DOI: 10.1534/genetics.104.033894

Roles of *RAD6* Epistasis Group Members in Spontaneous Polζ-Dependent Translesion Synthesis in *Saccharomyces cerevisiae*

Brenda K. Minesinger* and Sue Jinks-Robertson*,†,[1]

**Biochemistry, Cell and Developmental Biology Program of the Graduate Division of Biological and Biomedical Sciences and* †*Department of Biology, Emory University, Atlanta, Georgia 30322*

Manuscript received July 23, 2004
Accepted for publication January 14, 2005

ABSTRACT

DNA lesions that arise during normal cellular metabolism can block the progress of replicative DNA polymerases, leading to cell cycle arrest and, in higher eukaryotes, apoptosis. Alternatively, such blocking lesions can be temporarily tolerated using either a recombination- or a translesion synthesis-based bypass mechanism. In *Saccharomyces cerevisiae*, members of the *RAD6* epistasis group are key players in the regulation of lesion bypass by the translesion DNA polymerase Polζ. In this study, changes in the reversion rate and spectrum of the *lys2ΔA746* −1 frameshift allele have been used to evaluate how the loss of members of the *RAD6* epistasis group affects Polζ-dependent mutagenesis in response to spontaneous damage. Our data are consistent with a model in which Polζ-dependent mutagenesis relies on the presence of either Rad5 or Rad18, which promote two distinct error-prone pathways that partially overlap with respect to lesion specificity. The smallest subunit of Polδ, Pol32, is also required for Polζ-dependent spontaneous mutagenesis, suggesting a cooperative role between Polδ and Polζ for the bypass of spontaneous lesions. A third error-free pathway relies on the presence of Mms2, but may not require PCNA.

THE integrity of the genome is threatened not only by environmental agents such as UV and gamma rays, but also by DNA lesions generated spontaneously during normal cellular metabolism. Such spontaneous lesions include oxidative-based damage, base alkylation, loss of DNA bases, and chromosome breaks. Not only can endogenous DNA damage change the base-pairing properties of nucleotides, but also it creates noncoding lesions (Friedberg *et al.* 1995). Such noncoding lesions can block the forward progression of the replication fork, resulting in cell cycle arrest and/or cell death. Normally, DNA repair processes such as nucleotide excision repair and base excision repair remove blocking lesions before the replication machinery encounters them (Hoeijmakers 2001; Kunkel 2003). If, however, the damage escapes removal by a repair pathway, mechanisms exist to bypass the lesion in order to allow continued DNA replication. Such bypass can be accomplished in a relatively error-free manner via homologous recombination or template switching involving the sister chromatid. Alternatively, a blocking lesion can be bypassed by translesion synthesis, which employs a low-fidelity polymerase to replicate across the damage, often at the cost of increased mutations (Kunkel 2003).

Three translesion polymerases have been described in *Saccharomyces cerevisiae*: Polη, Rev1, and Polζ. Both Polη and Rev1 belong to the Y family of DNA polymerases, whose founding members include polymerases involved in the SOS response of *Escherichia coli* (Ohmori *et al.* 1999). *In vitro*, Polη (encoded by *RAD30*) can bypass thymine-thymine dimers and 8-oxo-G lesions in an error-free manner (Johnson *et al.* 1999; Haracska *et al.* 2000b; Yuan *et al.* 2000), while bypassing other lesions in an error-prone manner (Yuan *et al.* 2000). *In vivo*, lesion bypass by Polη has a variable effect on spontaneous mutagenesis; *rad30* mutants can exhibit an increase, a decrease, or no change in mutation rate, depending upon the assay system used (McDonald *et al.* 1997; Roush *et al.* 1998). *REV1* encodes a protein with limited polymerase activity *in vitro*, specifically incorporating cytosine across from both damaged and undamaged bases (Lawrence 2002). This polymerase activity, however, does not appear to be required for the role(s) of Rev1 in *in vivo* mutagenesis (Haracska *et al.* 2001; S. Wiley and S. Jinks-Robertson, unpublished observations). Rev1 interacts genetically with the third translesion polymerase in yeast, Polζ (Harfe and Jinks-Robertson 2000; Lawrence 2002). Polζ is composed of two subunits: a catalytic subunit encoded by *REV3* and an accessory factor encoded by *REV7* (Nelson *et al.* 1996). Like the other translesion polymerases, Polζ has no 3′-5′ proofreading activity and demonstrates low processivity, with half of the molecules dissociating from a DNA template after the addition of only three to four nucleotides (Nelson *et al.* 1996). *In vitro*, the fidelity of Polζ when bypassing DNA damage is lesion dependent; Polζ bypasses thymine dimers in an error-prone manner

[1]*Corresponding author:* Department of Biology, Emory University, 1510 Clifton Rd., Atlanta, GA 30322.
E-mail: sue.jinks-robertson@emory.edu

(Nelson *et al.* 1996), but thymine glycols in an error-free manner (Johnson *et al.* 2003). In addition to its capacity to place a nucleotide across from a damaged base, Polζ possesses the unusual ability to extend from a primer:template mispair (Johnson *et al.* 2000; Haracska *et al.* 2003), an activity that is absent in replicative DNA polymerases. Given that 50–75% of spontaneous mutagenesis in yeast has been attributed to Polζ (Quah *et al.* 1980), understanding how the activity of Polζ is regulated is an important step in elucidating the complex mechanisms underlying spontaneous mutagenesis.

The use of Polζ in bypassing spontaneous DNA lesions appears to be largely regulated by members of the *RAD6* epistasis group, which include *RAD6*, *RAD18*, *RAD5*, *MMS2*, *UBC13*, and *SRS2*. This epistasis group was originally defined in relation to induced DNA damage and was termed the postreplication repair (PRR) pathway. This designation specifically reflects the ability of many members of this pathway to convert the low-molecular-weight DNA formed in response to induced damage into larger DNA products without removal of the original lesion (Prakash 1981; Torres-Ramos *et al.* 2002). Further work revealed that the PRR pathway is composed of an error-prone and an error-free subpathway, both of which are under the control of Rad6 and Rad18 (Prakash 1981). The error-prone pathway depends on Polζ, while the error-free PRR pathway is composed of Rad5, Mms2, Ubc13, and Srs2 (Ulrich and Jentsch 2000; Broomfield *et al.* 2001; Ulrich 2001; Broomfield and Xiao 2002).

Although the term PRR has been used to describe *RAD6*-dependent lesion bypass of spontaneous as well as induced damage, use of this acronym may be misleading if used to describe spontaneous lesion bypass, as the roles of these proteins in bypassing spontaneous damage appear to differ from those utilized for bypassing induced damage (Liefshitz *et al.* 1998; Cejka *et al.* 2001). For example, Rad6 and Rad18 are only partially required for spontaneous mutagenesis, whereas they are completely required for mutagenesis in response to induced damage (Friedberg *et al.* 1995). In addition, the activity of postreplication repair has not been formally demonstrated to occur in the absence of induced damage. For the sake of clarity, we refer to spontaneous lesion bypass promoted by members of the *RAD6* epistasis group as the spontaneous lesion bypass (SLB) pathway, to differentiate it from damage-induced lesion bypass.

In contrast to the two-pathway model proposed for bypass of induced damage, genetic characterization of SLB suggests a three-pathway model, with two error-prone pathways and one error-free pathway (Figure 1). Elimination of Rad18 or Rad5 alone results in an increase in Polζ-dependent mutagenesis, while simultaneous loss of Rad18 and Rad5 results in a dramatic decrease in spontaneous mutagenesis, similar to a *rev3* mutant (Liefshitz *et al.* 1998; Cejka *et al.* 2001). One error-prone pathway (Rad18-dependent pathway) appears to be composed of Rad6, Rad18, Pol32, and Polζ, while the other (Rad5-dependent pathway) is composed of Rad5, Pol32, and Polζ. *RAD6* encodes a ubiquitin-conjugating enzyme (Jentsch *et al.* 1987) that physically associates with Rad18, a single-stranded DNA binding protein (Bailly *et al.* 1997). Deletion of *RAD6* results in a pleiotrophic phenotype, affecting sporulation (Morrison *et al.* 1988), telomere elongation (Huang *et al.* 1997), protein degradation (Dohmen *et al.* 1991), and damage-induced PRR (Prakash 1981), while deletion of *RAD18* appears to be specific for PRR (Prakash 1981). Rad6 and Rad18 form a stable complex, which can dimerize via the Rad18 subunit to promote Polζ-dependent translesion synthesis (Bailly *et al.* 1997). The second error-prone branch of the SLB pathway is thought to require a homodimer of the Rad5 protein (Ulrich and Jentsch 2000; Figure 1), an ssDNA-dependent ATPase with homology to the Swi/Snf family of chromatin remodeling factors (Johnson *et al.* 1992, 1994). Whether the Rad5- and Rad18-dependent error-prone pathways are truly functionally redundant, or whether each exhibits some lesion specificity, is not known. In addition to its self-association, Rad5 can interact with Mms2 and Ubc13 (Ulrich and Jentsch 2000), which together form a heterodimer possessing K63-linked ubiquitin-conjugating activity (Hofmann and Pickart 1999). Although Rad5 is capable of interacting with Mms2 and Ubc13, a requirement for these proteins in Rad5-dependent error-prone bypass of spontaneous damage has not been demonstrated.

The error-free branch of the SLB model appears to be composed of members of both of the error-prone arms and components of the DNA replication machinery (Figure 1). A heterotrimeric complex consisting of Rad6, Rad18, and Rad5 is formed via an association between Rad6-Rad18 and Rad5 (Ulrich and Jentsch 2000). Additionally, Rad5 interacts with Mms2 and Ubc13 to create a large multimeric complex to promote error-free lesion bypass. Such bypass may involve the DNA replication processivity factor PCNA (*POL30*), as an allele of the corresponding gene, *pol30-46*, has been implicated in an error-free lesion bypass mechanism in response to induced damage (Ayyagari *et al.* 1995; Torres-Ramos *et al.* 1996). Polδ, encoded by *POL3*, *POL31*, and *POL32*, has also been predicted to reside in the error-free arm on the basis of epistasis of *rad18* to the *pol3-13* allele in response to induced damage (Giot *et al.* 1997). Although the catalytic subunit (Pol3) of Polδ is implicated in error-free lesion bypass, the smallest subunit of Polδ, Pol32, has been associated with mutagenesis. *pol32* mutants exhibit decreased spontaneous and damage-induced mutagenesis, similar to a *rev3* mutant (Huang *et al.* 2000, 2002). However, it remains to be determined if the decrease in mutagenesis seen in a *pol32* mutant is the result of loss of Polζ activity.

