were only modestly reduced in *rev3* strains (Table 2). When corrected for the percentage of cells that had experienced an HO cut, the frequency of Trp^+ revertants of *trp1-fsT* among *REV3* (GRY1296) cells that had received a DSB had increased dramatically (4000-fold) to 210×10^{-8}. When adjusted for the percentage HO-cut, the *rev3* strain (SLH406) also showed a large increase in the frequency of revertants of the *trp1-fsT* allele among cells that had experienced a DSB to 130×10^{-8} (a 3000-fold increase). Thus *REV3* had virtually no effect on HO-induced reversion of the frameshift allele *trp1-fsT*.

DSB-induced mutation to revert the *trp1-fsA* allele was *REV3*- independent as well. Among cells that had repaired a DSB, the median frequency of Trp^+ revertants in the *REV3* strain GRY1292 was 50×10^{-8} (a 170-fold increase over the spontaneous frequency). The *rev3* strain SLH394 showed a similar increase to 48×10^{-8} among cells that had experienced a DSB (a 300-fold increase over the spontaneous frequency).

Spontaneous reversion of point mutations showed a greater *REV3* dependence than reversion of frameshift mutations: We investigated the role of *REV3* in spontaneous reversion of these and other alleles in haploid strains in the absence of the HO-expressing plasmid. The results obtained in isogenic pairs (*REV3* and *rev3*)

TABLE 3

Spontaneous reversion in haploids

Allele	*REV3* strains		*rev3* strains		Isogenic *rev3/REV3*
	Strain	Reversion frequency[a]	Strain	Reversion frequency[a]	
trp1-488	SLH147	15×10^{-9}	SLH113	1.2×10^{-9}	0.08
	SLH146	7.4×10^{-9}	SLH114	0.59×10^{-9}	0.08
ade2-101	GRY1281	67×10^{-9}	SLH404	0.79×10^{-9}	0.01
	GRY1279	47×10^{-9}	SLH391	7.0×10^{-9}	0.15
trp1-fsA	GRY1282	3.2×10^{-9}	SLH310	1.0×10^{-9}	0.31
	GRY1279	3.6×10^{-9}	SLH391	2.6×10^{-9}	0.72
trp1-fsT	GRY1283	1.1×10^{-9}	SLH403	0.68×10^{-9}	0.62
	GRY1281	1.8×10^{-9}	SLH404	1.2×10^{-9}	0.67
his3-192	SLH146	18×10^{-9}	SLH114	9.7×10^{-9}	0.54
	GRY1281	55×10^{-9}	SLH404	26×10^{-9}	0.47
	GRY1279	5.9×10^{-9}	SLH391	18×10^{-9}	3.05

[a] Frequencies represent median value of nine independent cultures.

of strains are presented in Table 3. The *trp1-488* nonsense allele reverted at a 12.5-fold lower frequency in the two *rev3* strains SLH113 and SLH114 than in the isogenic *REV3* strains SLH147 and SLH146. Reversion of the ochre mutation *ade2-101* was 85-fold lower in the *rev3* strain SLH404 than in *REV3* strain GRY1282, and sevenfold lower in *rev3* strain SLH391 than in *REV3* strain GRY1279.

We measured spontaneous reversion of three frameshift mutations as well. Reversion of *trp1-fsA* showed a 1.4- to 3.2-fold lower reversion frequency in *rev3* strains SLH310 and SLH391 than in *REV3* strains GRY1281 and GRY1279. The *trp1-fsT* allele reverted at roughly the same frequency in *rev3* and *REV3* strain (1.6-fold lower in *rev3* strains). Likewise, the frameshift allele *his3-192* showed little difference in reversion frequencies between *rev3* and *REV3* strains (between 2.1-fold lower to 3.05-fold higher in *rev3* strains).

We measured the spontaneous mutation frequency in the *CAN1* gene by measuring the frequency of canavanine resistance and observed only a 1.4- to 7-fold lower frequency in *rev3* strains compared to *REV3* (Table 4). Any effect of *rev3* on base substitution mutation rates may be obscured by a lack of effect on frameshift and Ty insertions. It remains to be seen whether the spectrum of *can1* mutations is altered in *rev3* strains.

DISCUSSION

The finding that defects in mismatch repair can lead to elevated levels of tumorigenesis (PARSONS *et al.* 1993; LEACH *et al.* 1993; reviewed in KUNKEL *et al.* 1997) raises the question of how mismatches are generated. In rapidly dividing cells, polymerase errors during S-phase DNA replication may be a likely source. However, in quiescent tissues, mismatches and mutations may be more likely to result from repair of DNA damage. There are three general classes of DNA damage repair described in yeast (for a review see FRIEDBERG 1988). The *RAD3* epistasis group of genes is needed for excision repair of UV-induced damage. Some of the genes in this group are yeast homologues of those defective in patients with Xeroderma pigmentosum (*RAD3* = XPD, *RAD25* = XPB, *RAD2* = XPG), who have an increased incidence of skin tumors (reviewed in ABOUSSEKHRA and WOOD 1994).

