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Transposon Tn*5* excision in yeast: Influence of DNA polymerases α, δ, and ε and repair genes

(recombination/DNA repair/*rad50, -52*)

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ABSTRACT **Interaction between short repeats may be a source of genomic rearrangements and deletions. We investigated possible interactions between short (9 base pairs) direct repeats in yeast by using our previously described system for analyzing bacterial transposon Tn*5* excision in yeast. Mutations of either *POL3* or *POL1*, the proposed structural genes for polymerases δ and α, respectively, yield high levels of excision at semipermissive temperatures. *pol2* (corresponding to polymerase ε) and *pol2 pol3* double mutants do not exhibit enhanced excision. A majority of excision events involve direct repeats and are precise; the remaining imprecise excisions occur within or in the vicinity of the repeats. The three DNA repair pathways identified by *rad1*, *rad6* and *rad18*, *rad50* and *rad52* mutations were examined for their possible role in Tn*5* excision; no enhancement was observed in mutants. However, the *pol3*-stimulated Tn*5* excision was reduced in *rad52* and *rad50* mutants. This suggests the potential for interaction between the systems for DNA double-strand break/recombinational repair and DNA synthesis. Based on the suggestion of Morrison *et al.* [Morrison, A., Araki, H., Clark, A. B., Hamatake, R. H. & Sugino, A. (1990) *Cell* 62, 1143–1151] that polymerases δ and α are responsible for lagging-strand synthesis and that polymerase ε is responsible for leading-strand synthesis, we suggest that Tn*5* excision is stimulated under conditions of altered lagging-strand synthesis, possibly due to extended opportunities for single-strand interactions between the inverted insertion sequence I550 repeats of Tn*5*.**

Short repeats of DNA sequence are a source of genomic rearrangements, duplications, and deletions. In bacteria, precise excision occurs between short, 9-base-pair (bp) direct repeats of a transposon target sequence. Excision is stimulated by the long, internal, inverted insertion sequences (IS). Molecular and genetic studies suggest that such deletion may result from "illegitimate" recombination between short repeats. The most likely mechanism is the "slippage" of a replicating strand (1–3). The same mechanism was proposed for formation of deletions between short direct repeats (4). Tn*5* and Tn*10* excision in *Escherichia coli* is influenced by genes involved in recombination (*recB, recC*), mismatch correction (*dam, mutH, mutL, mutS, uvrD*), single-stranded DNA-binding protein (*ssb*), and the proofreading exonuclease subunit of DNA polymerase III (*dnaQ*) (5, 6). Precise excision of a small, 50-bp palindromic Tn*10* remnant is increased in *polA* (DNA polymerase I) mutants (6).

Based on results from bacteria, transposon excision may provide a means for studying various DNA metabolic processes, including DNA repair, recombination, and replication. We therefore introduced Tn*5* into the yeast *Saccharomyces cerevisiae* and examined excision from the *LYS2* gene (7). The low excision rate (10^{-9}) was increased 100-fold in tex mutants (8). These and several other yeast DNA repair and metabolism mutants have been examined in order to understand the Tn*5* excision process. Mutations in the structural genes for two of three yeast DNA polymerases (polymerase α and δ, but not polymerase ε) greatly enhance the excision frequency. These observations are consistent with the proposed role for the three DNA polymerases (9) in chromosomal replication and lead us to suggest a replicative role in transposon excision.

From analysis of yeast mutants involved in repair, recombination, and recombinational repair, we conclude that the recombinational pathway for DNA double-strand break (DSB; ref. 10) repair is integral to the previously reported high-frequency Tn*5* excision phenotype of the *tex1* (*pol3*) mutant. However, we suggest that the two genes examined, *RAD52* and *RAD50*, may also function during replication, which for the case of Tn*5* can lead to enhanced excision.

MATERIALS AND METHODS

Plasmids and Strains. Genetic nomenclature is described in ref. 11; see refs. 9 and 12 for DNA polymerases. The plasmid pBL304, provided by P. Burgers, contained a 3.7-kilobase (kb) *Hin*dIII/*Mlu* I fragment with *POL3* (13) cloned between the *Hin*dIII and *Mlu* I sites of YCp50. The plasmid HL1 bearing the 12-kb fragment with *POL3* was derived from a yeast genomic DNA library (kindly supplied by P. Hieter) based on a p366 vector, an analog of YCp50 in which *URA3* is replaced by a 2.2-kb *LEU2* fragment. YRp14/*trp1-Δ1* (p13) plasmid was used (14) to make the deletion of *TRP1*.