The decision to perform lesion bypass of induced

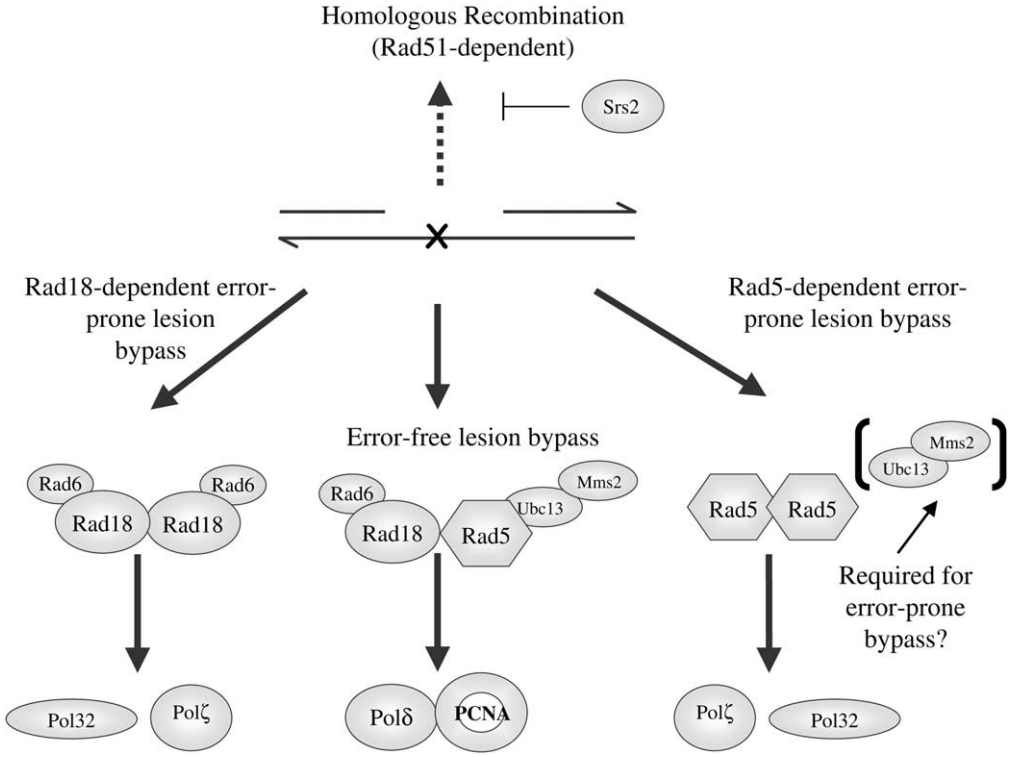

FIGURE 1.—Current model for lesion bypass promoted by the *RAD6* epistasis group in response to spontaneous damage. Spontaneous damage is indicated as an X. The model predicts two Polζ-dependent error-prone arms: one controlled by the Rad6-Rad18 complex and the other controlled by a homodimer of Rad5. The error-free pathway is composed of proteins involved in both error-prone arms, as well as proteins involved in DNA replication. See text for details; adapted from CEJKA *et al.* (2001).

damage via members of the *RAD6* epistasis group as opposed to recombination-based bypass appears to rely largely on the activity of Srs2 (reviewed in BROOMFIELD *et al.* 2001; SMIRNOVA and KLEIN 2003). *SRS2* encodes a 3′-5′ helicase with antirecombinase activity (RONG and KLEIN 1993) and *in vitro* can strip the Rad51 protein from the single-stranded nucleoprotein filament that promotes invasion of a duplex DNA molecule (KREJCI *et al.* 2003; VEAUTE *et al.* 2003). Thus, Srs2 has been proposed to inhibit Rad51-dependent recombination, thereby allowing members of the *RAD6* epistasis group to perform their functions in damage tolerance. Although Srs2 plays a key role in Polζ-dependent lesion bypass in response to induced damage (LIEFSHITZ *et al.* 1998), the role of Srs2 with regard to spontaneous damage is less clear.

Previously, we described a chromosomal-based, frameshift-specific reversion assay that can be used to monitor overall changes in spontaneous mutation rates as well as in patterns of mutagenesis. This system utilizes the *lys2ΔA746* −1 frameshift allele that reverts via second-site, compensatory +1 frameshift events. Different classes of net +1 frameshift mutations can be detected within an ∼150-bp "reversion window" defined by stop codons in alternate, incorrect reading frames (HARFE and JINKS-ROBERTSON 1999). In most genetic backgrounds, the occurrence of single-nucleotide insertions, termed "simple" events, predominates. However, in genetic backgrounds in which DNA damage repair mechanisms have been compromised, there is a striking increase in the proportion of "complex" events, which are composed of a single-nucleotide insertion coupled with a base-pair substitution (HARFE and JINKS-ROBERTSON 2000). The accumulation of these complex frameshifts requires Polζ; deletion of the catalytic subunit of Polζ (*REV3*), inactivation of the catalytic domain of Rev3, or the elimination of the processivity factor Rev7 completely abolishes complex frameshift events (HARFE and JINKS-ROBERTSON 2000; S. YELLUMAHANTI, B. MINESINGER and S. JINKS-ROBERTSON, unpublished results). The complex events detected by the *lys2ΔA746* assay system are a distinctive mutational signature of Polζ and thus provide a unique opportunity to identify factors affecting its activity in spontaneous mutagenesis. In this study, we use the *lys2ΔA746* assay system to analyze Polζ-dependent mutagenesis in the absence of key components of the *RAD6* epistasis group. The results obtained further refine the role of *RAD6* epistasis group members in the regulation of Polζ-dependent spontaneous mutagenesis and lend further support to the three-pathway model of spontaneous lesion bypass.

MATERIALS AND METHODS

Media and growth conditions: Yeast strains were grown nonselectively in YEP medium (1% yeast extract, 2% Bacto-peptone, 250 mg/liter adenine; 2% agar for plates), which was supplemented with either 2% dextrose (YEPD) or 2% glycerol and 2% ethanol (YEPGE). Selective growth was on synthetic complete medium containing 2% dextrose (SCD) and lacking the appropriate nutrient (SHERMAN 1991). YEPD plates containing 200 mg/liter Geneticin (G418; YEPD + G418) or 300 mg/liter hygromycin B (YEPD + hygromycin) were used to

TABLE 1

Strains used in this study

Strain	Relevant genotype	Reference
SJR1166	*rad1::hisG-URA3-hisG*	Harfe and Jinks-Robertson (2000)
SJR1177	*rad1::hisG*	Laboratory strain
SJR1272	*rev3::KAN*	Harfe and Jinks-Robertson (2000)
SJR1274	*rad1::hisG-URA3-hisG rev3::KAN*	Harfe and Jinks-Robertson (2000)
SJR1770	*rad1::hisG rev3::KAN*	This study
SJR1672	*rad1::hisG-URA3-hisG pol32Δ::HPH*	This study
SJR1681	*rad1::hisG srs2::hisG-URA3-hisG*	This study
SJR1827	*rad18::hisG-URA3-hisG*	This study
SJR1678	*rad1::hisG rad18::hisG-URA3-hisG*	This study
SJR1797	*rad1::hisG rev3::KAN rad18::hisG-URA3-hisG*	This study
SJR1867	*rad5Δ::HPH*	This study
SJR1868	*rad1::hisG rad5Δ::HPH*	This study
SJR1828	*mms2Δ::KAN*	This study
SJR1778	*rad1::hisG mms2Δ::KAN*	This study
SJR2063	*rad5Δ::HPH rad18::hisG-URA3-hisG*	This study
SJR2064	*rad1::hisG rad5Δ::HPH rad18::hisG-URA3-hisG*	This study
SJR2061	*mms2Δ::KAN rad18::hisG-URA3-hisG*	This study
SJR2062	*rad1::hisG mms2Δ::KAN rad18::hisG-URA3-hisG*	This study
SJR2069	*leu2-R rad1::hisG LEU2::pol30-46 pol30::hisG-URA3-hisG*	This study
SJR2239	*leu2-K LEU2::pol30-46 pol30::hisG-URA3-hisG*	This study
SJR2228	*leu2-K pol32Δ::HPH*	This study

All strains are congenic derivatives of SJR922 (*MATα ade2-101$_{oc}$ his3Δ200 ura3ΔNco lys2ΔA746*; Harfe and Jinks-Robertson 1999).

select Geneticin- and hygromycin-resistant transformants, respectively. Ura$^-$ derivatives of strains were selected on SCD-uracil plates containing 1 g/liter 5-fluoroorotic acid (5-FOA) and supplemented with uracil to a final concentration of 12 mg/ml (Boeke *et al.* 1987). All growth was at 30°.

Strain construction: A complete list of strains is given in Table 1. All strains are congenic derivatives of SJR922 (*MATα ade2-101$_{oc}$ his3Δ200 ura3ΔNco lys2ΔA746*; Harfe and Jinks-Robertson 1999) and were obtained by standard lithium acetate transformation (Gietz *et al.* 1995). Ura$^-$ derivatives of the *rad1* mutant strains SJR1166 and SJR1274 (Harfe and Jinks-Robertson 2000) were identified on 5-FOA medium to create strains SJR1177 and SJR1770, respectively. *MMS2* and *REV3* were deleted using a PCR-generated kanMX2 cassette (Wach *et al.* 1994; Swanson *et al.* 1999), and transformants were selected on YEPD + G418. *RAD5* and *POL32* were deleted using a PCR-generated hphMX4 cassette (Goldstein and McCusker 1999), and transformants were selected on YEPD + hygromycin. All PCR-mediated gene deletions contain a precise deletion of the published open reading frame with the exception of *rev3::KAN*, in which the first and last 60 nt of the coding region remain (Swanson *et al.* 1999). *Eco*RI-digested pJJ239 (provided by L. Prakash) and *Sac*I/*Sph*I-digested pPM690 (Lee *et al.* 1999) were used to disrupt *RAD18* and *SRS2*, respectively. SJR2239 (*leu2-K pol30::hisG-URA3-hisG pol30-46*) and SJR2069 (*leu2-R rad1 pol30::hisG-URA3-hisG pol30-46*) were constructed as follows. First, *Afl*II-digested pJH-188 and *Kpn*I-digested pJH189 (Lichten *et al.* 1987) were used to create *leu2-K* and *leu2-R* derivatives of SJR922 and SJR1177, respectively. Following selection for Ura$^+$ transformants, plasmid loss events were selected on 5-FOA and the Leu$^-$ phenotype was confirmed. The *pol30-46* allele was then introduced by transformation of *Hpa*I-digested pBL248-46 (obtained from P. Burgers), which targets the *pol30-46* allele to the *LEU2* locus. Finally, the wild-type copy of *POL30* was disrupted using *Kpn*I/*Mlu*I-digested pBL243 (obtained from P. Burgers). Phenotypic confirmation of gene disruptions was conducted when appropriate. All disruptions were confirmed by PCR.

Determination of spontaneous reversion rates: Cultures containing 5 ml YEPGE were inoculated with single colonies and grown to saturation (2–3 days) on a roller drum. Cells were pelleted, washed in 5 ml sterile ddH_2O and resuspended in 1 ml sterile ddH_2O. To assess cell viability and Lys$^+$ reversion, appropriate cell dilutions were plated on YEPD and SCD-lysine plates, respectively. Cells were counted after 2–3 days of growth for YEPD plates and after 2–4 days for SCD-lysine plates. Reversion rates were determined by the method of the median (Lea and Coulson 1949) and 95% confidence intervals of these rates were calculated as described in Spell and Jinks-Robertson (2004). Briefly, the total number of revertants for each culture was ranked in ascending order. Table B11 of Altman (1990) was used to determine, on the basis of the total number of cultures, the ranked culture numbers to be used for establishing the high and low confidence intervals. The total number of revertants from the appropriate high- and low-ranked cultures was used to calculate the values of the 95% high and low confidence intervals, respectively.

The reversion rate of a specific category of frameshift event was determined by multiplying the percentage of the event in the reversion spectrum by the corresponding overall mutation rate. To determine the 95% confidence intervals for the event, the high and low confidence intervals for the overall mutagenesis rate were multiplied by the percentage of times the specific event occurred in the spectrum.

Reversion spectra: Spontaneous Lys$^+$ revertants were obtained as described above. To ensure that each reversion event was independent, only one colony from each culture was analyzed. After purifying the revertant colony on SCD-lysine plates, genomic DNA was isolated using standard yeast protocols. Sequencing of an appropriate PCR product was performed as described in Harfe and Jinks-Robertson (1999).

