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Participation of Translesion Synthesis DNA Polymerases in the Maintenance of Chromosome Integrity in Yeast *Saccharomyces cerevisiae*

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Abstract—We employed a genetic assay based on illegitimate hybridization of heterothallic *Saccharomyces cerevisiae* strains (the α-test) to analyze the consequences for genome stability of inactivating translesion synthesis (TLS) DNA polymerases. The α-test is the only assay that measures the frequency of different types of mutational changes (point mutations, recombination, chromosome or chromosome arm loss) and temporary changes in genetic material simultaneously. All these events are manifested as illegitimate hybridization and can be distinguished by genetic analysis of the hybrids and cytoductants. We studied the effect of Polζ, Polη, and Rev1 deficiency on the genome stability in the absence of genotoxic treatment and in UV-irradiated cells. We show that, in spite of the increased percent of accurately repaired primary lesions, chromosome fragility, rearrangements, and loss occur in the absence of Polζ and Polη. Our findings contribute to further refinement of the current models of translesion synthesis and the organization of eukaryotic replication fork.

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Translesion synthesis (TLS) DNA polymerases belong to the specialized group of DNA polymerases whose main function is to promote replication of damaged DNA by inserting nucleotides across from and past DNA lesions. In yeast *Saccharomyces cerevisiae*, several genes control translesion synthesis: the *REV3* and *REV7* genes encode for the two subunits of DNA polymerase ζ (Polζ) [1], the *RAD30* gene encodes DNA polymerase η (Polη) [2, 3], and the *REV1* gene encodes a deoxycytidyl transferase, the enzyme that in addition to the polymerase activity has the ability to interact with Polζ, Polη, and a subunit of the replicative DNA polymerase δ [4, 5]. Polζ can synthesize with low efficiency across several types of lesions, such as thymine glycols, T-T (6-4) photoproducts, and abasic sites [6-8]. The main function of Polζ is extension of primers with a mismatched terminal nucleotide pair [9-11]. Polη is the primary TLS polymerase responsible for accurate bypass of *cis-syn* cyclobutane pyrimidine dimers, a major DNA lesion resulting from UV irradiation, and 7,8-dihydro-8-oxoguanine occurring during oxidative stress [3, 12]. However, Polη exhibits a low fidelity when bypassing other lesions and during replication of undamaged DNA *in vitro* [13-16]. Studies of Polη mutants have important clinical implications, because mutations in the gene encoding for Polη in humans are associated with a complex disorder xeroderma pigmentosum variant characterized by sunlight sensitivity [17, 18]. Rev1, which interacts with multiple DNA polymerases, is believed to play an important regulatory role in DNA polymerase switching and during DNA synthesis past lesions [8, 19]. Although the deoxycytidyl transferase activity of Rev1 is used during the bypass of some lesions [20-22], the essential function of Rev1 in TLS is structural rather than enzymatic.

Abbreviations: MD, minimal dextrose; TLS, translesion synthesis; YPD, yeast extract, peptone, dextrose.

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Inactivation of certain TLS polymerases leads to a significant drop of point mutation frequency. For instance, deletions of the *REV3*, *REV7*, and *REV1* genes reduce the spontaneous mutagenesis up to 70% and virtually abolish mutagenesis induced by many DNA damaging agents [23]. This suggests TLS to be a highly mutagenic process. Nevertheless, a lack of the TLS may result in destabilization of genetic material on a higher level and may provoke chromosome rearrangements and even chromosome loss. In higher eukaryotes, TLS DNA polymerases were shown to be involved in some processes other than translesion synthesis. REV1 was reported to induce immunoglobulin gene conversion and generate sister chromatid exchanges during DSB repair in higher eukaryotes independently from Polζ [24]. The hREV7 is thought to play an important role in the G2/M checkpoint. In higher eukaryotes, REV7 interacts with specific factors of anaphase promoting complex (APC/C) and with the spindle checkpoint protein MAD2 [25, 26]. These interactions may regulate the cell cycle progression and have provided the first link between a mutagenic DNA polymerase and chromosome segregation control [26, 27]. Studies of the chicken DT40 line have shown the contribution of the *REV* genes to the prevention of chromosomal fragility/instability, increased chromosomal rearrangements, and homologous recombination [24, 28, 29]. Rev3 was also shown to be involved in homologous recombination (HR)-dependent double-strand-break (DSB) repair after ionizing radiation. Inactivation of the *REV3* gene in chicken caused a significant increase in the level of chromosomal breaks after ionizing radiation. These data suggest the involvement of Rev3 in DNA synthesis during HR-mediated DSB repair [28]. Yeast Rev3 was also shown to participate in DNA synthesis during DSB repair [30, 31].

Thus, the contribution of TLS DNA polymerases to genome stability maintenance is very complex. Here we undertake a comprehensive study of the role of TLS DNA polymerases in promoting and preventing genome instability in the same experimental system. To test the role of each DNA polymerase in genome stability maintenance we used an approach described in a previous work and named the α-test for *S. cerevisiae* [32-34]. The α-test allows one to score a wide spectrum of changes in the genetic material. It simultaneously measures the frequency of gene mutation, recombination, loss of chromosome III, and chromosome arm loss. The distinctive feature of the α-test is its ability to measure the frequency of primary (non-inherited) lesions in the genetic material and to estimate the proportion of primary lesions that were precisely eliminated by repair after mating, and the proportion of lesions that led to inherited genetic changes [32, 33].

In this study, we provide evidence that the activity of Polζ and Polη prevents global chromosome rearrangements and chromosome loss, potentially by suppressing formation of double-strand breaks.