The *RAD52* epistasis group of genes is needed for recombinational repair and has been traditionally thought to be error-free, as an undamaged homologue

TABLE 4

Spontaneous mutation of *CAN1* in haploids

REV3 strains		*rev3* strains		Isogenic *rev3/REV3*
Strain	Frequency[a] Can^r	Strain	Frequency[a] Can^r	
SLH147	19×10^{-7}	SLH113	6.0×10^{-7}	0.32
SLH146	24×10^{-7}	SLH114	15×10^{-7}	0.63
GRY1283	14×10^{-7}	SLH403	2.0×10^{-7}	0.14
GRY1281	25×10^{-7}	SLH404	15×10^{-7}	0.60
GRY1282	7.4×10^{-7}	SLH310	1.7×10^{-7}	0.23
GRY1279	23×10^{-7}	SLH391	13×10^{-7}	0.57

[a] Frequencies represent median value of nine independent cultures.

is used as a template to repair the damaged or broken DNA. It was recently demonstrated that recombinational repair of an introduced double-strand break in mitotic cells is not error-free, but increases the rate of reversion of a nearby allele ~100-fold, from 10^{-9} to 10^{-7} (STRATHERN *et al.* 1995). However, the data presented here demonstrated that recombinational repair involving the *trp1-488* allele could be made nearly error-free by removing *REV3* function. Although *REV3* was needed for this DSB-associated mutagenesis, it was not needed for recombination.

The third class of DNA repair utilizes the *RAD6* epistasis group of genes, which includes *REV3*. These genes are needed for "error-prone" translesion repair across a damaged DNA template. It has long been known that treatment of cells with DNA damaging agents, such as chemicals or UV light, causes an increase in mutations (HAYNES and KUNZ 1981). These lesions are repaired by using the damaged DNA strand as a template for DNA replication. Many DNA polymerases are unable to continue synthesis past a DNA lesion. In *Escherichia coli*, stalled Pol III is modified by UmuD'C to allow it to resume DNA synthesis in a nonprocessive and error-prone mode (RAJAGOPALAN *et al.* 1992). The newly identified yeast DNA polymerase ζ, comprised of Rev3p and Rev7p, has the ability to synthesize past a thymine dimer (NELSON *et al.* 1996). This is in accordance with the observation that *REV3*, *REV7*, and *REV1* (which has sequence homology to UmuC) are required for UV-induced mutagenesis in *S. cerevisiae* (LAWRENCE *et al.* 1985; LARIMER *et al;* 1989; LEMONTT 1971).

It has been suggested that the only role of error-prone repair is in translesion synthesis (MORRISON *et al.* 1989). Surprisingly, we found that *REV3* can act during repair of a DSB, where the unbroken homologous chromosome is used as a template for DNA synthesis, and that the *REV3*-dependent synthesis can introduce errors. The lack of a proofreading exonuclease motif in *REV3* (MORRISON and SUGINO 1994) may result in elevated error rates when pol ζ acts on undamaged templates. We entertain the possibility that pol ζ acting on undamaged DNA templates plays a major role in spontaneous mutagenesis. It will be important to understand what features enable pol ζ to act on a DNA template.

Why might Rev3p be recruited to act in recombinational repair? Expression of the three essential DNA polymerases of *S. cerevisiae* is induced during S-phase (JOHNSON *et al.* 1985; MORRISON *et al.* 1990), while expression of *REV3* remains at a constant low level throughout the cell cycle (SINGHAL *et al.* 1992). Perhaps the stoichiometry of the different polymerases plays a role in determining which acts at different points in the cell cycle, with Rev3p being in greater relative abundance, and thus having a higher probability of acting at times other than S-phase. Alternatively, a DSB or some intermediate in recombinational repair might actively recruit Rev3p. In *E. coli* single-stranded DNA (formed as an intermediate in all models of recombination) acts as a signal that eventually leads to the activation of UmuD, allowing translesion synthesis to take place (FRANK *et al.* 1993). A third possibility is that intermediates in DSB repair are more susceptible to chemical modifications, requiring the action of a translesion polymerase.

We found that *REV3* was required for DSB-repair-induced reversion of a nonsense mutation but not for two frameshift alleles of *trp1*. To determine whether this represented a general property of pol ζ, we examined the effect of *REV3* on spontaneous reversion of several point mutations and frameshift alleles. For two point mutations, *trp1-488* and *ade2-101*, we found that spontaneous reversion showed a strong dependence on *REV3*. QUAH *et al.* (1980) reported that *rev3* strains showed decreased spontaneous reversion (three- to sevenfold less) of two nonsense alleles and one missense allele. In our studies *REV3* had a less dramatic effect on reversion of the three frameshift alleles tested. This small collection of alleles highlights the allele specificity of such reversion studies and suggests either that pol ζ more readily makes base substitution mutations than frameshift mutations, and/or that the other polymerase(s) that gives spontaneous mutations makes relatively more frameshift errors. While translesion polymerases might of necessity have a low fidelity of base selection to enable them to synthesize over damaged bases, it would be a useful property of a translesion polymerase to be able to retain frame. Mutations differentially affecting frameshifting *vs.* misincorporation have been reported for T4 DNA polymerase (RIPLEY and SHOEMAKER 1983; RIPLEY *et al.* 1983) and HIV reverse transcriptase (BEBENEK *et al.* 1995). ROCHE *et al.* (1994) examined the role of *REV3* in spontaneous mutation of a plasmid-borne *SUP4-o* gene. The overall mutation rate is reduced to 40% in *rev3* cells. In their analysis, total base substitution and single base frameshifts are equivalently reduced. In both the *REV3* and *rev3* backgrounds, nearly all the single base frameshifts occur in a sequence of five G's. However, they found that certain sites within the gene show marked differences in base substitution between *REV3* and *rev3* strains, indicating that sequence context (or perhaps secondary structure) influences the *REV3* effect. Perhaps differences in DNA structure (*SUP4* is a tRNA gene) or transcription (Pol II *vs.* Pol III) alter the mutability of different sites.