TABLE 2

***lys2ΔA746* reversion rates in wild-type and mutant strains**

Strain	All frameshifts		Complex frameshifts		Noncomplex frameshifts	
	Rate ($\times 10^{-9}$)	Rate relative to WT	Rate ($\times 10^{-10}$)	Rate relative to WT	Rate ($\times 10^{-10}$)	Rate relative to WT
WT	3.2 (2.7–3.7)[a]	1.0	1.8 (1.6–2.1)	1.0	30 (25–35)	1.0
rad18	6.3 (5.1–7.7)	2.0	15 (12–18)	8.3	48 (39–59)	1.6
rad5	5.0 (4.4–6.9)	1.6	14 (13–20)	7.8	38 (33–51)	1.3
mms2	5.2 (4.6–7.1)	1.6	11 (10–16)	6.1	39 (34–53)	1.3
pol30-46	4.4 (3.9–5.4)	1.4	4.3 (3.9–5.4)	2.4	40 (36–51)	1.3
pol32	7.3 (5.7–9.7)	2.3	0.79 (0.62–1.1)	0.44	72 (56–96)	2.4
rad18 rad5	3.5 (3.0–4.4)	1.1	0.37[b] (NA)	<0.21	35 (30–44)	1.2
rad18 mms2	5.4 (3.9–8.1)	1.7	16 (12–24)	8.9	38 (27–57)	1.3

The category of "all frameshifts" refers to the overall reversion rate. The rates of complex and noncomplex frameshifts were calculated by multiplying the percentage of times the specific frameshift event occurred by the corresponding overall reversion rate. WT, wild type; NA, not applicable.

[a] Values within parentheses are 95% confidence intervals.

[b] Maximum theoretical rate calculated as if one event had been detected.

Sequences were analyzed using the Sequence Manager Software (DNASTAR, Madison, WI) licensed from BIMCORE at Emory University. A table describing the nature of the complex insertions (cins) in each strain is available upon request.

Statistical analysis: Monte Carlo statistical analysis (Adams and Skopek 1987; Cariello *et al.* 1994) was performed to compare the distribution (number and location) of complex and simple frameshift events within two reversion spectra. A complex event is defined as a single-nucleotide insertion accompanied by a base-pair change and a simple frameshift is defined as the insertion of one nucleotide in the reversion window. Briefly, a table was created so that one column contained each nucleotide in the reversion window. In a homopolymer run, one cannot precisely determine where the frameshift occurred; therefore, any repeated sequence was considered as a single nucleotide. Each nucleotide (or nucleotide group) was assigned the designation of "C" for complex or "S" for simple and the number of complex or simple events that occurred at each site was recorded. The location of a given complex frameshift corresponded to the position where the frameshift event occurred, not to where the base substitution occurred. Probability (P) values <0.05 were considered significantly different.

RESULTS

Complex frameshifts increase in the absence of Rad18, Rad5, and Mms2: To investigate the role of members of the *RAD6* epistasis group with regard to spontaneous mutagenesis, we examined both reversion rates and spectra of the *lys2ΔA746* −1 frameshift allele in the absence of key members of this epistasis group. Previous studies demonstrated that a *rad18*, *rad5*, or *mms2* mutant exhibits a Polζ-dependent mutator phenotype (Roche *et al.* 1995; Liefshitz *et al.* 1998; Xiao *et al.* 1999; Cejka *et al.* 2001; Huang *et al.* 2002). Consistent with these results, elimination of Rad18, Rad5, or Mms2 resulted in a small but significant increase in the *lys2ΔA746* spontaneous reversion rate (Table 2). To determine the locations of these compensatory +1 frameshift mutations, we sequenced the "reversion window" of independent, Lys$^+$ revertant colonies and compiled these mutations to create a reversion spectrum. Frameshift events were classified into one of two broad categories: complex frameshifts, in which a simple insertion event was accompanied by a base-pair change, and noncomplex frameshifts, which included all other frameshift events. When necessary, the category of noncomplex frameshift events was further broken down into simple events, composed of the insertion of a single nucleotide; large deletion events in which large portions of the sequence were deleted (see below); and "other" events that did not fit into any previously described category. Our previous work has demonstrated that complex events are a unique mutational signature of Polζ-dependent mutagenesis (Harfe and Jinks-Robertson 2000) and, as such, provide a means with which to specifically monitor perturbations in Polζ mutagenic activity *in vivo*. Sequencing of revertants revealed that the percentage of complex frameshift events increased from ~6% in a wild-type strain to 23% in the *rad18* and *mms2* mutants and 28% in the *rad5* mutant (Figure 2). This translates into a six- to eightfold increase in the rate of complex events in a *rad5*, *rad18*, or *mms2* background. While the rate of noncomplex events was not significantly elevated in either a *rad5* or an *mms2* mutant, there was a slight increase in a *rad18* mutant (Table 2).

In addition to examining the proportion of complex and noncomplex frameshift events, we also compared the overall distribution of complex and simple frameshift events using a Monte Carlo-based method (see materials and methods; Adams and Skopek 1987; Cariello *et al.* 1994). Comparison of the distribution of frameshift events in a *rad18*, *mms2*, or *rad5* mutant to that of a wild-type spectrum indicated that each was significantly different from wild type ($P \leq 0.0006$). This

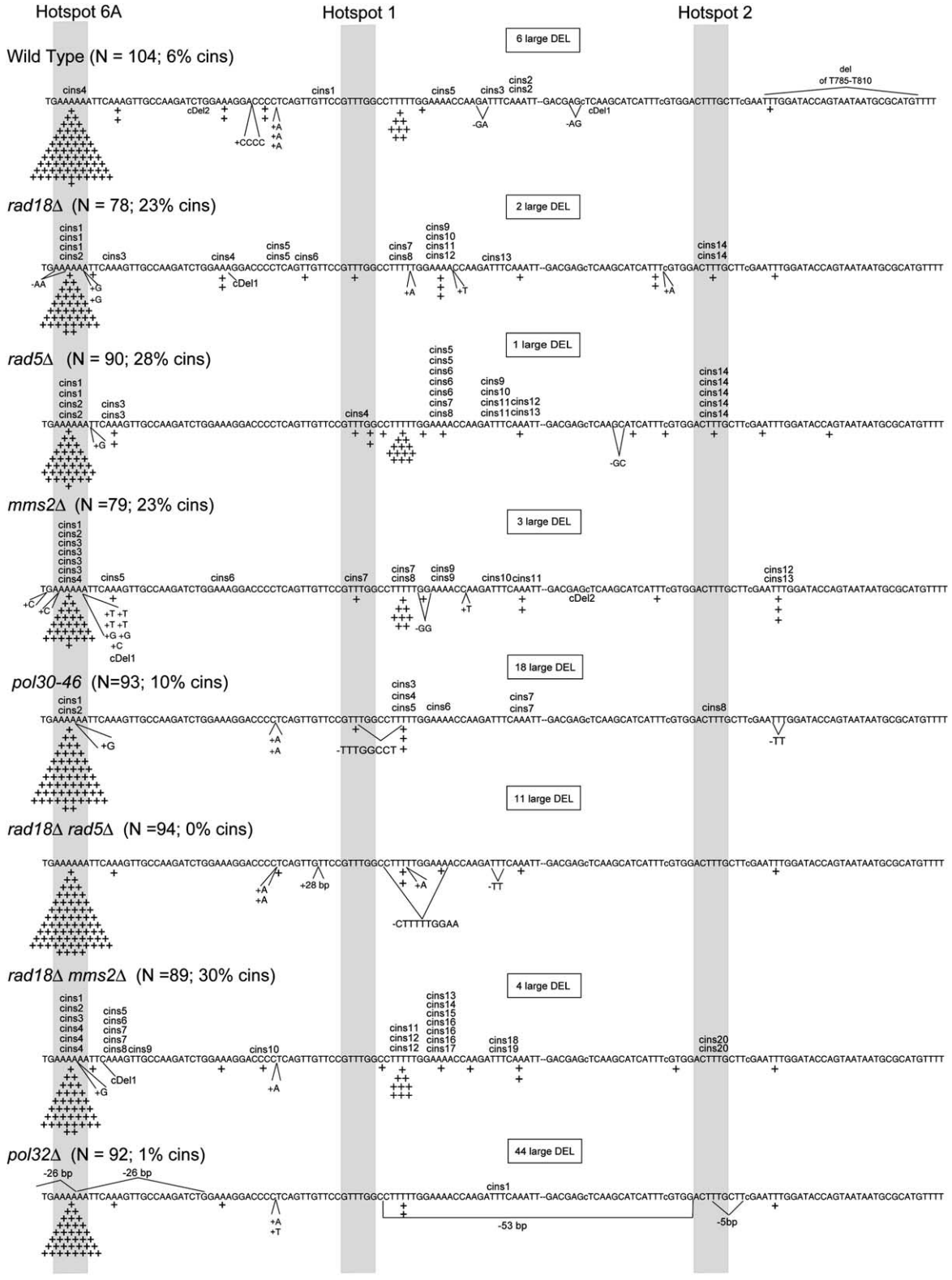

FIGURE 2.—Reversion spectra of wild-type and mutant strains. The total number of independent revertants sequenced as well as the percentage of total complex events for each strain is indicated next to the relevant strain genotype. The position of the nucleotide deleted to create the *lys2ΔA746* allele is indicated by dashes and the nucleotides changed to extend the reversion window are indicated by lowercase letters (see HARFE and JINKS-ROBERTSON 1999). Insertions of one nucleotide are shown as pluses, 2-bp deletions are shown as minuses, and complex deletions are shown as "cDel" below the sequence; complex insertions are indicated above the sequence as "cins." Individual complex events that had identical sequence changes are given identical numbers within each spectrum. The number of events created by the deletion of 95 or 131 bp between a 10- or 7-bp direct repeat, respectively, are indicated as "large DEL" and are boxed above each spectrum. The sequences for hotspots 6A, 1, and 2 are indicated by the shaded areas. The wild-type spectrum was published previously (HARFE and JINKS-ROBERTSON 1999).

is consistent with previous reports in which significant differences in forward mutation spectra were observed in *mms2*, *rad18*, and *rad5* mutants compared to a wild-type strain (Kunz *et al.* 1991; Xiao *et al.* 1999; Huang *et al.* 2002). While the distribution of frameshift events in the *mms2* mutant was similar to the distributions in both the *rad5* ($P = 0.2$) and the *rad18* ($P = 0.09$) mutants, the frameshift distributions in the *rad5* and *rad18* mutants were not similar ($P = 0.005$).

The function of Mms2 is not required in the Rad5-dependent error-prone subpathway of SLB: The initial assignment of Mms2 and Ubc13 to the error-free branch of the PRR pathway was primarily based on epistasis analysis with other *RAD6*-pathway members with regard to sensitivity to various DNA-damaging agents (Broomfield *et al.* 1998; Hofmann and Pickart 1999), which may or may not reflect the ways in which the cell deals with spontaneous damage. With regard to spontaneous lesions, the only evidence to place Mms2 and Ubc13 in the error-free pathway is that *mms2* and *ubc13* mutants have increased spontaneous Polζ-dependent mutagenesis (Hofmann and Pickart 1999; Xiao *et al.* 2000). However, an increase in spontaneous Polζ-dependent mutagenesis is also seen in *rad18* or *rad5* mutants, which are also defective in the error-prone pathways (Huang *et al.* 2000, 2002; Cejka *et al.* 2001). Given that the Mms2-Ubc13 complex physically interacts with Rad5 (Ulrich and Jentsch 2000; Ulrich 2003) and that Rad5 is active in both an error-prone and an error-free pathway of SLB (Liefshitz *et al.* 1998; Cejka *et al.* 2001), it is possible that Mms2-Ubc13 also functions in both pathways in response to spontaneous damage. If Mms2-Ubc13 is required for all functions of Rad5 in SLB, then one would expect the mutation rates and spectra of a *rad5* and an *mms2* mutant to be the same. Indeed, the overall mutation rates, the complex frameshift rates, and the distribution of frameshift events were indistinguishable between the *rad5* and *mms2* mutants (Figure 2; Table 2), which would be consistent with Mms2 functioning in both the error-free and the error-prone Rad5-dependent pathways.

To further examine possible differences between Rad5 and Mms2 loss, *lys2ΔA746* reversion rates and spectra were obtained in a *rad18* background. *rad18 rad5* strains have low, *rev3*-like levels of mutagenesis (Liefshitz *et al.* 1998; Cejka *et al.* 2001). Thus, we hypothesized that if Mms2 was needed for all functions of Rad5, *rad18 mms2* mutants would also display low levels of mutagenesis. In agreement with previous studies, *rad18 rad5* mutants had a significant decrease in the frameshift rate relative to each of the single mutants in our assay system (Table 2). Strikingly, complex frameshift events were completely absent in the *rad18 rad5* double mutant (Figure 2). This represents at least a 38-fold reduction in complex events in the *rad18 rad5* double mutant relative to the single mutants and at least a 4.8-fold reduction relative to a wild-type strain. These results are consistent with a model in which Polζ-dependent mutagenesis occurs via distinct Rad5- and Rad18-dependent error-prone pathways.