MATERIALS AND METHODS

Strains and growth conditions. The yeast strains used in the study are listed in Table 1. Strains were grown in rich media (yeast extract, peptone, dextrose (YPD)) or minimal media (minimal dextrose (MD)) containing the corresponding selective amino acids required for selection. All liquid and solid media were prepared according

Table 1. *Saccharomyces cerevisiae* strains used

Strain	Genotype	Application
D926	*MATα//MATα leu2Δ//leu2Δ lys2Δ//lys2Δ ura3Δ//ura3Δ his4Δ// his4Δ thr4Δ//thr4Δ CYH^s//CYH^s*	partner for copulation
K5-99-35C-D924	*MATα ura3Δ leu2Δ met15Δ lys5 his3 cyh^r*	strain-tester for illegitimate hybridization
K5-99-35C-D924-*rad30Δ*	*MATα ura3Δ leu2Δ met15Δ lys5 his3 cyh^r rad30Δ::KanMX4*	
K5-99-35C-D924-*rev3Δ*	*MATα ura3Δ leu2Δ met15Δ lys5 his3 cyh^r rev3Δ::LEU2*	
K5-99-35C-D924-*rev3Δ-rad30Δ*	*MATα ura3Δ leu2Δ met15Δ lys5 his3 cyh^r rev3Δ::LEU2 rad30::KanMX4*	
K5-99-35C-D924-*rev7Δ*	*MATα ura3 leu2Δ met15Δ lys5 his3 cyh^r rev7Δ::KanMX4*	
K5-99-35C-D924-*rev1Δ*	*MATα ura3 leu2Δ met15Δ lys5 his3 cyh^r rev1Δ::KanMX4*	
26B-D924	*MATα uraΔ3 leu2Δ met15Δ kar1-1 cyh^r*	strain-tester for cytoduction
26B-D924-*rad30Δ*	*MATα ura3Δ leu2Δ met15Δ kar1-1 cyh^r rad30Δ::kanMX4*	
26B-D924-*rev3Δ*	*MATα ura3Δ leu2Δ met15Δ kar1-1 cyh^r rev3Δ::LEU2*	
26B-D924-*rev3Δ-rad30Δ*	*MATα ura3Δ leu2Δ met15Δ kar1-1 cyh^r rev3Δ::LEU2 rad30::KanMX4*	
26B-D924-*rev7Δ*	*MATα ura3 leu2Δ met15Δ kar1-1 cyh^r rev7Δ::KanMX4*	
26B-D924-*rev1Δ*	*MATα ura3 leu2Δ met15Δ kar1-1 cyh^r rev1Δ::KanMX4*	
78A-P2345	*MATα his5*	testers for mating type determination
2G-P2345	*MAT*a *his5*	
2A-P143	*MATα ura3Δ ade8Δ*	

to standard protocols [35]. Media with 24 ml/liter glycerol as the sole source of carbon was used to determine respiratory competence. The media for cytoductants selection was MD lacking glucose and containing ethyl alcohol (20 ml/liter) and cycloheximide (5 mg/liter). Yeast cultures were grown at 30°C.

Strain construction. Strains with a deletion of the *REV3* gene were constructed as previously described [36]. The *RAD30*, *REV7*, and *REV1* genes were disrupted by a selectable *KanMX* cassette as described [37]. The *REV7* and *REV1* gene deletions were confirmed by decreased UV-induced mutagenesis [38]. The *RAD30* deletion was confirmed by increased sensitivity to UV irradiation [39].

Measurement of illegitimate hybridization and cytoduction frequency. For illegitimate hybridization, 50 μl of fresh overnight culture of the tester strain (K5-99-35C-D924 or its derivates) were transferred to selective media containing histidine, threonine, leucine, and uracil for hybrid growth. In the case of UV light treatment, plates with the tester strain were irradiated with UV (25 J/m^2). An aliquot (100 μl) of the partner strain D926 was then added. At the same time, the cultures of the tested strain were diluted appropriately and plated on YPD to determine the number of viable cells. Surviving colonies were scored after two days of incubation, and hybrids were scored after three days.

For cytoduction, fresh overnight independent cultures of strains to be tested were concentrated 10 times (~10^9 cells per ml). The aliquots (100 μl) of each strain (recipient and donor) were transferred onto rich media. Two strains mixed together were incubated for 2 days and then replica plated onto the selective media containing uracil, leucine, methionine, and ethyl alcohol (20 ml/liter) and cycloheximide (5 mg/liter) for cytoductant growth. To determine the survival of the recipient strain, cell cultures were diluted to appropriate cell densities and plated on YPD. Surviving colonies were scored after 2 days of incubation, and cytoductants were scored after 10 days. For UV-induced cytoduction, 100 μl of the recipient strain (26B-D924 and its derivates) was irradiated with UV (25 J/m^2) on plates. An equivalent amount of the donor strain was then added to the treated recipient strain.

The frequency of hybridization and cytoduction was calculated as $F = (Ma)/(Nb)$, where M is the number of hybrids/cytoductants, N is the number of survivors, and a and b are the corresponding dilution factors. The significance of differences between samples was evaluated by the Mann–Whitney test with $P = 0.05$ [40]. For each experiment, at least nine independent cultures were used. Phenotypes of illegitimate hybrids and cytoductants were determined by replica plating on media lacking selected amino acids. Based on their phenotypes, cytoductants and hybrids were placed into appropriate classes. The frequency of each class was calculated by multiplying the proportion of each class by the overall frequency of hybrids or cytoductants.

RESULTS

Genetic system for study of influence of genotoxic factors on stability of genetic material. To study the effects of inactivation of TLS DNA polymerases on genome stability, we utilized a test system that allows for the detection of a wide spectrum of genetic events. The test system used in the study was named the α-test [32, 33]. The criterion of genome instability in the α-test is the frequency of a mating type switch from mating type "α" to mating type "a". Each event of a mating type switch may be scored in selective conditions. In the α-test two different strains of the same mating type are mixed on selective media, which supports the growth of "illegitimate" hybrids only but not parental strains. Such "illegitimate" hybridization is possible when one of the mating cells had changed its mating type from "α" to "a". Different changes of genetic material may lead to a mating type switch in yeast strains of the mating type "α". The mating type of *S. cerevisiae* cells is controlled by locus *MAT* on the right arm of chromosome III near the centromere [41-43]. The *MAT* locus determines the "a" or "α" cell type of haploid cells. Additional elements of the *S. cerevisiae* mating system are two cassettes (*HMR*a and *HMR*α), which contain silent genetic information for the "a" and "α" mating type, respectively (Fig. 1a). Normally, switching of the mating type occurs via the "cassette mechanism": information from *HMR*a or *HMR*α replaces the one in the *MAT* locus [43, 46]. In addition, illegitimate hybridization between "α"-type cells of heterothallic strains may be caused by the loss of chromosome III, loss of the chromosome arm, recombination between the *MAT* locus and the cassette *HMR*a, point mutations, and temporary changes (DNA lesions) in the *MAT*α locus [33, 47]. The frequency of illegitimate hybridization increases after DNA damaging treatment due to the disturbance of the *MAT* locus expression [46]. All the events could be scored in the α-test by analyzing the phenotypes of illegitimate hybrids if both arms of chromosome III are marked (Fig. 1 (a and b) and Table 2) [33].