Strains are presented in Table 1. The strain *POL*$^+$-DM corresponds to the replacement transformant of *pol3-t*-DM by the 11-kb *Eco*RI fragment with *POL3* from the plasmid HL1.

Different *rad* and *pol* mutants were created from *POL*$^+$-DM and *pol3-t*-DM strains by the technique of one-step gene disruption (16) and were confirmed genetically. *pol2-1* disruptions were confirmed by Southern blot analysis. The following deletion/disruption constructs were used to make mutations in the corresponding genes: *REV3, REV3* with a *LEU2* insertion (17); *RAD1*, the 6.96-kb *Sal* I fragment of plasmid L962 (constructed by R. Keil) with the replacement of the 2.1-kb *Stu* I/*Cla* I fragment of *RAD1* (70% of the coding sequence) by the 3.2-kb *LEU2 Bgl* II fragment with *RAD6*; RAD6, *Hpa* I-digested plasmid pDEL21 where all of *RAD6* is replaced by *URA3* (18); *RAD18*, an *Eco*RI/*Xho* II fragment of the plasmid rad18-del-*TRP1* (A. Morrison, per-

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Abbreviations: IS, insertion sequence; DSB, double-strand break.

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Table 1. Yeast strains used in this study and source

Strain	Genotype or parents	Ref. or source
POL$^+$-DM	*MATα lys2*-Tn*5–13 ura3 leu2-2 trp1-Δ1**	This study
pol3-t-DM†	*MATα lys2*-Tn*5–13 ura3 leu2-2 trp1-Δ1 pol3-t*	This study
2749-2-2	*MATa pol3-2 his7 leu2*	L. Hartwell
DAG2	2749-2-2 × *POL*$^+$-DM	This study
2789-27-1	*MATa pol1-1 leu2 ura3 can1 sap3 his7*	L. Hartwell
DAG3	2789-27-1 × *POL*$^+$-DM	This study
pol1-17-ss111	*MATa pol1-17 ura3-52 trp1-289 ade2-101 tyr1 gal2 can1*	15
5d-D512	*MATα lys2*-Tn*5–13 leu2-2* Pol$^+$	8
DAG9	*pol1-17*-ss111 × 5d-D512	This study
5a-D512	*MATa lys2*-Tn*5–13 leu2-2 pol3-t*	8
CH265-7c-rev3	*MATα rev3::LEU2 his3-Δ1 arg4-17 trp1 leu2-3,112 ura3-52*	A. Morrison
D627	CH265-7c-rev3 × 5a-D512	This study
17c-DAG8	*MATα pol1-17 pol3-t ade2-101 lys2*-Tn*5–13 leu2-2*	This study
3a-D395	*MATa pol3-t lys2*-Tn*5–13 leu2-2*	8
lt13-f2-α′1	*MATα lys2*-Tn*5–13 leu2-2* Pol$^+$	8

The genotypes of the haploid strains and the strain numbers of haploids used for crosses are shown.
*The *trp1-Δ1* deletion was created by gene replacement using plasmid YRp14/trp1-Δ1.
†Former name was 2trp-14a-D575.

sonal communication) in which *RAD18* is replaced by *TRP1; RAD50*, an 11.5-kb *Eco*RI/*Bgl* II fragment of the plasmid pNKY83, containing a *HISG-URA3-HISG* insert replacing the entire *RAD50* gene (19); *RAD52*, a 3141-bp *Sph* I/*Sal* I fragment of the pDU1 plasmid in which the major part of the *RAD52* reading frame between the *Bst*EII and *Pst* I sites is replaced by a 2058-bp *URA3 Bcl* I/*Bam*HI fragment; and *POL2*, a 4.0-kb *Eco*RI fragment of the plasmid P2A5 in which the 1.2-kb *URA3 Bam*HI fragment is inserted in the *Bgl* II site of *POL2* (9).