We next analyzed the *rad18 mms2* strain. If Mms2 is required for all functions of Rad5 in SLB, then a *rad18 mms2* mutant should be equivalent to a *rad18 rad5* mutant. Although the overall reversion rate of a *rad18 mms2* mutant was not significantly different from that of the *rad18 rad5* mutant, the rate of complex frameshift accumulation in the *rad18 mms2* background was dramatically different from that in a *rad18 rad5* background (Figure 2; Table 2). The rate of complex frameshift events was at least 43-fold higher in a *rad18 mms2* mutant than in the *rad18 rad5* mutant, with complex events accounting for 30% of the reversion events in the *rad18 mms2* strain (Figure 2). A comparison of the distribution of frameshift events in a *rad18 rad5* mutant *vs.* a *rad18 mms2* mutant by Monte Carlo analysis revealed highly significant differences ($P < 0.0001$). These data provide a clear distinction between the functions of Rad5 and Mms2 in response to spontaneous damage and are consistent with the existence of a Rad5-dependent, Mms2-independent error-prone bypass pathway.

Polζ-dependent mutagenesis in *rad18*, *mms2*, and *rad5* mutants in the absence of nucleotide excision repair: To more thoroughly investigate the role of Rad5, Rad18, and Mms2 in spontaneous mutagenesis, the *lys2ΔA746* reversion system was sensitized by deleting the corresponding genes in a nucleotide excision repair (NER)-defective (*rad1*) background. We anticipated that the resulting increase in the amount of unrepaired spontaneous damage would create more opportunities for Polζ-dependent bypass of DNA lesions, thus allowing enhanced detection of subtle changes in frameshift accumulation. Indeed, elimination of *rad5*, *rad18*, or *mms2* in a *rad1* background significantly increased the *lys2ΔA746* reversion rate two- to threefold when compared to either the corresponding NER-proficient parent or the *rad1* single mutant (Table 3). While the proportion of complex frameshift events did not change when *RAD5* or *RAD18* was deleted in a *rad1* background (Figure 3; 29% in *rad1 rad5 vs.* 28% in *rad5*; 29% in *rad1 rad18 vs.* 23% in *rad18*; 28% in *rad1*), the percentage of complex frameshift events nearly doubled in the *rad1 mms2* double mutant (51%) relative to the *mms2* or *rad1* single mutant (23% or 28%, respectively). These results are consistent with the involvement of Mms2 in only an error-free pathway. Multiplying the percentage of complex frameshift events of the double-mutant strains by their respective mutation rates revealed that the double mutants had complex frameshift rates three- to sevenfold greater than those of the corresponding single mutants (Tables 2 and 3).

To confirm that the increases in complex events seen in these double-mutant strains were due to Polζ-dependent activity, we eliminated *REV3* from the *rad1 rad18* mutant. In agreement with earlier observations (Huang

TABLE 3

Reversion rates of the *lys2ΔA746* allele in NER-defective strains

Strain	All frameshifts		Complex frameshifts		Noncomplex frameshifts	
	Rate ($\times 10^{-9}$)	Rate relative to *rad1*	Rate ($\times 10^{-10}$)	Rate relative to *rad1*	Rate ($\times 10^{-10}$)	Rate relative to *rad1*
rad1	4.1 (3.9–4.7)[a]	1.0	11 (11–13)	1.0	30 (28–34)	1.0
rad1 rad18	14 (11–21)	3.5	41 (32–62)	3.7	99 (78–150)	3.3
rad1 rad18 rev3	3.3 (2.0–4.0)	0.80	0.34[b] (NA)	<0.031	33 (20–52)	1.1
rad1 rad5	15 (10–19)	3.7	43 (29–55)	3.9	110 (71–140)	3.7
rad1 mms2	14 (9.8–18)	3.4	71 (50–91)	6.5	69 (48–89)	2.3
rad1 rad18 mms2	20 (13–56)	4.9	49 (32–138)	4.5	150 (98–420)	5.0
rad1 pol30-46	7.7 (5.0–10)	1.9	9.9 (6.5–13)	0.90	67 (44–87)	2.2
rad1 pol32	3.7 (2.8–9.2)	0.91	0.40 (0.3–1.0)	0.036	37 (28–91)	1.2
rad1 srs2	5.3 (3.6–6.2)	1.3	7.5 (5.1–8.8)	0.68	46 (31–53)	1.5

The category of "all frameshifts" refers to the overall reversion rate. The rates of complex and noncomplex frameshifts were calculated by multiplying the percentage of times the specific frameshift event occurred by the corresponding overall reversion rate. NA, not applicable.

[a] Values within parentheses are 95% confidence intervals.

[b] Maximum theoretical rate calculated as if one event had been detected.

et al. 2002), the increased mutation rate of the *rad1 rad18* strain was dependent upon Rev3 (Table 3). Accordingly, the *rad1 rad18 rev3* strain was devoid of complex frameshift events (Figure 3). In the triple mutant, we estimate that the rate of complex insertions was at least 25-fold lower than that in a *rad1* mutant and at least 120-fold lower than that in a *rad1 rad18* mutant.

In addition to determining the rates of different classes of frameshifts, the overall distribution of frameshift events in the double mutants was compared to those of the appropriate SLB-defective or NER-defective single mutants. By Monte Carlo analysis, neither the *rad1 rad18* nor the *rad1 mms2* double mutant was similar to the relevant single-mutant strains (data not shown). Although the distribution of complex and simple events in a *rad1 rad5* mutant differed significantly from the distribution in a *rad1* strain ($P = 0.002$), it was not significantly different from that of a *rad5* strain ($P = 0.05$).

Work in a NER-proficient background indicated that Mms2 was not required for all functions of Rad5, and we were interested in determining if this held true in response to an increased load of unrepaired spontaneous damage. Similar to their NER-proficient counterparts, the overall mutation rates and rates of complex frameshift formation of the *rad1 rad5* and *rad1 mms2* mutants were not statistically different. However, the distribution of frameshift events by Monte Carlo analysis revealed significant differences between *rad1 mms2* and *rad1 rad5* strains ($P < 0.0001$).

In the absence of NER, Rad18 and Rad5 are required for accumulation of complex frameshift events at discrete locations in the *lys2ΔA746* reversion window: One striking feature of the *rad1* spectrum is the accumulation of complex frameshift events primarily in two distinct locations, termed hotspots 1 and 2. These locations are thought to be sites of a specific type (or class) of spontaneous damage that remains in the genome in the absence of NER, thus making it a substrate for Polζ-dependent translesion synthesis (Harfe and Jinks-Robertson 2000; B. Minesinger, A. Abdulovic and S. Jinks-Robertson, unpublished results). In stark contrast to the *rad1 mms2* and the *rad1 rad5* spectra, each of which contained complex frameshifts at hotspots 1 and 2, there were no events at these locations in the *rad1 rad18* spectrum (Figure 3). Relative to a *rad1* strain, both the *rad1 mms2* and the *rad1 rad5* mutants had an ~2-fold increase in the rate of complex events at hotspots 1 and 2, while the *rad1 rad18* strain had at least a 6.7-fold decrease in the rate of these events (Table 4). The lack of complex events at hotspots 1 and 2 in the *rad1 rad18* mutant (as well as a *rad1 rad18 mms2* mutant; see Figure 3) suggests that Polζ requires the presence of Rad18 to bypass the relevant lesion(s) that accumulates near hotspots 1 and 2 in the absence of NER. Alternatively, Polζ may still bypass the lesion(s) in the absence of Rad18, but does so in a manner undetectable in our assay system.

In addition to changes in the distributions of complex events at hotspots 1 and 2, there was also a striking difference in the accumulation of complex events at a 6A run located at the 5′ end of the reversion window in the *rad1 rad5* strain compared to either the *rad1 rad18* or the *rad1 mms2* strain (Figure 3). Complex events at the 6A run accounted for ~11% of the total reversion events in the *rad1 rad18* and *rad1 mms2* strains, whereas these events composed <1.2% of the *rad1 rad5* spectrum (assuming one event). This corresponds to at least an eightfold greater rate of complex events at the 6A run in the *rad1 rad18* or the *rad1 mms2* strain compared to the *rad1 rad5* mutant (Table 4). Interestingly, the

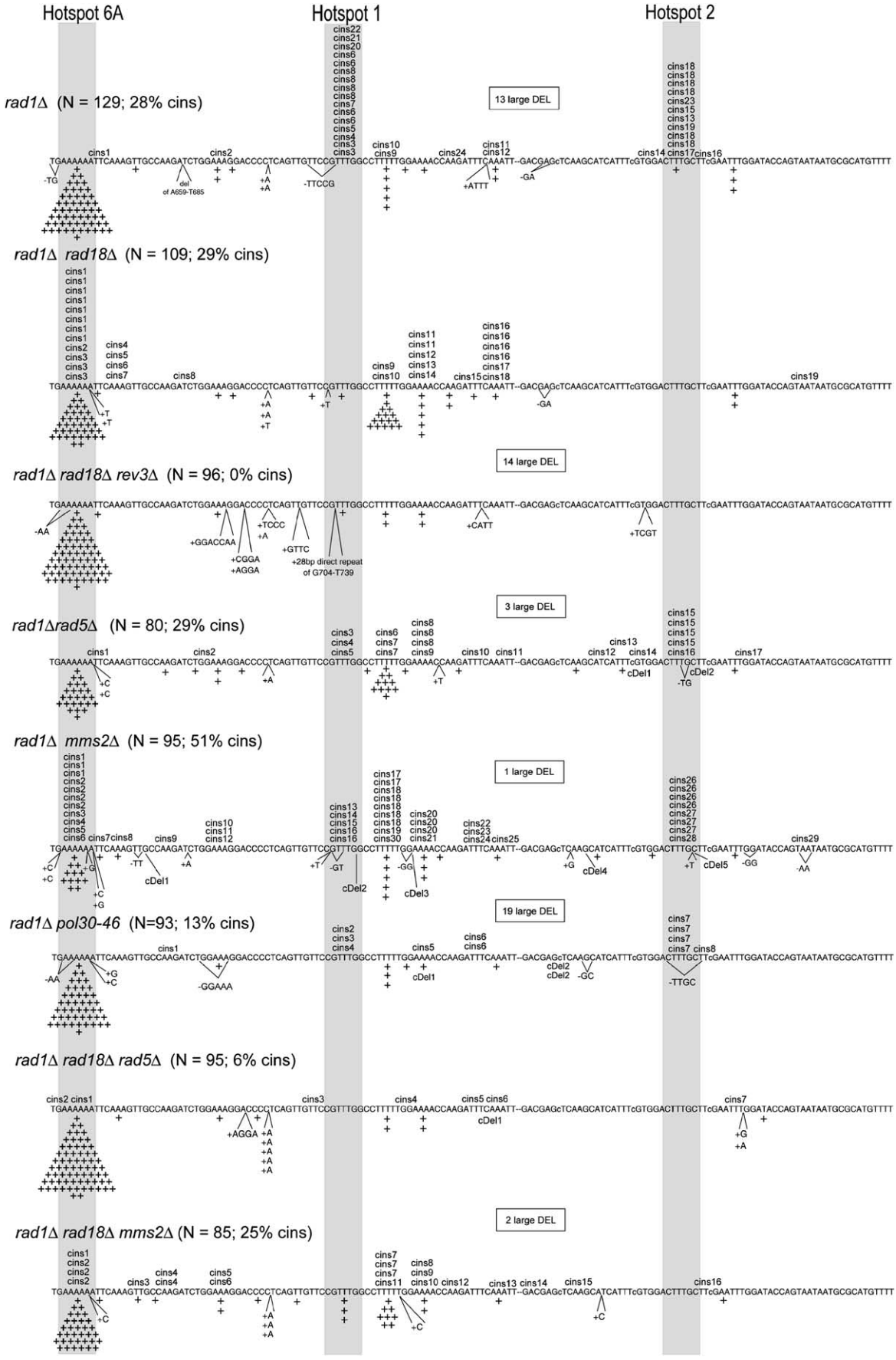

FIGURE 3.—Reversion spectra of strains defective in NER and SLB. See Figure 2 for details.