Two studies (LAWRENCE and CHRISTENSEN 1979 and LAWRENCE *et al.* 1984) examined the effect of *REV3* on UV-induced reversion of point mutations and frameshift alleles. Reversion of nine point mutant alleles was studied. Seven of these alleles show a strong *REV3*-dependence for UV-induced reversion (30- to 100-fold). However, two of the point mutations show only a twofold *REV3* effect. These two *REV3*-independent alleles show higher reversion rates than the other point alleles, suggesting that they may be "hotspots"

for a *REV3*-independent, UV-induced mutation mechanism. UV-induced reversion of eight frameshift alleles of *cyc1* and *his4* was examined by these authors as well. *REV3*-dependence for five of these alleles range from three- to 17-fold, while reversion of three frameshift alleles show a stronger *REV3* dependence (25- to 200-fold). Thus, these authors found a major role for *REV3* in UV-induced reversion of base substitution mutations with variable effects on frameshift mutations.

Recombination is greatly enhanced during meiosis with substantial evidence that it is initiated by DSBs (CAO *et al.* 1990; KEENEY *et al.* 1997). MAGNI and VON BORSTEL (1963) noticed an increase in mutation during meiosis and termed it the "meiotic effect." A small, significant correlation between recombination and mutation was observed. Recently, expression of *REV3* was reported to be somewhat induced during meiosis (SINGHAL *et al.* 1992). When our strains were put through meiosis, we found a similar association between recombination and mutation in meiosis (unpublished results). It remains to be determined whether *REV3* plays a role in the meiotic effect.

Our result that the *REV3* polymerase can act near a DSB, but is not essential for recombinational repair, can be interpreted in two ways. *REV3* might normally act at all DSBs, with a modest error rate. Since filling in gaps left by recombination is essential to restore a broken chromosome, another polymerase(s) would be recruited in the absence of *REV3*. Alternatively, *REV3* might normally act at only a small subset of DSBs, with a high rate of infidelity. The loss of these events when *REV3* is absent would be too small to be detected as a recombination defect. Our finding that DSB-induced mutation to revert the frameshift alleles *trp1-fsA* and *trp1-fsT* is *REV3*-independent demonstrates that yet another polymerase is able to act, with a low fidelity, in DSB repair. We have initiated a mutant hunt to identify other genes that affect the fidelity of synthesis during DSB repair with the goal of identifying other polymerases and accessory factors needed for recombinational repair.

Our observation that pol ζ can act as a mutator on undamaged templates raises the question of whether there are circumstances where localized replication infidelity could be advantageous. We suggest that selective hypermutation could be accomplished by recruiting a low fidelity polymerase to copy undamaged templates. Adaptive mutation in *E. coli* is one area in which the data are most consistent with elevated mutagenesis of subsets of the genome rather than a global phenomenon (FOSTER and CAIRNS 1992) and may reflect mutagenic repair acting on recombination intermediates (HARRIS *et al.* 1994) involving Pol III (FOSTER *et al.* 1995). Adaptive reversion of a *lys2* frameshift mutation (STEELE and JINKS-ROBERTSON 1992) and a *his4* missense mutation (HALL 1992) have been reported in *S. cerevisiae*. The role of pol ζ in this process has not yet been determined.

Localized mutagenesis plays a role in both antigenic variation and immunoglobulin gene maturation. Base substitution frequencies as high as 3% have been reported associated with the recombination-mediated activation of surface glycoprotein gene in trypanosomes (LU *et al.* 1993). One model for the origin of these base substitutions is the involvement of a low fidelity polymerase in these gene conversion events (LU *et al.* 1994). Similarly, subsequent to the diversity provided by immunoglobulin gene rearrangement and imprecise gene segment joining, there is an elevated mutation rate localized to the variable region of rearranged immunoglobulin genes in mice and humans (reviewed in NEUBERGER and MILSTEIN 1995). Sequences homologous to *REV3* exist in higher eukaryotes. Perhaps pol ζ contributes to somatic hypermutation adding to the diversity of immunoglobulin and T-cell receptor genes by being recruited for localized synthesis on undamaged templates. In summary, our results suggest that damaged bases in the template are not a prerequisite for DNA synthesis by pol ζ or other error prone polymerases and raises the possibility that they have a role in programmed localized mutagenesis.

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