Genetic Procedures and Molecular Studies. Transposon excision was assayed by using the lys2::Tn*5–13* insertion (7, 8). Methods for determining the excision frequency have been described (8). A cell suspension (≈10^6 cells per ml) was plated by using a 120-pin replicator to yeast extract/peptone/dextrose. Each drop contained 1000–2000 cells. After growth at different temperatures, the plates were replica plated to medium lacking lysine and then incubated at 20°C. Revertant colonies were counted after 14–15 days. To estimate the number of cells on replica imprints, cells in imprints lacking papillae were resuspended in water and counted with a hemocytometer. Where a decrease in excision frequency per plated cell was detected compared to a wild-type strain, the excision frequency per survivor was also measured in order to demonstrate that the decrease was not due simply to a decrease in survival. The rate of excision based on 8–17 independent cultures was estimated by the method of Lea and Coulson (20).

DNA Sequencing. For sequence determination of precise and imprecise excisions of Tn*5–13*, the 388-bp *Bam*HI/*Xho* I region of *LYS2* (where the Tn*5–13* insertion resided) was cloned into plasmid pLL12-BX (8) by gap filling. Plasmid DNA was prepared by the alkaline denaturation method (21) and sequenced with modified T7 polymerase by two-step dideoxynucleotide sequencing (22). We used the 19-mer oligonucleotide primer ("Impuls" Novosibirsk, U.S.S.R.) CAGTGAACTAACGCACCAT complementary to the sequence near the *Bam*HI site 179 bp from the site of insertion.

RESULTS

Tn*5* Excision in DNA Polymerase Mutants. When the bacterial transposon Tn*5* is introduced into the yeast *LYS2* gene, it is rarely excised (Table 2). The temperature-sensitive *tex1* mutation increased excision nearly 100-fold at semipermissive temperatures (8). Based on the *tex1* phenotype, including mutability and chromosome loss, several cell-cycle mutants defective in DNA metabolism were examined for allelism. In the current work, the *tex1* mutation was found to be an allele of *POL3* (formerly *CDC2*), the structural gene for DNA polymerase δ (13, 23), and it was renamed *pol3-t*. The allelism was ascertained from crosses with *pol3-2* and the temperature sensitivity of *tex1* was complemented by *POL3* contained in plasmids pBL304 and HL1.

A role for replication in Tn*5* excision was further revealed in studies with *pol3-2* and two alleles of *POL1* (formerly *CDC17*), the structural gene for DNA polymerase α (all mutants are temperature sensitive for growth at 37°C). Tn*5–13* excision frequencies were determined in haploid isolates obtained from tetrads of the appropriate diploids. Effects of the *pol1* and *pol3* alleles were determined after pregrowth at different temperatures since the excision increase is temperature dependent (8). Similar to results with *pol3-t*, excision at the semipermissive temperature of 30°C was increased up to 100-fold over wild-type levels in all *pol1* and *pol3* segregants (Table 2). Except for some *pol3-2* segregants, wild-type excision frequencies were observed after pregrowth at 20°C. The *pol3-t*-DM mutant also exhibited high excision frequencies at 25°C. The *pol1 pol3* double mutant 17c-DAG8, which grows poorly at 30°C, exhibited an excision frequency comparable to that of the single mutants at 25°C.

In addition to DNA polymerases α and δ, DNA polymerase ε is also proposed to be required for replicative synthesis (9). Neither the nonlethal disruption mutation *pol2-1* (Table 2) nor a temperature-sensitive mutation *pol2-ts* (provided by A. Sugino; E.P., unpublished results) increased Tn*5* excision frequency. Another gene, *REV3*, encodes a predicted protein sequence similar to that of DNA polymerase α and is likely to be a nonessential polymerase involved in mutagenesis (17). A disruption mutation of this gene did not increase Tn*5* excision.

While the *pol2* and *rev3* mutations did not increase Tn*5* excision, a decrease was not excluded; decreased excision would have been difficult to detect because the wild-type level is low, ≈10^{-9}. We therefore examined their effects on excision in a *pol3* background, wherein decreases should be readily detected. When the *rev3::LEU2* mutation was combined with the *pol3-t* mutation, it did not significantly alter the high excision frequency observed for *pol3* mutants after pregrowth at 30°C (Table 2). Wild-type levels were observed after growth at 20°C. Contrary to this result, *pol2-1* reduced the high excision of the *pol3-t* mutant to wild-type levels at 25°C. (Because the double mutant exhibited poor growth at 30°C, excision frequencies were determined after pregrowth at 20°C or 25°C.) To assess further the effect of *pol2-1*, the