Moreover, a modification of the α-test (selective system of illegitimate cytoduction) allows us to detect primary lesions in the genetic material that are repaired after mating and do not result in heritable changes [45, 48]. Cytoduction is uncompleted hybridization, when the cytogamy is not followed by nuclear fusion and diploid cell formation (Fig. 1c and Table 2). Analysis of the phenotype of illegitimate cytoductants allows us to distinguish between the primary lesions and inherited changes of genetic material occurring both spontaneously and induced by genotoxic agents. Unfortunately, the system of cytoduction does not allow us to register the genetic events that are lethal in haploids, such as chromosome or chromosome arm loss. Therefore, these two variants of the α-test are complementary and together allow us to register a wide spectrum of genetic events [45].

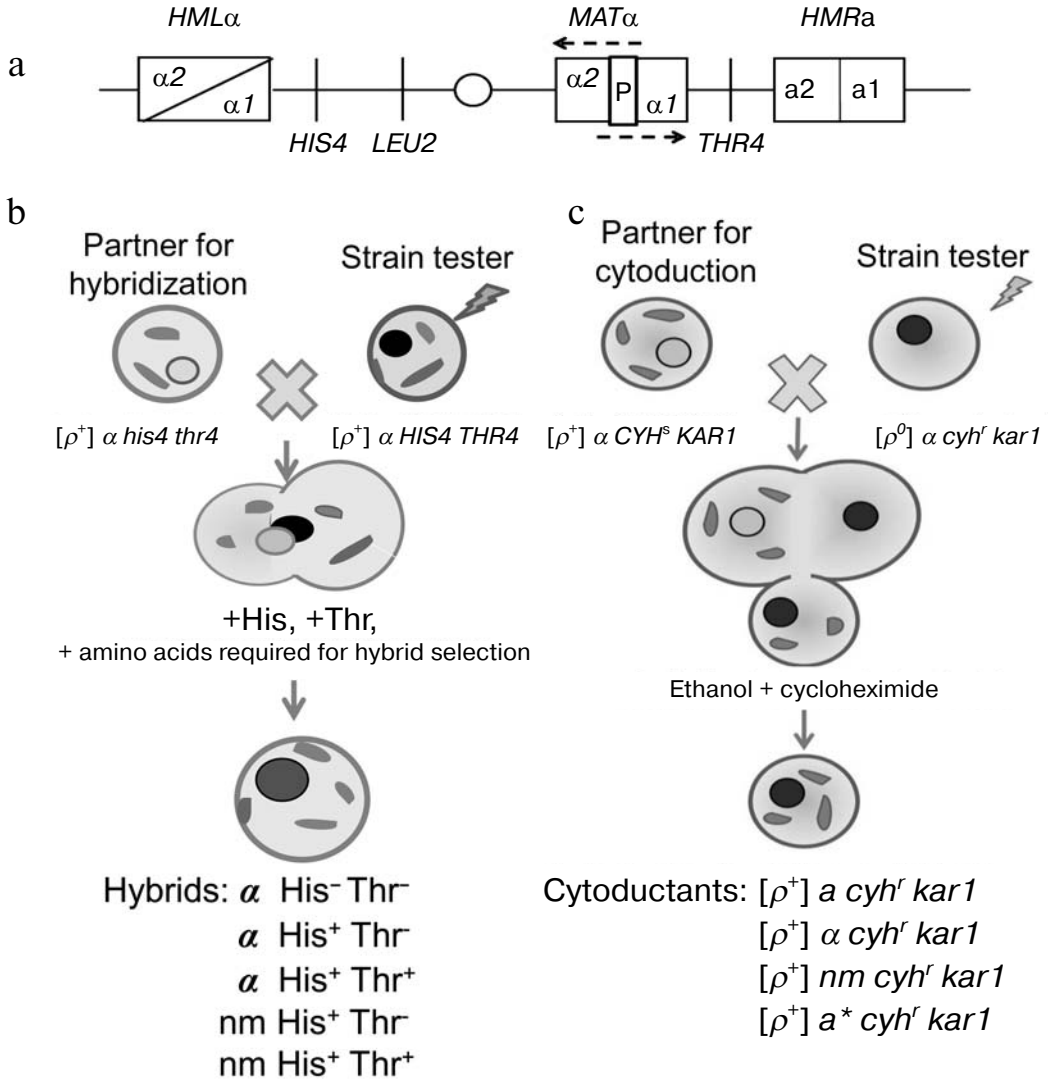

Fig. 1. Genetic aspects of the α-test. a) Structure of *S. cerevisiae* chromosome III showing details of the *MAT* locus and markers on the right and left arms of the chromosome used in illegitimate hybridization experiments. P, bidirectional promoter of the *MAT* locus. Arrows designate the directions of transcription of the *MATα1* and *MATα2* genes in the *MAT* locus [44]. b) Scheme of the selective system of illegitimate hybridization. The strain tester bears wild-type alleles of the *HIS4* and *THR4* genes. The partner for hybridization bears mutant alleles or deletions of these genes. Illegitimate hybrids are selected on media containing the appropriate amino acids for hybrid growth (see "Materials and Methods") including histidine and threonine to allow for the growth of hybrids with lost chromosome III or rearrangements. The five possible phenotypes of hybrids are listed. The interpretation of these phenotypes is shown in Table 2. c) Scheme of the selective system of illegitimate cytoduction [45]. The tester strain, which is exposed to the genotoxic agent, bears allele of resistance to the antibiotic cycloheximide (*cyh*^{r}) for nucleus selection and mutation *kar1*, which prevents karyogamy. The tester strain also lacks mitochondria, which results in [ρ^0] phenotype (inability to utilize certain sources of carbon, e.g. ethanol) and serves as a cytoplasm marker. The partner for cytoduction bears markers of sensitivity to cycloheximide (*CYH*^{s}), a wild-type allele of the *KAR1* gene, and has functional mitochondria [ρ^+]. Cells with mixed cytoplasm and bearing the nucleus of the tester strain are selected on the medium containing ethyl alcohol and cycloheximide (see "Materials and Methods").