TABLE 4

Rates of complex events at discrete locations in the *lys2ΔA746* allele

Strain	Rate at hotspots 1 and 2 ($\times 10^{-10}$)	Rate at 6A run ($\times 10^{-10}$)
WT	0.30[a] (NA)[b]	0.31 (0.26–0.36)
rad1	8.6 (8.2–9.8)	0.39[a] (NA)
rad18	1.6 (1.3–2.0)	3.2 (2.6–3.9)
rad1 rad18	1.3[a] (NA)	16 (13–24)
rad5	3.5 (3.1–4.7)	2.3 (2.0–3.2)
rad1 rad5	15 (10–19)	1.9[a] (NA)
mms2	0.63 (0.56–0.87)	4.4 (3.9–6.1)
rad1 mms2	19 (13–25)	15 (11–20)
rad1 pol30-46	5.8 (3.8–7.5)	NA
rad1 srs2	1.7 (1.2–2.0)	NA

The rates of complex events at hotspots 1 and 2 and at the 6A run were calculated by multiplying the percentage of times the specific frameshift event occurred by the corresponding overall reversion rate. WT, wild type; NA, not applicable.

[a] Maximum theoretical rate calculated as if one event had been detected.

[b] Values within parentheses are 95% confidence intervals.

discrepancy between the rates of complex frameshifts at the 6A run of *rad5*, *rad18*, and *mms2* mutants was evident only in the absence of NER, suggesting that in the absence of NER, lesion(s) that accumulate near the 6A run require Rad5 for efficient Polζ-dependent mutagenesis.

Fitness defects are apparent when both *RAD18* and *RAD5* are deleted in a NER-defective background: To further characterize the roles of Rad18, Rad5, and Mms2 in the presence of an increased spontaneous damage load, we created *rad1 rad18 mms2* and *rad1 rad18 rad5* strains. Although the *rad1 rad18 mms2* mutant demonstrated a nearly fivefold increase in the overall and complex *lys2ΔA746* reversion rate relative to the *rad1* single mutant, these rates were not statistically different from those of the *rad1 mms2* or *rad1 rad18* strain (Table 3). Complex reversion events in the triple mutant composed 25% of the spectrum (Figure 3), similar to that of the *rad1 rad18* strain (29%) and less than half that of the *rad1 mms2* strain (51%). By Monte Carlo analysis, the distribution of simple and complex frameshift events in a *rad1 rad18 mms2* reversion spectrum differed significantly from the *rad1* and *rad1 mms2* spectra ($P \leq 0.0001$), but was not different from the *rad1 rad18* spectrum ($P = 0.055$). Notably, complex reversion events were completely absent at hotspots 1 and 2 in *rad1 rad18 mms2* triple mutant, similar to the *rad1 rad18* spectrum (Figure 3), strengthening the argument that Rad18 is required for bypass of a lesion(s) accumulating near these locations.

Examination of a *rad1 rad18 rad5* strain revealed an unexpected fitness defect. Plating efficiencies (percentage of colonies formed per total cell number) of the *rad1 rad18 rad5* mutant were at least 5-fold lower than that of a *rad1* strain and at least 2.5-fold lower than that of a *rad1 rad18* strain (data not shown). The precise plating efficiency and reversion rate were difficult to quantify accurately in this mutant, as the percentage of viable cells varied considerably between cultures. In addition to inconsistent plating efficiency, colony sizes were variable in the *rad1 rad18 rad5* mutant background (data not shown). This defect in fitness was observed only in the *rad18 rad5* mutant in the absence of NER, which suggests that when NER and all three branches of the SLB pathway are disabled simultaneously, the remaining DNA repair/bypass pathways are unable to fully compensate for the loss, leading to an increase in cell death or loss of cell proliferation. Despite the decrease in fitness, we examined the *lys2ΔA746* reversion spectrum of the *rad1 rad18 rad5* strain. Given that the *rad18 rad5* mutant did not accumulate complex reversion events, we were surprised to detect complex events when *RAD18* and *RAD5* were deleted in a *rad1* background (Figure 3). The unexpected presence of complex events suggests that Polζ may be able to promote translesion synthesis without Rad18 and Rad5 in response to an increased load of spontaneous damage. However, given the fitness defect of this strain, it is possible that other repair or replication-associated processes may be perturbed, resulting in the complex events detected in the *rad1 rad18 rad5* mutant spectrum. An investigation of whether Polζ was responsible for the complex events seen in the *rad1 rad18 rad5* mutation spectrum would require the creation of a *rad1 rad18 rad5 rev3* quadruple-mutant strain, which was not attempted due to the poor growth of the triple-mutant strain.

***pol30-46* mutants have increased overall spontaneous mutation rates, but *pol30-46* spectra are not equivalent to *mms2* spectra:** On the basis of epistasis analysis with *mms2* and *pol30-46* mutants in response to exogenous damage, Ulrich (2001) and Broomfield and Xiao (2002) have placed PCNA downstream of *MMS2* in the error-free bypass pathway. *pol30-46* mutants demonstrate an increase in spontaneous mutagenesis of 2- to 7-fold relative to a wild-type strain (Ayyagari *et al.* 1995; Torres-Ramos *et al.* 1996); whether Polζ contributes to the *pol30-46* mutator phenotype has not been determined. Therefore, we examined the *lys2ΔA746* reversion rates and spectra of *pol30-46* strains in the presence and absence of NER. If PCNA resides downstream of *MMS2* in the error-free SLB pathway, we hypothesized that spontaneous mutagenesis in a *pol30-46* mutant would be similar to that in an *mms2* mutant; namely, *pol30-46* strains should have an overall increase in mutation rate and a specific increase in the rate of complex frameshift events similar to that of *mms2* strains. Indeed, *pol30-46* mutants had an increased overall mutation rate that was slightly, but significantly (1.4-fold), greater than that of a wild-type strain, similar to previous reports

(Ayyagari *et al.* 1995; Torres-Ramos *et al.* 1996; Table 2). This rate was indistinguishable from that of an *mms2* strain. While the rate of complex frameshift events increased by 2.4-fold in the *pol30-46* mutant relative to a wild-type strain, the rate of complex frameshift events in the *pol30-46* mutant *decreased* by >2-fold relative to the *mms2* strain. Using Monte Carlo analysis, we found that the distribution of frameshifts in the *pol30-46* spectrum was significantly different from that in the *mms2* spectrum ($P = 0.0023$), but not from that in the wild-type spectrum ($P = 0.24$). Similar results were obtained when mutation rates and spectra were compared between the NER-defective *rad1 pol30-46* and *rad1 mms2* strains (Table 3; Figure 3). While the overall mutation rates of the *rad1 pol30-46* and the *rad1 mms2* strains were not significantly different, the rate of complex events was 7-fold lower in the *rad1 pol30-46* strain than in the *rad1 mms2* strain (Table 3). Monte Carlo analysis revealed significant differences between the distribution of frameshift events when comparing the *rad1 mms2* and *rad1 pol30-46* strains ($P < 0.0001$), but not when comparing the *rad1* and the *rad1 pol30-46* strains ($P = 0.36$). Taken together, these data indicate that even though *POL30* and *MMS2* both play a role in response to spontaneous damage, they are not equivalently required for the same process (see discussion).

Additional analysis of the *pol30-46* mutation spectra in both the presence and the absence of NER revealed an unusually high number of large deletion events, in which 95 or 131 bp were deleted between either a 10- or a 7-bp direct repeat, respectively. The rate of large deletion events in a *pol30-46* strain (8.5×10^{-10}) was nearly fivefold greater than that in a wild-type strain (1.8×10^{-10}). These results were similar to the fourfold increase in large deletion events detected in the *rad1 pol30-46* strain (16×10^{-10}) relative to the *rad1* strain (4.1×10^{-10}) and suggest that processive DNA replication may be perturbed in *pol30-46* mutants.

Pol32, a subunit of Polδ, is required for complex frameshift events: In addition to PCNA, another protein involved in DNA replication, Pol32, has been placed in the *RAD6* epistasis group on the basis of sensitivities of *pol32* mutants to DNA-damaging agents (Huang *et al.* 2000). Pol32 is a small, nonessential subunit of the replicative DNA polymerase Polδ (Gerik *et al.* 1998) and appears to help tether Polδ to PCNA (Burgers and Gerik 1998; Lu *et al.* 2002). *POL32* has been implicated in Polζ-dependent mutagenesis, as *pol32* mutants display defects in damage-induced mutagenesis and have reduced levels of spontaneous mutagenesis (Gerik *et al.* 1998; Huang *et al.* 2000, 2002). However, it is not clear whether the defects in spontaneous mutagenesis are the direct result of a perturbation in Polζ-dependent mutagenesis or the result of a decrease in another type of mutagenesis. We, therefore, created a *rad1 pol32* strain and compared the *lys2ΔA746* reversion rate and spectrum to those of the *rad1* parent strain. Although the overall reversion rate was not significantly affected (Table 3), there was a dramatic change in the reversion spectrum upon deletion of *POL32*. Of 94 reversion events sequenced, only 1 event was complex (1.1%) in the *rad1 pol32* double mutant compared to 36 complex events among 129 sequenced (28%) in the *rad1* parent strain (Figure 4). This corresponds to an ~30-fold decrease in the rate of accumulation of complex events in the *rad1 pol32* double mutant relative to the *rad1* strain. In addition to a decrease in complex insertions and consistent with the findings of Huang *et al.* (2002), there was also a 2.4-fold increase in the rate of large deletion events in the absence of *POL32*. The significant increase in large deletion events detected in the *rad1 pol32* strain was not a reflection of DNA damage repair capacity of the cell, as NER-proficient *pol32* strains also demonstrated a significant 30-fold increase in the rate of large deletion events relative to a wild-type strain (Figure 2). These data indicate that in the absence of *POL32*, Polζ-dependent mutagenesis is compromised and that replication processivity is perturbed.

Srs2 decreases Polζ-dependent spontaneous mutagenesis at hotspots 1 and 2 in a *rad1* strain: *SRS2* was originally uncovered in a screen for mutations that suppress the damage sensitivity of a *rad6* mutant (Lawrence and Christensen 1979). Srs2 is a nonessential 3′-5′ helicase (Rong and Klein 1993) that appears to regulate the use of the *RAD6* pathway in response to exogenous damage by preventing Rad51-dependent recombination (Schiestl *et al.* 1990; Veaute *et al.* 2003). Although a role for Srs2 in the induced damage response is clear, evaluation of mutation rates in *srs2* mutants has not detected a role for *SRS2* in response to spontaneous damage (Liefshitz *et al.* 1998). It is possible, however, that changes in spontaneous Polζ-dependent mutagenesis in *srs2* mutants may be too subtle to detect by conventional mutation rate calculations. We, therefore, examined both the overall and the complex frameshift mutation rate of the *lys2ΔA746* allele in an *srs2* strain.

If lesions accumulating in a *rad1* background were substrates for Rad51-dependent recombination as well as Polζ-dependent translesion synthesis, one would predict that loss of Srs2 should lead to a decrease in the accumulation of complex frameshift events in an *srs2* mutant, as more lesions would be bypassed by a recombination-based mechanism. Consistent with this idea, the rate of complex frameshift events in the *rad1 srs2* strain was slightly but significantly less than that in a *rad1* strain (Table 3). A much larger decrease in rate of complex frameshift events was seen at hotspots 1 and 2, with a fivefold decrease in the rate of complex frameshifts in the *rad1 srs2* strain relative to the *rad1* strain (Figure 4; Table 4). This suggests that in the absence of NER, loss of Srs2 prevents Polζ-dependent bypass of at least a subset of the lesion(s) accumulating at or near hotspots 1 and 2.