Table 2. Effects of mutations in DNA polymerase genes on Tn5 excision

POL genotype	Reversion frequency × 10^{-8} 20°C	25°C	30°C	Fraction of revertants that are Adp$^+$*	Strains tested
Pol$^+$	0–0.3	<1†	0–0.4	0.16 (0.05–0.38)	Segregants‡ of D512, DAG2, DAG3, DAG9; haploids *POL*$^+$-DM, lt13-f2-α'1
pol1-1	0–5.6	ND	6.9–19.3	0.12 (0.04–0.28)	6 *pol1* segregants of DAG3
pol1-17	0–0.8	ND	1.2–33	ND	9 *pol1* segregants of DAG9
pol3-2	0–54	ND	6.0–88	0.03 (0.006–0.09)	11 *pol3* segregants of DAG2
pol3-t	0–1.2	35 ± 11§	5.0–52	0.08 (0.03–0.16)	22 *pol3* segregants of D512; haploids *pol3-t*-DM, 3a-D395
pol1-17 pol3-t	0.07 (0–0.6)	11 ± 4	ND	ND	17c-DAG8
rev3::LEU2	0–0.4	ND	0–0.4	ND	10 *rev3 POL3* segregants of D627
pol2-1	<1	0.26 (0–0.6)	<1	ND	*pol2-1* derivative of *POL*$^+$-DM
pol3-t rev3::LEU2	0–0.5	ND	21–100	ND	4 *rev3 pol3* segregants of D627
pol3-t pol2-1	0.12 (0.03–0.3)	0.5 (0.2–0.9)	ND	ND	*pol2-1 pol3-t*-DM

Frequencies correspond to revertants per plated cell. The mean numbers (based on at least four experiments) ± SD or 95% confidence limits (for low frequencies) are presented in all cases where the frequency was determined for one strain. Confidence limits are given in parentheses. When frequencies were determined for two or more strains, the highest and the lowest frequency are presented. ND, not determined.

*The fraction of Lys$^+$ Adp$^+$ revertants was determined in the following strains: *Pol*$^+$, strain lt13-f2-α'1; *pol1-1*, haploid segregants 1a, 10c and 22b from DAG3; *pol3-2*, haploid segregants 1b and 2c from DAG2; *pol3-t*, strain 3a-D395. The 95% confidence limits are given in parentheses. All revertants were selected after pregrowth at 30°C.

†The excision frequency was determined only for strain *POL*$^+$-DM.

‡The following numbers of haploid Pol$^+$ segregants were used for frequency determinations: D512, 22 segregants; DAG2, 11 segregants; DAG3, 10 segregants; DAG9, 9 segregants. Data for the D512 Pol$^+$ and *pol3-t* segregants are from ref. 8.

§Determined for the *pol3-t*-DM strain only.

rate of excision per generation was determined using the method of the median (ref. 20; see *Materials and Methods*). The rate for the *pol3-t*-DM strain was 14 × 10^{-8} after pregrowth at 25°C, while the double mutant *pol2-1 pol3-t*-DM strain exhibited a rate of 1.1 × 10^{-8} after pregrowth at both 20°C and 25°C.

The Role of Different Repair Pathways in Tn5 Excision. Because repair processes might also play a role in Tn5 excision, we tested the effects of mutations (in isogenic backgrounds) blocking each of three genetically defined yeast repair pathways (Table 3): *rad1*, excision repair; *rad6* and *rad18*, mutagenesis; *rad50* and *rad52*, recombinational/double-strand break repair (24). Reversion frequencies of the *pol3-t rad50* and *pol3-t rad52* strains were measured after pregrowth at 25°C because of poor growth at 30°C. Frequencies for all other strains were determined after pregrowth at 30°C.

None of the repair mutations in a Pol$^+$ background increased the excision frequency; decreases could not be detected because of the low excision frequency in Pol$^+$ strains. However, as described above, decreases could be identified by incorporating a mutation into a *pol3-t* background. The presence of a *rad50* or *rad52* mutation in combination with *pol3-t* reduced Tn5 excision to that observed in Pol$^+$ strains. The rate of excision per generation for the double mutants was 1.2 × 10^{-8} and 1.0 × 10^{-8}, respectively. The other *rad* mutations had no effect on excision frequency in *pol3-t* strains.