Effect of inactivation of TLS DNA polymerases on frequency of illegitimate hybridization and cytoduction. The frequency of hybridization and cytoduction reflects the number of primary lesions that led to a disturbance of the *MAT* locus expression and, hence, mating type switching. Therefore, analysis of the frequency of induced and spontaneous hybridization or cytoduction allows us to estimate the activity of different genotoxic agents. In the present work, we investigated the effect of inactivation of TLS polymerases on illegitimate hybridization and cytoduction in the absence of genotoxic treatment and after UV irradiation. UV irradiation is a common environmental genotoxicant and a widely used model DNA-damaging agent that induces the formation of cyclobutane pyrimidine dimers and 6-4-photoproducts [49]. TLS polymerases are known to provide temporal

Table 2. Genetic events that lead to illegitimate mating of the *MATα his4 thr4* strain to *MATα HIS4 THR4* and phenotypes of the resulting hybrids and cytoductants [31]

Genetic event	Phenotype of cytoductants	Phenotype of illegitimate hybrids
Conversion between *HMR*a and *MAT* locus	a	n/m His^+Thr^+
Reciprocal recombination between *MAT* locus and *HMR*a	lethal	n/m His^+Thr^-
Loss of right arm of chromosome III	–"–	α His^+Thr^-
Loss of chromosome III	–"–	α His^-Thr^-
Mutations in *MATα* (*matα1* or *matα2*)	n/m	α His^+Thr^+
Mutations in *MATα* (both in *MATα1* and *MATα2*, or in the bidirectional promoter, *MATα* deletions)	recessive "a" or Alf-phenotype (from "a"-like fakers)	α His^+Thr^+
Transient lesions in the *MATα* locus (both in *MATα1* and *MATα2*, or in the bidirectional promoter)	α	α His^+Thr^+

Note: n/m, non-mating phenotype.

tolerance to UV irradiation, as well as to various chemical agents [23]. We used the α-test to determine the consequences for the genome stability of inactivating this DNA damage tolerance pathway.

Normally, the frequency of spontaneous illegitimate hybridization in the wild type strain varies from 10^{-6} to 10^{-7} and the frequency of spontaneous illegitimate cytoduction is 10^{-7} to 10^{-8}. In accordance with earlier studies [45, 46], we observed that UV light increases illegitimate hybridization and cytoduction in the wild-type strain up to 5- and 3.5-fold, respectively, in comparison to the spontaneous frequency (Fig. 2 legend and Table 3, respectively). The effects of *rev3Δ*, *rev7Δ*, *rad30Δ*, and *rev1Δ* mutations on the frequency of spontaneous and UV-induced hybridization and cytoduction are shown in Fig. 2 and Table 3, respectively. The absolute frequencies of illegitimate hybridization varied widely between different experiments, but the observed effects of TLS mutations were similar in each experiment. Figures 2-4, therefore, show the increase/decrease in the frequency of genetic events relative to the wild-type strain rather than the absolute frequency of these events.

We observed no significant effect of Polζ or Polη deficiency on the frequency of spontaneous illegitimate hybridization (Fig. 2a). However, UV-induced frequency of illegitimate hybridization was approximately 8-fold higher in the *rev3Δ*, *rev7Δ*, and *rad30Δ* strains and approximately 20-fold higher in the double *rev3Δ rad30Δ* mutant in comparison to a UV-treated wild-type strain (Fig. 2b). In contrast, inactivation of the *REV1* gene decreased the frequency of both spontaneous and UV-induced illegitimate hybridization 12- and 66-fold in comparison to the wild-type strain, respectively (Fig. 2b). Moreover, unlike in other studied strains, the UV treatment does not increase the frequency of illegitimate hybridization of the *rev1Δ* strain in comparison to its frequency of spontaneous hybridization. The genetic analysis of the rare hybrids obtained in the *rev1Δ* background further showed that this mutation decreased the frequency of all (both spontaneous and UV-induced) genetic events that we can distinguish in the illegitimate hybridization assay (chromosome loss, chromosome arm loss, reciprocal recombination, and gene conversion).

We also examined the effect of inactivation of the *REV3*, *REV7*, and *RAD30* genes in the selective system of illegitimate cytoduction. Only *RAD30* deletion showed an increased frequency of spontaneous cytoduction (approximately 2.5-fold), while deletion of the other

Table 3. Frequency of spontaneous and UV-induced illegitimate cytoduction in wild-type strain K5-99-35C-D924 and its derivates, $\times 10^{-7}$

Strain	Spontaneous frequency of cytoduction	UV-induced frequency of cytoduction (25 J/m^2)
WT	4 (3-5)	18 (14-38)
rev3Δ	3 (1-5)	18 (9-26)
rev7Δ	5 (5-11)	**106 (83-115)**
rad30Δ	**10 (8-17)**	**75 (54-110)**
rev3Δ rad30Δ	9 (1-10)	**100 (37-250)**

Note: All frequencies are medians and 95% confidence intervals. Frequencies that significantly differ from the wild-type strain are marked in bold letters.