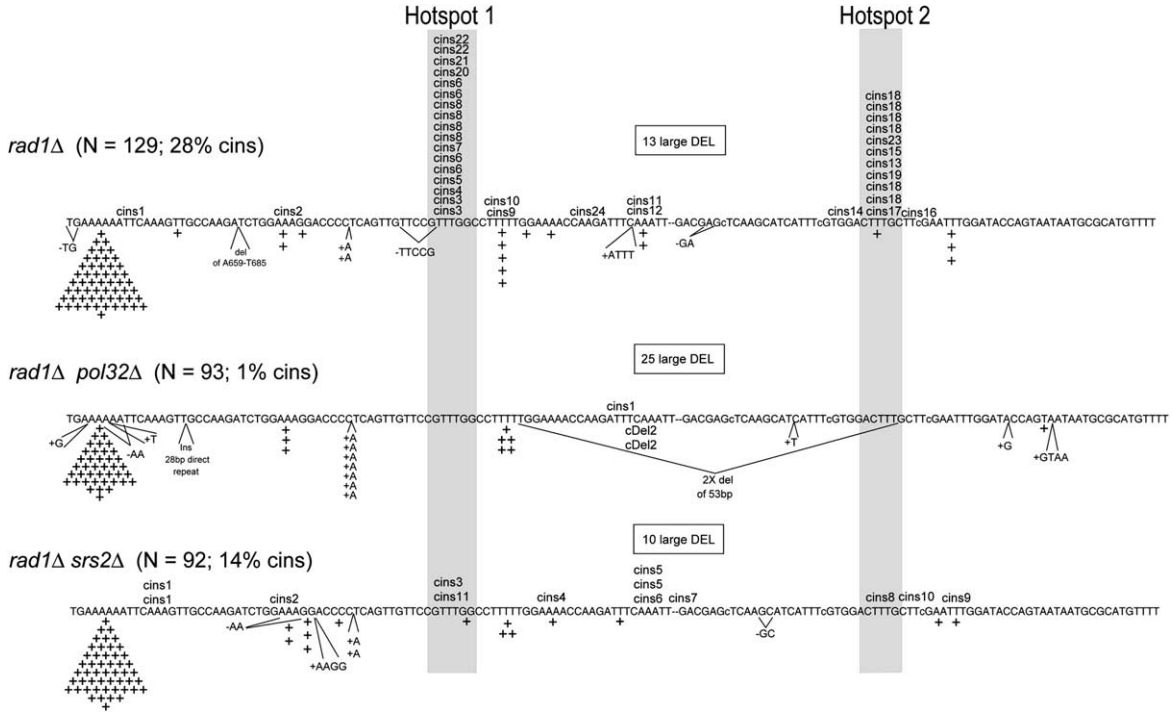

FIGURE 4.—Reversion spectra of repair-defective strains. See Figure 2 for details.

DISCUSSION

Reversion of the chromosomal *lys2ΔA746* allele occurs via a wide variety of net +1 frameshifts within an ~150-bp reversion window. Complex reversion events, in which one or more base substitutions accompany the selected frameshift, are of particular significance, as our previous studies have demonstrated that these events are completely dependent on the activity of the Polζ translesion DNA polymerase (HARFE and JINKS-ROBERTSON 2000). In this work, we have used this unique mutational signature to probe the relationship between members of the *RAD6* epistasis group and spontaneous Polζ-dependent mutagenesis.

The current three-pathway model for SLB by members of this epistasis group is based primarily on rates of *CAN1* forward mutations, which are assumed to be mostly base substitution events. Two distinct error-prone arms have been proposed—one controlled by the Rad6-Rad18 heterodimer and the other by a Rad5 homodimer—as well as an error-free arm composed of Rad6, Rad18, Rad5, Mms2, Ubc13, PCNA, and Polδ (LIEFSHITZ *et al.* 1998; CEJKA *et al.* 2001). Both error-prone pathways appear to involve translesion synthesis by Polζ, while the error-free pathway is speculated to involve a strand-switching mechanism in which the newly synthesized strand of the sister chromatid is used as a template to bypass the lesion. As predicted by this model, we observed an increase in the *lys2ΔA746* reversion rate in the absence of Mms2, Rad18, or Rad5, each of which is in the error-free arm. Although the increase in overall reversion rate was twofold at most, the types of mutations that occurred in a *rad18*, *mms2*, or *rad5* mutant were dramatically different from those in a wild-type strain. Specifically, there were proportionally many more complex events in the mutant spectra than in the wild-type spectrum. Relative to wild type, the rates of Polζ-dependent complex frameshift events increased six- to eightfold, while the rates of noncomplex frameshifts changed little, if any, in the mutants. Whereas elimination of *rad5* or *rad18* alone caused a striking increase in complex frameshifts, simultaneous deletion of *rad5* and *rad18* resulted in the complete disappearance of these events. The frameshift spectra thus clearly demonstrate not only that Rad5 and Rad18 participate in an error-free pathway, but also that each protein individually regulates a Polζ-dependent error-prone bypass mechanism.

The *lys2ΔA746* reversion rate increases observed when Rad18, Rad5, or Mms2 was eliminated in a NER-compromised *rad1* background were also consistent with a three-pathway model for spontaneous lesion bypass. In contrast to the *RAD1* background, however, where only the rates of complex events were elevated, both complex and noncomplex events were elevated to the same extent in the *rad1 rad18* and *rad1 rad5* double mutants. At least in the case of the *rad1 rad18* mutant, the rate elevation of both types of events was dependent on the presence of Rev3, indicating that Polζ can generate noncomplex as well as complex frameshifts in the *lys2ΔA746* assay. Relative to the *rad1* single mutant and unlike the *rad1 rad5* or *rad1 rad18* mutant, complex events were elevated more than noncomplex events in the *rad1 mms2* mutant, consistent with the involvement

of Mms2 only in an error-free bypass pathway (see below for further discussion).

The examination of mutation spectra when *RAD6* epistasis group genes were deleted in a NER-defective background proved to be particularly informative. Normally in a *rad1* background, complex events compose 28% of the mutant spectrum and accumulate at two distinct hotspots (hotspots 1 and 2). These hotspots are believed to correspond to sites of a discrete type of spontaneous damage (most likely an oxidative lesion; B. Minesinger, A. Abdulovic and S. Jinks-Robertson, unpublished observations) that is specifically removed by the NER pathway. In the absence of NER, the damage presumably is bypassed by a mechanism that leads to the observed accumulation of the Polζ-dependent complex events. (However, it is formally possible that these mutations reflect the primary extension of base mispairs by Polζ, rather than by a Polζ-dependent lesion bypass.) The presence of the distinct hotspots in a *rad1* background allowed not only these specific complex events to be monitored but also additional complex hotspots to become evident upon deletion of *RAD18*, *RAD5*, or *MMS2*. In the NER-defective background, *rad18* mutants failed to accumulate any complex events at hotspot 1 or 2, while the rate of these events increased twofold upon elimination of Rad5 or Mms2. These data indicate that complex events at hotspots 1 and 2 are generated specifically by the Rad18-dependent error-prone pathway; either the Rad5-dependent error-prone pathway does not bypass the underlying lesion or bypass occurs in a manner not detected by the *lys2ΔA746* assay system. In contrast to the *rad1 rad18* mutant, the *rad1 rad5* strain did not accumulate complex events at a novel hotspot within a 6A run, implying that the Rad18 error-prone pathway does not generate these events. Thus, although both the Rad5- and the Rad18-dependent error-prone pathways promote Polζ-dependent lesion bypass, the distinct mutational signatures indicate that these pathways are not simply functionally redundant. In *E. coli*, the sequence context of a defined lesion appears to determine the specific constellation of proteins required for translesion synthesis (Wagner *et al.* 2002). Our data might reflect a similar context-specific bypass of a common lesion or could reflect the bypass of different lesions by the Rad5- *vs.* Rad18-dependent error-prone pathway. Alternatively, the changes in the distributions of complex events could reflect distinct roles for Rad5 and/or Rad18 when bypassing lesions during normal DNA replication *vs.* gap filling or during leading- *vs.* lagging-strand DNA synthesis.

The third branch of the three-pathway model of SLB is an error-free arm regulated by a heterodimer of Rad6-Rad18 and a complex of Rad5-Ubc13-Mms2. Although Ubc13-Mms2 forms a stable complex with Rad5 (Ulrich and Jentsch 2000; Ulrich 2003), the possibility that Ubc13-Mms2 might also be involved in the Rad5-, Polζ-dependent error-prone bypass pathway has not been addressed. The indistinguishable *lys2ΔA746* reversion rates of *rad5* and *mms2* mutants (Tables 2 and 3) would be consistent with a coincident requirement for Rad5 and Ubc13-Mms2 in both error-free and error-prone pathways of SLB. Examination of the corresponding reversion spectra, however, does not support a role for Ubc13-Mms2 in the Rad5-dependent error-prone pathway. This is most evident in a *rad18* background, where deletion of *MMS2 vs. RAD5* generated reversion spectra containing 30% complex events *vs.* 0% complex events, respectively (Figure 2). In addition to the clear separation of the roles of Rad5 and Mms2 with regard to spontaneous mutagenesis, strains completely lacking Rad5 are more sensitive to UV than are strains harboring a mutant Rad5 protein that is unable to physically interact with the Mms2-Ubc13 heterodimer (Ulrich 2003). This suggests that there also may be an Mms2-Ubc13 independent role of Rad5 in induced DNA damage tolerance. Given the homology of Rad5 to the Swi/Snf family of chromatin remodeling factors (Johnson *et al.* 1994) and its genetic interaction with histone H2B (Martini *et al.* 2002), it is possible that the Mms2-Ubc13 independent activity of Rad5 may involve chromatin remodeling.

Although there were no complex events among the 94 *lys2ΔA746* revertants analyzed in the *rad18 rad5* double-mutant background, 7% (7/95) of the revertants isolated in the *rad1 rad18 rad5* triple-mutant background harbored a complex frameshift. These data suggest either that repair/bypass factors other than Polζ can generate these events or that Polζ retains some function even in the absence of both Rad18 and Rad5. In relation to the latter possibility, it has been reported that deletion of *RAD5* and *RAD18* in a recombination-defective strain (*rad51* mutant) does not reduce the *CAN1* mutation rate to that of a *rad51 rev3* strain, suggesting that a portion of Polζ activity remains (Liefshitz *et al.* 1998). The facilitator (if any) of this Polζ-dependent activity in the absence of Rad18 and Rad5 is not known, but may depend upon Rad6, independent of its association with Rad18 (Cejka *et al.* 2001). While the relationship between the complex events detected in the triple-mutant strain and Rev3 was not determined due to the poor health of the *rad1 rad18 rad5* strain, it should be noted that in every spectrum examined to date, complex events completely disappear upon deletion of *REV3*.