Table 3. Effect of mutations in different repair pathways on Tn5 excision

RAD genotype	Reversion frequency × 10^{-8} *POL*$^+$	*pol3-t*
Rad$^+$	0.02 (0–0.11)	38 ± 17
rad1	<1	71 ± 23
rad6	<1	40 ± 21
rad18	<1	71 ± 30
rad50	<1	1.5 (0.9–2.3)
rad52	<1	0.13 (0.05–0.4)

Frequencies were measured for strains *pol3-t*-DM and *POL*$^+$-DM and for *rad* deletion and disruption mutants of these strains. Mean numbers ± SD or mean numbers and 95% confidence limits (for low frequencies) are presented. The confidence limits are given in parentheses.

Imprecise Excision of Tn5 Is Also Increased by *pol1* and *pol3* Mutations. Two types of Lys$^+$ chromosomal revertants can be distinguished genetically. Those with decreased levels of the *LYS2* gene product, aminoadipate reductase, are able to grow (Adp$^+$) on medium containing α-aminoadipate as the sole nitrogen source (25), whereas wild-type *LYS2* strains are unable to grow (Adp$^-$) on this medium. Therefore, Lys$^+$ Adp$^+$ revertants of *lys2*::Tn5 are presumptive imprecise excisions and Lys$^+$ Adp$^-$ revertants are presumptive precise excisions. This was tested by sequencing revertants in the region of the expected excision. The three Lys$^+$ Adp$^-$ revertants from a *pol3-t* background were generated by precise excision of Tn5. [Previously, we reported precise excision of Tn5–*13* based on sequencing a limited number of *LYS2* revertants of a *lys2*::Tn5 insertion mutation located on a multicopy YEp plasmid (7).] Among seven independent Lys$^+$ Adp$^+$ isolates examined, all arose from imprecise excision (Fig. 1); three were from a Pol$^+$ and the rest were from a *pol3-t* background. The breakpoints for all seven sequenced imprecise excisions were situated on short direct repeats in Tn5 and *LYS2* sequences near the original Tn5 ends (see Fig. 1).

Using the criterion of growth on α-aminoadipate, we determined the frequency of Adp$^+$ revertants in the various polymerase mutants (Table 2). The frequencies of Adp$^+$ revertants are comparable among all excision events in Pol$^+$, *pol1-1*, and *pol3-t*. While the Adp$^+$ fraction in *pol3-2* appears somewhat reduced, there are still considerable imprecise events. Thus, DNA polymerase defects can increase the frequency of both precise and imprecise Tn5 excision, and the ratio of the two in Pol$^+$ and mutants is similar.

DISCUSSION

The insertion of transposon Tn5 into the yeast genome has provided the opportunity to investigate interactions between DNA regions containing small repeats. Using various DNA polymerase mutations, we have concluded that replicative components play an important role in Tn5 excision. The products of the *POL1*, *POL2*, and *POL3* genes have been concluded to be DNA polymerases involved in replication (ref. 9 and references therein). Because several mutant alleles were examined, the observed differences between the vari-