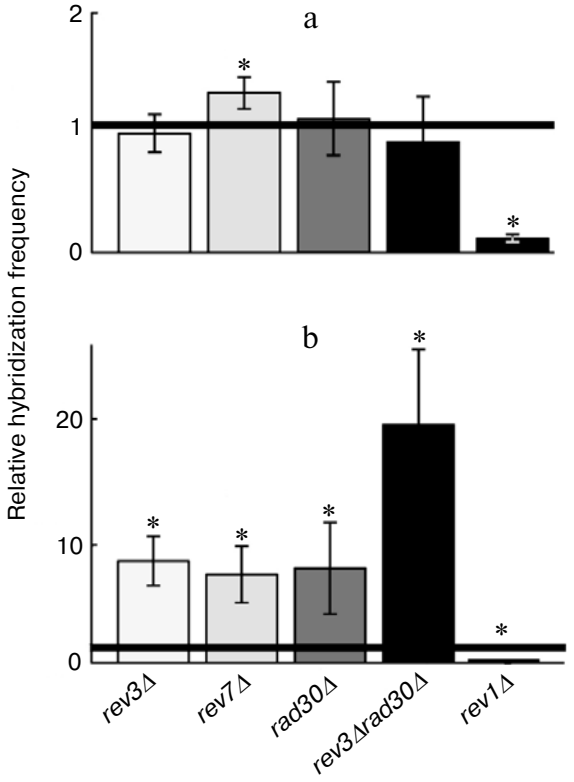

Fig. 2. Relative frequency of illegitimate hybridization in *rev3Δ*, *rev7Δ*, *rad30Δ*, *rev3Δ rad30Δ*, and *rev1Δ* derivatives of K5-99-35C-D924 strain in the absence of genotoxic factors (a) and that induced by UV-irradiation (25 J/m^2) (b). The hybridization frequency in the wild-type strain is designated as 1 and indicated by the bold horizontal line. All frequencies are means and standard errors for five experiments. The spontaneous frequencies in the wild-type strain were 130 (60-250), 45 (30-50), 170 (113-400), 168 (51-200), and 30 (20-46) ($\times 10^{-6}$). The corresponding frequencies of illegitimate hybridization in the UV-treated wild type strain were 280 (190-610), 270 (170-300), 310 (230-1110), 230 (210-450), and 96 (70-130) ($\times 10^{-6}$). Asterisks indicate a statistically significant difference from the wild-type strain as determined by sign test.

genes had no significant effect (Table 3). In contrast, after exposure to UV light, the frequency of cytoduction was increased 7-fold in the *rev7Δ* strain, 4.6-fold in the *rad30Δ* strain, and 6.5-fold in the double *rev3Δ rad30Δ* mutants (Table 3). Because Rev1 inactivation had a strong negative effect on the illegitimate mating, we were unable to measure the frequency of such a rare event as cytoduction in the *rev1Δ* strain.

Effect of TLS polymerase inactivation on chromosome or chromosome arm loss. Previous findings that the inactivation of the *REV* genes (*REV1*, *REV3*, and *REV7*) cause chromosome loss and breaks in vertebrates [24, 27] inspired us to study the effect of inactivation of TLS polymerases on chromosome stability in yeast. Illegitimate mating of *S. cerevisiae* strains of the same mating type "α" could occur upon the loss of the right arm of chromosome III or the loss of the whole chromosome III [43]. The frequency of these events is strain-dependent and could be increased after DNA-damaging treatment [33, 43]. In our case, exposure of the wild-type strain to UV light increased the frequency of chromosome arm loss (approximately 2-fold) and had no influence on the frequency of chromosome loss (Table 4).

We observed that inactivation of Polζ and Polη does not induce significant spontaneous chromosome loss (Fig. 3a). After UV irradiation, the frequency of chromosome loss was 4.5-fold higher in strains with single *REV3*, *REV7*, and *RAD30* deletions than in the wild type strain. Simultaneous deletion of *REV3* and *RAD30* leads to a more dramatic (approximately 15-fold) increase in the frequency of UV-induced chromosome loss (Fig. 3a) in comparison to a UV-treated wild-type strain. We also investigated the effect of inactivation of TLS polymerases on the frequency of spontaneous and UV-induced chromosome arm loss. The *REV3* and *REV7* gene deletions led to an increase in chromosome arm loss, both spontaneously and after UV treatment (Fig. 3, c and d). While inactivation of the catalytic and regulatory subunits of Polζ had a similar effect on the frequency of spontaneous chromosome arm loss (2-fold higher than in the wild-type strain), the *REV3* deletion had a stronger effect on the chromosome arm loss after UV light treatment compared to the *REV7* deletion (10- and 3-fold, respectively) (Fig. 3d). The frequency of chromosome arm loss was elevated 6.5-fold in the UV-treated *rev3Δrad30Δ* strain as well (Fig. 3, c and d).

Inactivation of the *REV1* gene led to a 20- and 40-fold decrease in the spontaneous frequency of chromosome and chromosome arm loss, respectively. The UV-induced frequency of both events was decreased 30-fold in comparison to the UV-treated wild-type strain.

Table 4. Frequency of genetic events that led to illegitimate hybridization of wild-type strain K5-99-35C-D924, $\times 10^{-6}$

UV, J/m^2	Chromosome III loss	Chromosome III arm loss	Conversion	Recombination	Mutations and primary lesions	Hybrids analyzed
0	160 (124-230)	9 (7-13)	2 (1-3)	1.0 (0.5-1.5)	4 (3-6)	1362
25	160 (98-190)	22 (13-25)	3 (2-4)	3 (2-4)	160 (100-190)	1325

Note: All frequencies are medians for 21 cultures with 95% confidence intervals in parentheses.