PCNA interacts with multiple proteins involved in DNA synthesis and DNA repair, presumably targeting/recruiting these proteins as needed to sites of nascent DNA synthesis (Maga and Hubscher 2003). It recently has been shown that post-translation ubiquitination and/or sumoylation of PCNA is important for activating the error-prone and error-free arms of the *RAD6* pathway (Hoege *et al.* 2002; Stelter and Ulrich 2003; Haracska *et al.* 2004). Current data suggest that either Ubc9-Siz1-dependent sumoylation or Rad6-Rad18-dependent monoubiquitination of amino acid K164 of PCNA (to-

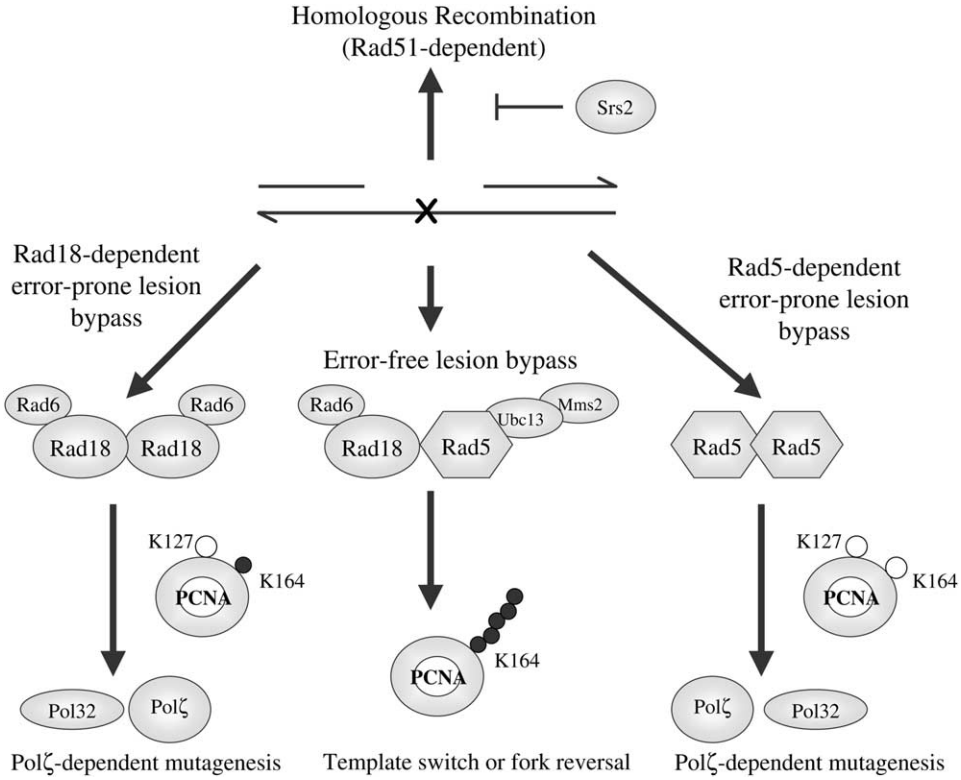

FIGURE 5.—Proposed three-pathway model for spontaneous lesion bypass by *RAD6* epistasis group members. Bypass of spontaneous damage (indicated as X) can be processed by Rad6-Rad18-controlled or Rad5-controlled error-prone pathways, which have overlapping specificities for lesion bypass. The Rad18-controlled error-prone pathway requires simultaneous K127 sumoylation and K164 ubiquitination of PCNA, whereas the Rad5-controlled error-prone pathway requires simultaneous sumoylation of both K164 and K127 of PCNA. Mms2 is not required for the Rad5-dependent error-prone arm. An error-free pathway is composed of Rad6, Rad18, Rad5, Mms2, and Ubc13 and requires multi-ubiquitination of K164 of PCNA. SUMO is depicted as an open circle; ubiquitin is depicted as a solid circle. See text for details.

gether with sumoylation of K127) leads to Polζ-dependent translesion synthesis. Error-free lesion bypass appears to be triggered when the Rad5-Ubc13-Mms2 complex adds multiple K63-linked ubiquitin proteins to the initial monoubiquitin moiety. These data have led to the proposal of a two-pathway model for lesion bypass in response to induced DNA lesions: an error-prone, Polζ-dependent pathway regulated by Rad6-Rad18 and an error-free pathway that requires the Rad5-Ubc13-Mms2 complex in addition to the Rad6-Rad18 complex. While a similar model has been proposed to explain the bypass of spontaneous lesions, a two-pathway model does not take into account the Rad5-dependent error prone pathway, which, suggested by others (LIEFSHITZ *et al.* 1998; CEJKA *et al.* 2001) and clearly shown here, does not involve the Mms2-Ubc13 complex. Incorporating the data of STELTER and ULRICH (2003) into a three-pathway model, we suggest that simultaneous sumoylation of PCNA at K127 and K164 in the absence of *RAD18* (disabling the error-free pathway) leads to Rad5-dependent error-prone bypass, whereas simultaneous sumoylation at K127 and monubiquitination at K164 (*i.e.*, in an *mms2*, *ubc13*, or *rad5* mutant) leads to Rad18-dependent error-prone bypass (Figure 5). An important area of future studies will be to determine the relevance of the various PCNA modifications to the Rad5- *vs.* Rad18-dependent error-prone pathways of spontaneous lesion bypass.

An additional connection between PCNA and lesion bypass has come from analysis of the *pol30-46* allele, which was uncovered by double alanine-scanning mutagenesis (AYYAGARI *et al.* 1995). In response to induced damage, *pol30-46* appears to affect the error-free lesion bypass pathway downstream of Mms2 (AYYAGARI *et al.* 1995; XIAO *et al.* 2000). Little is understood, however, about how this allele affects the bypass of spontaneous DNA lesions. The reversion rate of the *lys2ΔA746* allele was similar in the *pol30-46* and *mms2* mutant strains, and we expected the reversion spectra to also be similar. They were significantly different, however, leading to an estimated twofold reduction in the overall rate of complex frameshifts in the *pol30-46* mutant relative to the *mms2* mutant. Significant differences were also detected in the rate of complex frameshift events in the *pol30-46* mutant and the *mms2* mutant when NER was compromised, with a sevenfold decrease in complex frameshift events in the *rad1 pol30-46* strain relative to the *rad1 mms2* strain. The *pol30-46* allele thus clearly affects the bypass of spontaneous and induced lesions in fundamentally different ways. It should also be noted that *pol30-46* strains may have replication processivity defects (an attribute that had not been detected previously for this allele), as large deletion events increased significantly in *pol30-46* strains relative to the parental strains.

In addition to examining the role of PCNA in response to spontaneous Polζ-dependent mutagenesis, we have also investigated the role of Polδ in this process. Polδ is composed of one nonessential (Pol32) and two essential (Pol3 and Pol31) subunits (GERIK *et al.* 1998).

Mutations in *POL3* and *POL32* are epistatic with *rad6* mutations in terms of DNA damage sensitivity and cause defects in induced mutagenesis in response to UV and MNNG damage (Gerik *et al.* 1998; Haracska *et al.* 2000a; Huang *et al.* 2000). Similar decreases in spontaneous mutagenesis have been detected using the *CAN1* forward mutation assay, where deletion of *POL32* resulted in a weak antimutator phenotype in a wild-type background and completely eliminated the spontaneous mutator phenotype of strains defective in NER (*rad1* and *rad10*) and in lesion bypass (*rad6*, *rad18*, and *mms2*), but not in recombination (*rad51* and *rad52*; Huang *et al.* 2000, 2002). Deletion of *REV3* conferred similar phenotypes in these backgrounds, suggesting an essential role for Pol32 in the Polζ-dependent bypass of spontaneous DNA lesions. Although loss of *POL32* did not decrease the spontaneous reversion rate of the *lys2Δ A746* allele in a *rad1* background, it did completely eliminate complex frameshifts in this assay, indicating that it is indeed essential for Polζ-dependent lesion bypass. Given that the Pol32 subunit of Polδ interacts with PCNA, Polα, and Srs2 (Huang *et al.* 2000; Johansson *et al.* 2004), Pol32 could be involved in sensing, signaling, or coordinating the replacement of Polδ with Polζ at sites of blocking damage. Alternatively, Pol32 could be required for Polδ to insert a nucleotide opposite a lesion, with the resulting mispaired terminus then being extended by Polζ (see Haracska *et al.* 2001, 2003).

The results reported here highlight the complexity of DNA damage tolerance pathways in yeast and underscore differences between lesion bypass in response to spontaneous *vs.* induced DNA damage. Data obtained using the *lys2ΔA746* reversion assay are consistent with a three-pathway model for the bypass of spontaneous lesions by the *RAD6* epistasis group (Figure 5). The analysis of reversion spectra in appropriate mutant strains not only has confirmed functional differences between Rad5 and Mms2, but also has revealed clear differences between the Rad18- and Rad5-dependent error-prone bypass pathways. Given that many of the proteins examined in this study are highly conserved, these results will likely be relevant to understanding the role of lesion bypass in spontaneous damage tolerance in higher eukaryotes.

We thank Peter Burgers for generously providing the *pol30-46* insertion and PCNA-disruption plasmids, Louise Prakash for *RAD18* and *SRS2* disruption plasmids, and Rachelle Spell and the members of the S.J.-R. laboratory for critically reading this manuscript. This work was supported by grant GM-64769 to S.J.-R. from the National Institutes of Heath. B.K.M. was partially supported by the Graduate Division of Biological and Biomedical Sciences of Emory University.

LITERATURE CITED

Adams, W. T., and T. R. Skopek, 1987 Statistical test for the comparison of samples from mutational spectra. J. Mol. Biol. **194:** 391–396.

Altman, D. G., 1990 *Practical Statistics for Medical Research.* CRC Press, New York.

Ayyagari, R., K. J. Impellizzeri, B. L. Yoder, S. L. Gary and P. M. Burgers, 1995 A mutational analysis of the yeast proliferating cell nuclear antigen indicates distinct roles in DNA replication and DNA repair. Mol. Cell. Biol. **15:** 4420–4429.

Bailly, V., S. Lauder, S. Prakash and L. Prakash, 1997 Yeast DNA repair proteins Rad6 and Rad18 form a heterodimer that has ubiquitin conjugating, DNA binding, and ATP hydrolytic activities. J. Biol. Chem. **272:** 23360–23365.

Boeke, J. D., J. Trueheart, G. Natsoulis and G. R. Fink, 1987 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 164–175.

Broomfield, S., and W. Xiao, 2002 Suppression of genetic defects within the *RAD6* pathway by *srs2* is specific for error-free postreplication repair but not for damage-induced mutagenesis. Nucleic Acids Res. **30:** 732–739.

Broomfield, S., B. L. Chow and W. Xiao, 1998 *MMS2*, encoding a ubiquitin-conjugating-enzyme-like protein, is a member of the yeast error-free postreplication repair pathway. Proc. Natl. Acad. Sci. USA **95:** 5678–5683.

Broomfield, S., T. Hryciw and W. Xiao, 2001 DNA postreplication repair and mutagenesis in *Saccharomyces cerevisiae.* Mutat. Res. **486:** 167–184.

Burgers, P. M., and K. J. Gerik, 1998 Structure and processivity of two forms of *Saccharomyces cerevisiae* DNA polymerase δ. J. Biol. Chem. **273:** 19756–19762.

Cariello, N. F., W. W. Piegorsch, W. T. Adams and T. R. Skopek, 1994 Computer program for the analysis of mutational spectra: application to p53 mutations. Carcinogenesis **15:** 2281–2285.

Cejka, P., V. Vondrejs and Z. Storchova, 2001 Dissection of the functions of the *Saccharomyces cerevisiae RAD6* postreplicative repair group in mutagenesis and UV sensitivity. Genetics **159:** 953–963.

Dohmen, R. J., K. Madura, B. Bartel and A. Varshavsky, 1991 The N-end rule is mediated by the UBC2 (Rad6) ubiquitin-conjugating enzyme. Proc. Natl. Acad. Sci. USA **88:** 7351–7355.

Friedberg, E. C., G. C. Walker and W. Siede, 1995 *DNA Repair and Mutagenesis.* ASM Press, Washington, DC.

Gerik, K. J., X. Li, A. Pautz and P. M. Burgers, 1998 Characterization of the two small subunits of *Saccharomyces cerevisiae* DNA polymerase δ. J. Biol. Chem. **273:** 19747–19755.

Gietz, R. D., R. H. Schiestl, A. R. Willems and R. A. Woods, 1995 Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast **11:** 355–360.

Giot, L., R. Chanet, M. Simon, C. Facca and G. Faye, 1997 Involvement of the yeast DNA polymerase δ in DNA repair *in vivo.* Genetics **146:** 1239–1251.