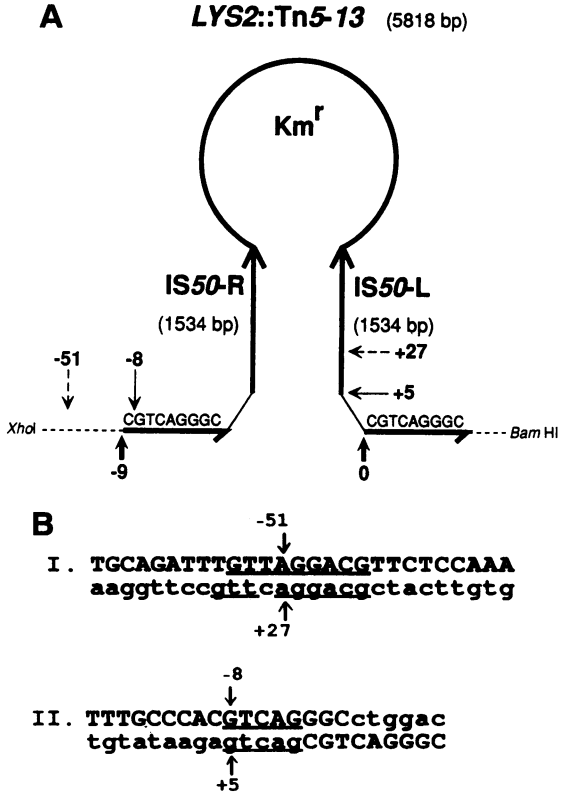

FIG. 1. The breakpoints of Tn*5–13* excision events. (*A*) Location of the Tn*5–13* insertion. Tn*5–13* is located 160 bp from the *Xho* I site in the middle of *LYS2* (7). Only the sequence of the duplicated 9-bp target is shown. The pair of thick arrows shows an example of breakpoints for precise excision. The pairs of thin and broken arrows show the positions of two types of imprecise excision breakpoints. (*B*) Short repeats in the breakpoints of imprecise excision in the strains 3a-D395 (*pol3-t*) and lt13-f2-α'1 (Pol$^+$). Short repeats and surrounding sequences are shown. *LYS2* and Tn*5* sequences are shown by capital and lowercase letters, respectively. Repeats are underlined. Type I excision was demonstrated in one revertant of 3a-D395. Type II excision was found in three revertants of 3a-D395 and in three revertants of lt13-f2-α'1. All revertants were obtained after pregrowth at 30°C.

ous polymerases appear to be related to their general functions and not to allele specificity. The *tex1* mutant (8) carries a mutation in the structural gene for polymerase δ (*pol3*). Mutations of this gene and of that for polymerase α (*pol1*) greatly increased excision at semipermissive temperatures, whereas the polymerase ε mutations (*pol2*) did not. Furthermore, the polymerase ε mutations blocked the increase observed in polymerase δ mutants.

Of the three genetically identified repair pathways examined (24, 26), only the *rad50* and *rad52* mutants greatly depressed the enhanced excision observed in *pol3* mutants. *RAD50* and *RAD52* are required for the repair of DSBs (ref. 27; J. Nitiss and M.A.R., unpublished data), which is accomplished by recombinational repair (10).

While these results may implicate DSBs and DSB recombinational repair systems in Tn*5* excision, we suggest that the *RAD50* and *RAD52* gene products may have functions in DNA metabolism other than recombination and that DSBs may not be responsible for the observed increases in Tn*5* excision. Based on genetic and physical evidence, both *rad52* and *rad50* mutations interfere with meiosis but at very different stages; unlike *rad50*, *rad52* mutants exhibit substantial meiotic recombination (28–32). *RAD52* may also have a replicative function, as indicated by the base pair substitution mutator phenotype of *RAD52* mutants (33, 34). [We did not consider the reported nuclease function of *RAD52* (35) because attempts by us (E.P., J. W. Drake, and M.A.R., unpublished data) and others to duplicate those results have been unsuccessful.]

The observed differences between the various replicative DNA polymerase mutants and the effects of the *rad50* and *rad52* mutants lead us to summarize our results in light of the suggestion of Morrison *et al.* (9) that polymerases α, δ, and ε may function in leading- and lagging-strand synthesis. The model is based solely on the reported activities of DNA polymerases α, δ, and ε. An important feature is that altered lagging-strand synthesis could generate local regions of single-stranded DNA. The model (Fig. 2) incorporates proposed mechanisms for Tn*5* excision in *E. coli* (3), wherein complementary intrastrand pairing of inverted repeats leads to a hairpin structure that might delay DNA synthesis and possibly lead to replication errors. For example, forward slippage of a replicating strand (Fig. 2*D*) might result from localized loss of pairing of the newly synthesized 3′ end followed by pairing with the direct repeat on the other side of the Tn*5* sequence. This would cause the loss of Tn*5* in one of the two newly synthesized strands. Initial experiments (D.A.G., unpublished data) suggest that the long inverted repeats in Tn*5* play an important role in excision in yeast, because excision of the transposon IS*1* inserted into *LYS2* is not increased by *pol3-t*. IS*1* also has small (8–11 bp) direct repeats (36) but the inverted repeats are only 23 bp of imperfect homology.