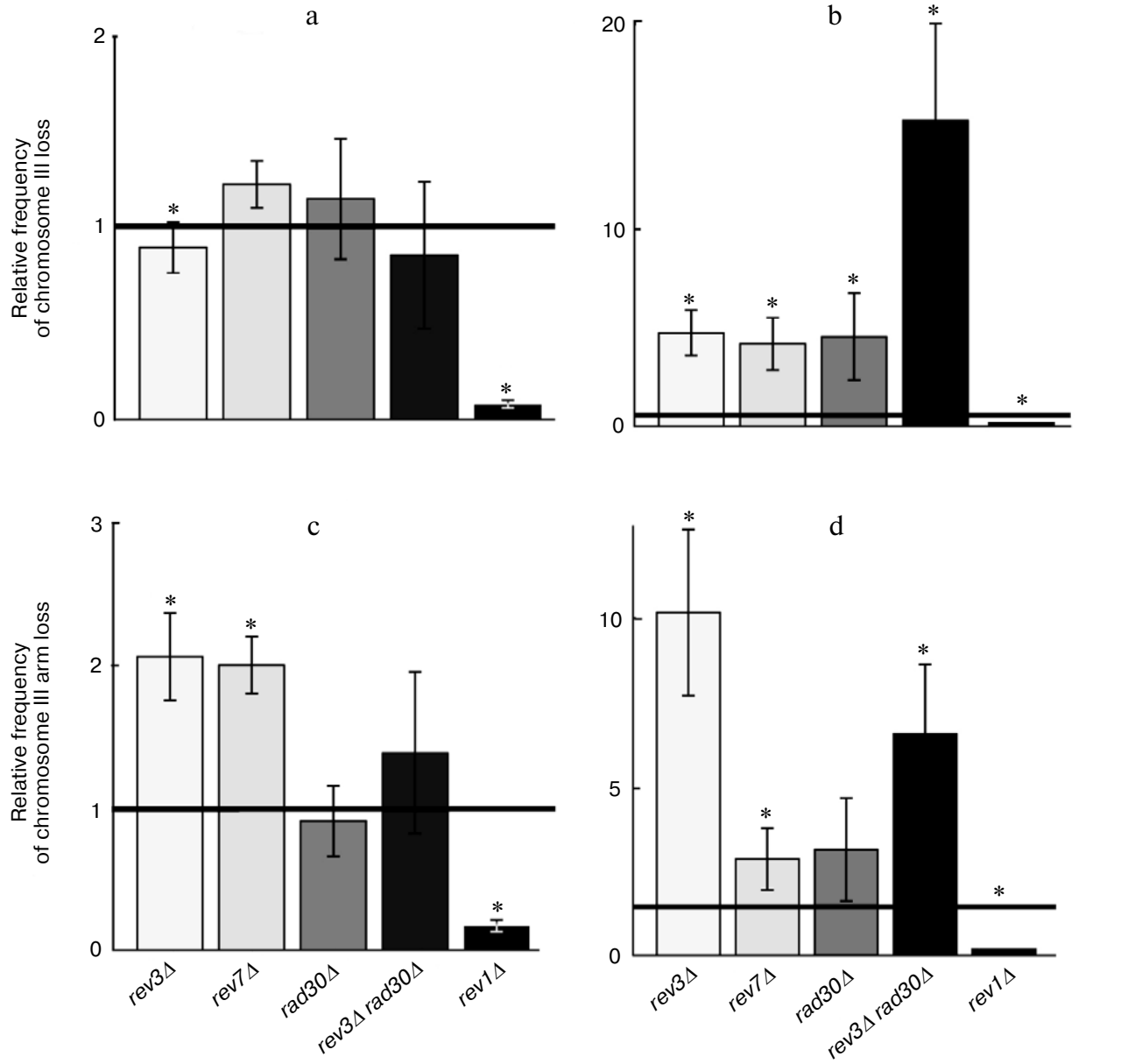

Fig. 3. Relative spontaneous (a, c) and UV-induced (25 J/m^2) (b, d) frequency of chromosome III loss (a, b) and chromosome III arm loss (c, d) in TLS DNA polymerase mutants. Here and in Fig. 4, all frequencies are means and standard errors for five experiments. All symbols are as shown in Figs. 2 and 4.

Effect of TLS polymerase inactivation on gene conversion and reciprocal recombination. Polζ and Polη were suggested to participate in DNA synthesis during recombinational events in yeast and in higher eukaryotes [28, 30, 31, 50]. In the selective system of illegitimate hybridization, it is possible to determine the frequency of gene conversion and reciprocal recombination events that triggered the hybridization. Here we examined the effect of Polζ, Polη, and Rev1 inactivation on these events in the a-test.

Inactivation of the *REV7* gene slightly increased the frequency of spontaneous gene conversion and reciprocal recombination (Fig. 4). In contrast, the *REV3* deletion had a negligible effect on the gene conversion and, in fact, decreased the reciprocal recombination almost 2-fold (Fig. 4). We did not observe any significant effect of Polη inactivation on the frequency of spontaneous gene conversion or reciprocal recombination. Simultaneous inactivation of Polη and Polζ, however, decreased the frequency of spontaneous reciprocal recombination 3.5-fold (Fig. 4c). Both conversion and reciprocal recombination are known to be induced by certain types of DNA damage. We observed that UV irradiation did not significantly affect the frequency of gene conversion, but it increased the frequency of reciprocal recombination 3-fold (Table 3). The frequency of gene conversion and reciprocal