Goldstein, A. L., and J. H. McCusker, 1999 Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae.* Yeast **15:** 1541–1553.

Haracska, L., S. Prakash and L. Prakash, 2000a Replication past O^6-methylguanine by yeast and human DNA polymerase η. Mol. Cell. Biol. **20:** 8001–8007.

Haracska, L., S. L. Yu, R. E. Johnson, L. Prakash and S. Prakash, 2000b Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase η. Nat. Genet. **25:** 458–461.

Haracska, L., I. Unk, R. E. Johnson, E. Johansson, P. M. Burgers *et al.*, 2001 Roles of yeast DNA polymerases δ and ζ and of Rev1 in the bypass of abasic sites. Genes Dev. **15:** 945–954.

Haracska, L., S. Prakash and L. Prakash, 2003 Yeast DNA polymerase ζ is an efficient extender of primer ends opposite from 7,8-dihydro-8-oxoguanine and O^6-methylguanine. Mol. Cell. Biol. **23:** 1453–1459.

Haracska, L., C. A. Torres-Ramos, R. E. Johnson, S. Prakash and L. Prakash, 2004 Opposing effects of ubiquitin conjugation and SUMO modification of PCNA on replicational bypass of DNA lesions in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **24:** 4267–4274.

Harfe, B. D., and S. Jinks-Robertson, 1999 Removal of frameshift intermediates by mismatch repair proteins in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **19:** 4766–4773.

Harfe, B. D., and S. Jinks-Robertson, 2000 DNA polymerase ζ introduces multiple mutations when bypassing spontaneous DNA damage in *Saccharomyces cerevisiae.* Mol. Cell **6:** 1491–1499.

Hoege, C., B. Pfander, G. L. Moldovan, G. Pyrowolakis and S.

Jentsch, 2002 *RAD6*-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature **419:** 135–141.
Hoeijmakers, J. H. J., 2001 Genome maintenance mechanisms for preventing cancer. Nature **411:** 366–374.
Hofmann, R. M., and C. M. Pickart, 1999 Noncanonical *MMS2*-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. Cell **96:** 645–653.
Huang, H., A. Kahana, D. E. Gottschling, L. Prakash and S. W. Liebman, 1997 The ubiquitin-conjugating enzyme Rad6 (Ubc2) is required for silencing in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:** 6693–6699.
Huang, M. E., A. de Calignon, A. Nicolas and F. Galibert, 2000 Pol32, a subunit of the *Saccharomyces cerevisiae* DNA polymerase δ, defines a link between DNA replication and the mutagenic bypass repair pathway. Curr. Genet. **38:** 178–187.
Huang, M. E., A. G. Rio, M. D. Galibert and F. Galibert, 2002 Pol32, a subunit of *Saccharomyces cerevisiae* DNA polymerase δ, suppresses genomic deletions and is involved in the mutagenic bypass pathway. Genetics **160:** 1409–1422.
Jentsch, S., J. P. McGrath and A. Varshavsky, 1987 The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature **329:** 131–134.
Johansson, E., P. Garg and P. M. Burgers, 2004 The Pol32 subunit of DNA polymerase δ contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. J. Biol. Chem. **279:** 1907–1915.
Johnson, R. E., S. T. Henderson, T. D. Petes, S. Prakash, M. Bankmann *et al.*, 1992 *Saccharomyces cerevisiae RAD5*-encoded DNA repair protein contains DNA helicase and zinc-binding sequence motifs and affects the stability of simple repetitive sequences in the genome. Mol. Cell. Biol. **12:** 3807–3818.
Johnson, R. E., S. Prakash and L. Prakash, 1994 Yeast DNA repair protein Rad5 that promotes instability of simple repetitive sequences is a DNA-dependent ATPase. J. Biol. Chem. **269:** 28259–28262.
Johnson, R. E., S. Prakash and L. Prakash, 1999 Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Pol η. Science **283:** 1001–1004.
Johnson, R. E., M. T. Washington, L. Haracska, S. Prakash and L. Prakash, 2000 Eukaryotic polymerases ι and ζ act sequentially to bypass DNA lesions. Nature **406:** 1015–1019.
Johnson, R. E., S.-L. Yu, S. Prakash and L. Prakash, 2003 Yeast DNA polymerase ζ (zeta) is essential for error-free replication past thymine glycol. Genes Dev. **17:** 77–87.
Krejci, L., S. Van Komen, Y. Li, J. Villemain, M. S. Reddy *et al.*, 2003 DNA helicase Srs2 disrupts the Rad51 presynaptic filament. Nature **423:** 305–309.
Kunkel, T. A., 2003 Considering the cancer consequences of altered DNA polymerase function. Cancer Cell **3:** 105–110.
Kunz, B. A., X. L. Kang and L. Kohalmi, 1991 The yeast *rad18* mutator specifically increases G-C→T-A transversions without reducing correction of G-A or C-T mismatches to G-C pairs. Mol. Cell. Biol. **11:** 218–225.
Lawrence, C. W., 2002 Cellular roles of DNA polymerase ζ and Rev1 protein. DNA Repair **1:** 425–435.
Lawrence, C. W., and R. B. Christensen, 1979 Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of *Saccharomyces cerevisiae rad6* mutants. J. Bacteriol. **139:** 866–876.
Lea, D. E., and C. A. Coulson, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **119:** 264–284.
Lee, S. K., R. E. Johnson, S. L. Yu, L. Prakash and S. Prakash, 1999 Requirement of yeast *SGS1* and *SRS2* genes for replication and transcription. Science **286:** 2339–2342.
Lichten, M., R. H. Borts and J. E. Haber, 1987 Meiotic gene conversion and crossing over between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. Genetics **115:** 233–246.
Liefshitz, B., R. Steinlauf, A. Friedl, F. Eckardt-Schupp and M. Kupiec, 1998 Genetic interactions between mutants of the 'error-prone' repair group of *Saccharomyces cerevisiae* and their effect on recombination and mutagenesis. Mutat. Res. **407:** 135–145.
Lu, X., C. K. Tan, J. Q. Zhou, M. You, L. M. Carastro *et al.*, 2002 Direct interaction of proliferating cell nuclear antigen with the small subunit of DNA polymerase δ. J. Biol. Chem. **277:** 24340–24345.
Maga, G., and U. Hubscher, 2003 Proliferating cell nuclear antigen (PCNA): a dancer with many partners. J. Cell Sci. **116:** 3051–3060.
Martini, E. M., S. Keeney and M. A. Osley, 2002 A role for histone H2B during repair of UV-induced DNA damage in *Saccharomyces cerevisiae*. Genetics **160:** 1375–1387.
McDonald, J. P., A. S. Levine and R. Woodgate, 1997 The *Saccharomyces cerevisiae RAD30* gene, a homologue of *Escherichia coli* dinB and umuC, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism. Genetics **147:** 1557–1568.
Morrison, A., E. J. Miller and L. Prakash, 1988 Domain structure and functional analysis of the carboxy-terminal polyacidic sequence of the Rad6 protein of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 1179–1185.
Nelson, J. R., C. W. Lawrence and D. C. Hinkle, 1996 Thymine-thymine dimer bypass by yeast DNA polymerase ζ. Science **272:** 1646–1649.
Ohmori, H., E. C. Friedberg, R. P. P. Fuchs, M. F. Goodman, F. Hanaoka *et al.*, 1999 The Y family of DNA polymerases. Mol. Cell **8:** 7–8.
Prakash, L., 1981 Characterization of postreplication repair in *Saccharomyces cerevisiae* and effects of *rad6*, *rad18*, *rev3* and *rad52* mutations. Mol. Gen. Genet. **184:** 471–478.
Quah, S.-K., R. C. von Borstel and P. J. Hastings, 1980 The origin of spontaneous mutation in *Saccharomyces cerevisiae*. Genetics **96:** 819–839.
Roche, H., R. D. Gietz and B. A. Kunz, 1995 Specificities of the *Saccharomyces cerevisiae rad6*, *rad18*, and *rad52* mutators exhibit different degrees of dependence on the *REV3* gene product, a putative nonessential DNA polymerase. Genetics **140:** 443–456.
Rong, L., and H. L. Klein, 1993 Purification and characterization of the SRS2 DNA helicase of the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **268:** 1252–1259.
Roush, A. A., M. Suarez, E. C. Friedberg, M. Radman and W. Siede, 1998 Deletion of the *Saccharomyces cerevisiae* gene *RAD30* encoding an *Escherichia coli* DinB homolog confers UV radiation sensitivity and altered mutability. Mol. Gen. Genet. **257:** 686–692.
Schiestl, R. H., S. Prakash and L. Prakash, 1990 The *SRS2* suppressor of *rad6* mutations of *Saccharomyces cerevisiae* acts by channeling DNA lesions into the *RAD52* DNA repair pathway. Genetics **124:** 817–831.
Sherman, F., 1991 Getting started with yeast. Methods Enzymol. **194:** 3–21.
Smirnova, M., and H. L. Klein, 2003 Role of the error-free damage bypass postreplication repair pathway in the maintenance of genomic stability. Mutat. Res. **532:** 117–135.
Spell, R. M., and S. Jinks-Robertson, 2004 Determination of mitotic recombination rates by fluctuation analysis in *Saccharomyces cerevisiae*, pp. 3–12 in *Genetic Recombination Protocols*, edited by A. S. Waldman. Humana Press, Totowa, NJ.
Stelter, P., and H. D. Ulrich, 2003 Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature **425:** 188–191.
Swanson, R. L., N. J. Morey, P. W. Doetsch and S. Jinks-Robertson, 1999 Overlapping specificities of base excision repair, nucleotide excision repair, recombination, and translesion synthesis pathways for DNA base damage in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 2929–2935.
Torres-Ramos, C. A., B. L. Yoder, P. M. Burgers, S. Prakash and L. Prakash, 1996 Requirement of proliferating cell nuclear antigen in *RAD6*-dependent postreplicational DNA repair. Proc. Natl. Acad. Sci. USA **93:** 9676–9681.
Torres-Ramos, C. A., S. Prakash and L. Prakash, 2002 Requirement of *RAD5* and *MMS2* for postreplication repair of UV-damaged DNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **22:** 2419–2426.
Ulrich, H. D., 2001 The *srs2* suppressor of UV sensitivity acts specifically on the *RAD5*- and *MMS2*-dependent branch of the *RAD6* pathway. Nucleic Acids Res. **29:** 3487–3494.
Ulrich, H. D., 2003 Protein-protein interactions within an E2-RING finger complex. Implications for ubiquitin-dependent DNA damage repair. J. Biol. Chem. **278:** 7051–7058.
Ulrich, H. D., and S. Jentsch, 2000 Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. EMBO J. **19:** 3388–3397.
Veaute, X., J. Jeusset, C. Soustelle, S. C. Kowalczykowski, E. Le

Cam *et al.*, 2003 The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature **423:** 309–312.

Wach, A., A. Brachat, R. Pohlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:** 1793–1808.

Wagner, J., H. Etienne, R. Janel-Bintz and R. P. P. Fuchs, 2002 Genetics of mutagenesis in *E. coli*: various combinations of translesion polymerases (Pol II, IV and V) deal with lesion/sequence context diversity. DNA Repair **1:** 159–167.

Xiao, W., B. L. Chow, T. Fontanie, L. Ma, S. Bacchetti *et al.*, 1999 Genetic interactions between error-prone and error-free postreplication repair pathways in *Saccharomyces cerevisiae*. Mutat. Res. **435:** 1–11.

Xiao, W., B. L. Chow, S. Broomfield and M. Hanna, 2000 The *Saccharomyces cerevisiae RAD6* group is composed of an error-prone and two error-free postreplication repair pathways. Genetics **155:** 1633–1641.

Yuan, F., Y. Zhang, D. K. Rajpal, X. Wu, D. Guo *et al.*, 2000 Specificity of DNA lesion bypass by the yeast DNA polymerase η. J. Biol. Chem. **275:** 8233–8239.

Communicating editor: M. Hampsey