According to Morrison *et al.* (9), the polymerase α gene probably synthesizes RNA primers and short adjacent DNA segments at the replication origin on the leading strand and at

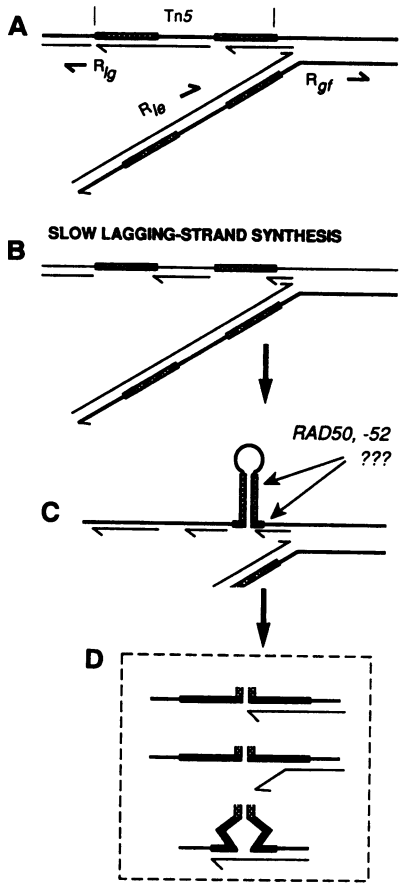

FIG. 2. DNA synthesis and bypass/excision of Tn*5*. Presented is a segment of replicating DNA with a Tn*5* element contained between the small repeats (designated as short solid blocks). Details of the model are described in text.

the beginning of Okazaki fragments on the lagging strand. Polymerase δ then completes the synthesis of these fragments. Leading-strand replication is suggested to be accomplished by polymerase ε. In the model described in Fig. 2, the rate of advancement of the growing fork is designated R_{gf}; rates of leading- and lagging-strand synthesis are designated R_{le} and R_{lg}. Under normal growth conditions, all three parameters are equal. We propose that delays in completing replication of the lagging strand without coordinated delays of leading-strand synthesis ($R_{lg} < R_{le} = R_{gf}$) could produce longer-lived single-stranded regions. These regions would provide an opportunity for Tn*5* to adopt the requisite secondary structure. [This proposal about delayed lagging-strand synthesis does not conflict with the observation that about one-third of the yeast genome remains unreplicated in a *pol3* mutant at the nonpermissive temperature (37).] The results with the *pol2* and the *pol2 pol3* mutants are consistent with our hypothesis. If it is assumed that lagging-strand and leading-strand syntheses are coordinated ($R_{lg} = R_{le} = R_{gf}$), defects in the leading-strand polymerase should not yield single-stranded regions and thus there would be no increased excision. The double mutant might result in a coordinately low rate of synthesis at the semipermissive temperature. However, we cannot exclude the fact that polymerase ε is involved directly in the last step of excision (Fig. 2*D*).

The *RAD50* and *-52* gene products play roles in homologous recombination and recombinational repair, either directly or through genes they control. Possibly they promote or stabilize the pairing of the inverted repeats; this would facilitate replication past the base of the hairpin. Alternatively, they might provide a homologous pairing activity that transfers the 3′ stalled end at the base of the palindrome (Fig. 2) to the next repeat, thereby allowing replication to continue. A similar function has already been proposed for the T4 recombination protein UvsX in replication past DNA lesions (38, 39). The view that Tn*5* can adopt a unique structure is supported by recent results demonstrating that Tn*5* undergoes mitotic gene conversion at least 10 times more frequently than do other *lys2* alleles (point mutations; 1- to 3-kb deletions; Ty1 insertion) (D.A.G. and A.L.M., unpublished data).

The excision of Tn*5* resulting in prototrophy appears to involve interaction between a repeated or related regions. Two categories of excision events, precise and imprecise, could be identified genetically. The majority (>85%) of events in POL^+ and pol^- mutants are due to precise excision (ref. 7 and this report). Imprecise excision breakpoints also occur between short repeats (Fig. 1): 5 bp of perfect homology in six cases, and 9 bp with 6 bp of perfect homology plus a 1-bp insert in one case. The imprecisely excised revertants demonstrate that different in-frame deletions in the region of Tn*5*–*13* insertion can still yield functional aminoadipate reductase. Because it is improbable that the only in-frame deletions that are compatible with enzyme function are those with ends in direct repeats (25), the direct repeats are likely to play an important role in Tn*5* excision. Excision events that cause out-of-frame changes would not be identified. Our results demonstrating imprecise excision are consistent with previous reports with bacteria for both Tn*5* and Tn*10*: breakpoints have been identified corresponding to positions of limited homology with one of the direct repeats or between regions of limited homology near the direct repeats (40, 41).

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