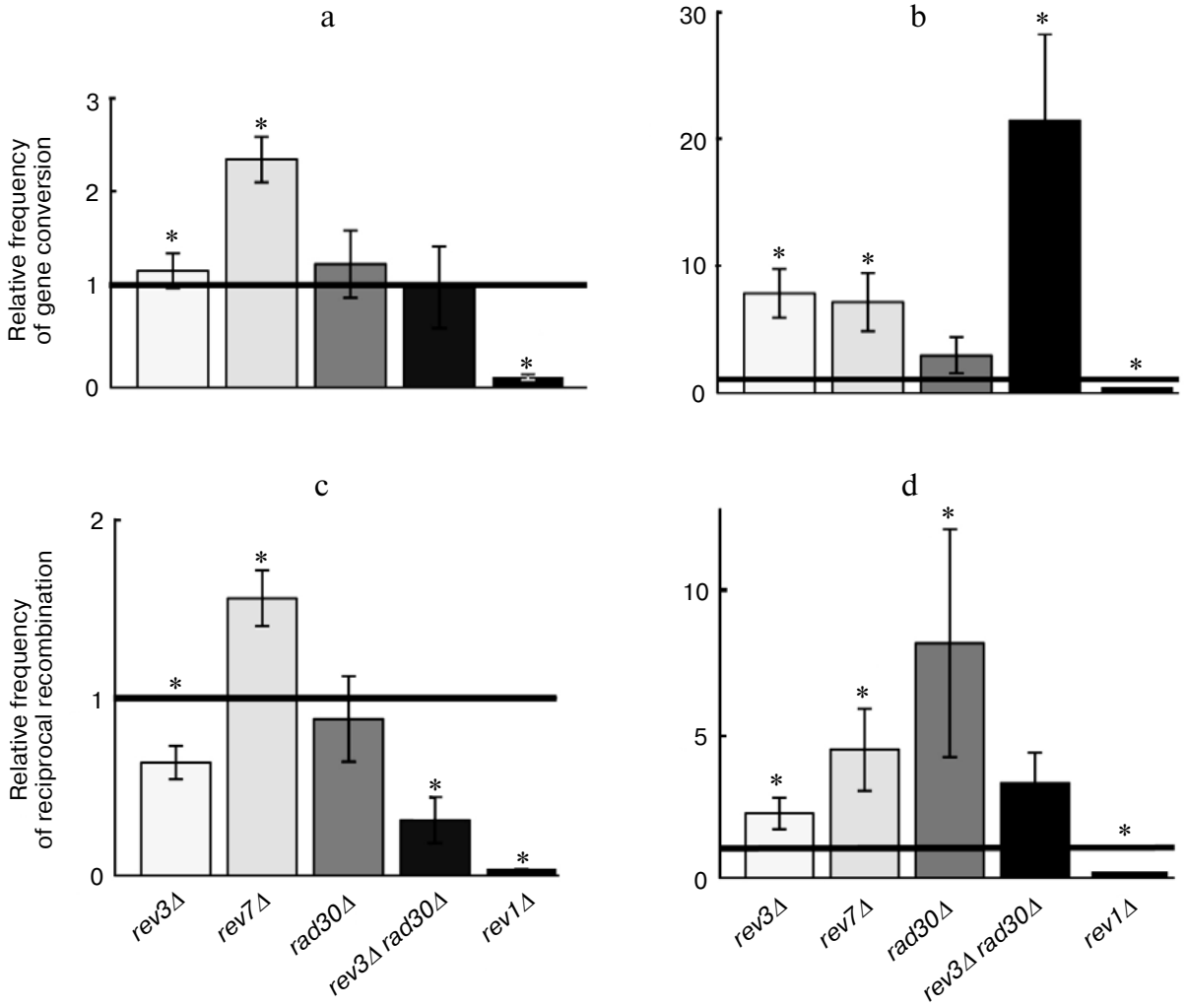

Fig. 4. Relative frequency of gene conversion (a, b) and reciprocal recombination (c, d) in TLS DNA polymerase mutants in the absence of genotoxicant treatment (a, c) and UV-induced frequencies (25 J/m^2) (b, d). All frequencies are means and standard errors for five experiments.

recombination events, however, is changed in some TLS DNA polymerase mutants. Inactivation of either subunit of Polζ increased the UV-induced gene conversion frequency approximately 7-fold (Fig. 4, a and b), but only *REV7* deletion increased the reciprocal recombination frequency after UV irradiation (Fig. 4, c and d). Inactivation of Polη showed no effect on the frequency of UV-induced gene conversion, but inactivation of the *RAD30* gene led to the 8-fold increase in UV-induced reciprocal recombination frequency (Fig. 4). The frequency of UV-induced gene conversion and reciprocal recombination in the double *rev3*Δ *rad30*Δ mutants was elevated 20- and 3-fold, respectively (Fig. 4).

Inactivation of Rev1 dramatically decreased of both spontaneous and UV-induced frequencies of gene conversion and reciprocal recombination (Fig. 4).

Ratio of inherited and non-inherited changes of genetic material in strains with *rev3Δ*, *rev7Δ*, *rad30Δ*, and *rev3Δ rad30Δ* deletions. Primary lesions in DNA that triggered illegitimate mating can either be accurately repaired or transformed into mutations. While mutations and non-inherited lesions in the *MAT* locus of tested strains could not be distinguished in illegitimate diploids, this could be done in the selective system of cytoduction by analysis of cytoductant classes. The cytoductants preserving their original mating type "α" are thought to result from the phenotypic expression of primary lesions that disappeared after mating due to correct repair.

We investigated the effect of TLS inactivation on the ratio of inherited (gene mutations and conversion) and non-inherited (primary lesions) changes in the genetic material using the selective system of cytoduction. While inactivation of Polη (*rad30Δ*) had only a slight effect on this ratio, the inactivation of Polζ (*rev3Δ* or *rev7Δ*) greatly increased the proportion of UV-induced primary (non-inherited) lesions in the *MAT* locus (Table 5). The effect of the double *rev3Δ rad30Δ* mutation was not different from the single *rev3Δ* and *rev7Δ* mutations. Thus, the

Table 5. Percentage of primary lesions that were transformed to mutations (inherited changes) and precisely repaired (non-inherited changes) in TLS DNA polymerase mutants

Strain	Changes, %			
	inherited	non-inherited	inherited	non-inherited
	without UV		UV treatment (25 J/m^2)	
WT	33.0 ± 3.4	67.0 ± 3.2	44.6 ± 3.8	55.4 ± 3.8
rev3Δ	35.0 ± 5.2	65.0 ± 5.2	11.0 ± 4.5	89.0 ± 3.8
rev7Δ	28.2 ± 4.4	71.8 ± 4.5	7.4 ± 0.3	92.6 ± 3.3
rad30Δ	32.0 ± 3.6	68.0 ± 3.6	35.0 ± 3.8	64.2 ± 3.8
rev3Δ rad30Δ	41.4 ± 4.4	58.6 ± 4.4	8.7 ± 0.3	91.3 ± 3.6

percent of non-inherited changes in genetic material is much higher in the absence of Polζ, consistent with the previously established role of this polymerase in mutagenic TLS [23].

DISCUSSION

Translesion synthesis is thought to be a highly mutagenic process that helps cells to tolerate replication-blocking damage even at the expense of decreased accuracy of replication. Because of its ability to register different types of genetic events, the test system used in this study allowed us to form a comprehensive view of the role of TLS in genome stability maintenance. We were able to estimate the ratio of accurately repaired primary lesions to lesions that caused gene mutations, recombination, or chromosome rearrangements both in the wild-type strain and mutants carrying different TLS defects. We provide evidence that TLS protects yeast cells from global chromosome rearrangements and chromosome loss in the presence of genotoxic factors.

According to the current concept of illegitimate αxα mating in yeast, it may occur when the transcription in the *MATα* locus of one of the copulating cells is repressed because of the presence of a lesion in it. The cell switches the mating type to the opposite (reversibly or irreversibly) and becomes able to copulate with another "α" cell. The lesion then can be repaired precisely after the completion of mating or become an inherited change if it is transformed into a mutation. In other cases, unrepaired primary lesions may provoke a double-strand break. Recombinational repair could rescue cells from such toxic double-strand breaks, but it would lead to genetic changes (gene conversion or large chromosomal deletions due to reciprocal recombination in our case). The double-strand breaks could also lead to chromosome or chromosome arm loss.

Investigation of TLS polymerase mutants revealed that *REV3*, *REV7*, and *RAD30* deficiency leads to an increased frequency of illegitimate hybridization of UV-irradiated cells. According to the current mating type switching model, this suggests that the inactivation of TLS polymerases increases the frequency of events that repress transcription of the *MATα*. An interesting possibility is that, in the absence of TLS, lesions may exist longer in DNA impairing the *MAT* locus transcription. Although TLS proteins are not expected to participate in lesion removal, it is conceivable that TLS deficiency increases the time during which lesions remain in the single-stranded DNA region where they cannot be repaired. In addition to blocking transcription, lesions in DNA could result in transcriptional mutagenesis due to inaccurate lesion bypass by the RNA polymerase [51, 52]. Transcriptional mutagenesis could potentially contribute to the mating type switching in yeast. In addition to the lesions themselves, stalled replication complexes could potentially impede the *MATα* locus transcription. Replicative DNA polymerases are blocked by DNA lesions [53]. It is conceivable that the number or the lifetime of the stalled replication complexes could increase if they are not acted upon by TLS in a timely manner. The stalled replication forks could significantly disturb the progression of transcription complexes. In the α-test, the double *rev3Δ rad30Δ* mutants show an additive increase in the frequency of illegitimate mating in comparison to the single-mutant effects. This is consistent with earlier data suggesting the participation of Polζ and Polη in the bypass of different types of lesions [7].

According to one of the current TLS models (the gap-filling model), the replicative polymerase stalling at a lesion leads to a quick re-initiation of replication downstream of the lesion [54]. TLS polymerases are then recruited to bypass the lesion and to fill the gap remaining between the lesion and the site of replication re-initiation. Thus, the absence of TLS polymerases could poten-

tially result in the persistence of single-stranded DNA regions. The increase in UV-induced chromosome loss and chromosome arm loss observed upon TLS polymerase inactivation (Fig. 3) may be a consequence of the accumulation of single-stranded gaps in DNA and their conversion to double-strand breaks. In addition, regions of single-stranded DNA are highly susceptible to spontaneous base damage [55, 56]. If such regions, indeed, accumulate in the TLS mutants, the additional DNA damage that they suffer could contribute to the increased frequency of the *MATα* inactivation and illegitimate mating. Cooperation of two TLS DNA polymerases helps cells to handle different DNA lesions that may lead to the accumulation of single-stranded DNA and double-strand breaks and, thus, chromosome fragility and loss.

In addition to increasing the UV-induced chromosome and chromosome arm loss, the inactivation of either subunit of Polζ increased the frequency of spontaneous chromosome arm loss (Fig. 3, b-d). We propose that spontaneous chromosome arm loss occurs in the Polζ-deficient cells due to events similar to those occurring after UV irradiation. Polζ is well known to participate in the bypass of endogenously generated DNA lesions [57-64]. It also can contribute to the replication of undamaged DNA when the fork progression is impeded for reasons other than DNA lesions [65, 66]. It is, therefore, likely that occasional re-priming of DNA synthesis and the accumulation of single-stranded gaps are also features of the normal DNA replication. As in the case of UV irradiation, the absence of Polζ could result in an increased accumulation of single-stranded DNA, double-strand breaks and, subsequently, increased chromosome arm loss. Replication fork impediment does not only provoke chromosome aberrations and chromosome loss due to the formation of double-strand breaks. Chromosome rearrangements can also result from homologous recombination repair of double-strand breaks [67]. The increased frequency of spontaneous and UV-induced gene conversion in the Polζ-deficient strains is consistent with the idea that Polζ participates in the avoidance of double-strand breaks in the DNA. Interestingly, the inactivation of Polζ and Polη had quite different effects on the frequency of UV-induced gene conversion and reciprocal recombination. This might reflect the participation of these TLS polymerases in the bypass of different types of lesions, and the processing of these lesions by distinct recombination pathways in the absence of these polymerases.

In several cases, we observed that the inactivation of catalytic (Rev3) and accessory (Rev7) subunits of Polζ had different effects on the genome stability. The frequency of spontaneous gene conversion and both spontaneous and UV-induced reciprocal recombination were significantly higher in the *rev7Δ* mutants in comparison to *rev3Δ* mutants (Fig. 4). At the same time, inactivation of Rev3 conferred a much stronger increase in the frequency of chromosome III arm loss than Rev7 inactivation (Fig. 3, c and d). This observation may suggest that Rev3 and Rev7 could function independently of each other. The Rev7 homolog in higher eukaryotes is involved in chromosome segregation control [26, 27]. The unique function of Rev7 observed in our study is unlikely to be related to chromosome segregation, because the frequency of chromosome loss is equally increased in the *rev3Δ* and *rev7Δ* mutants (Fig. 3, a and b).

Interestingly, we observed that the inactivation of Rev1 impedes illegitimate copulation of yeast cells. We believe that this impediment could be related to the cell cycle-specific regulation of *REV1* expression. It was shown recently that, unlike other TLS polymerases, Rev1 is preferentially produced in the G2 phase of the cell cycle [68]. This suggests that Rev1 may be required for damage tolerance pathways specific to the G2 phase. The Rev1-deficient cells could possibly have an altered cell cycle, which could affect their ability to copulate. In support of this idea, Jansen et al. reported that Rev1$^-$ chicken cells are characterized by a prolonged G2 phase of the cell cycle [69].

Numerous genetic syndromes, cancer, and autoimmune diseases are associated with decreased fidelity of DNA replication. One of the approaches that are being developed for the prevention and treatment of such diseases is the inhibition of uncontrolled DNA replication and selective inhibition of mutagenic DNA polymerases, such as TLS polymerases [70]. TLS is a major source of spontaneous and, particularly, genotoxicant-induced point mutations that could contribute to cancer development. This view is supported by a recent study demonstrating that the inactivation of *Rev1* results in a significant reduction in the incidence of tumors in mice exposed to benzo[a]pyrene [71]. The potential of using Polζ as a target for gastric and colorectal cancer prevention and therapy has also been discussed [72]. On the other hand, the *REV3* deficiency has been previously found to induce chromosome instability in chicken, mouse, and mammalian cells [28, 29, 73]. The present study further illustrates that the function of TLS polymerases is important for limiting chromosome instability in yeast, suggesting that this function is widespread among eukaryotes. Consistent with the important role of Polζ in the prevention of large genome rearrangements, *REV3L*$^{-/-}$ mice have recently been found to have increased susceptibility to spontaneous tumorigenesis [74]. The selective inhibition of Polζ, thus, may not be an innocuous approach for cancer prevention and therapy because of the potential to provoke chromosome rearrangements. While it might be efficient for suppressing certain types of cancer in which the accumulation of point mutation plays a major role, the undesired side effect of such a treatment is likely to be the increase in other types of genomic instability and, subsequently, other types of cancer.

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