The Rad27 (Fen-1) Nuclease Inhibits Ty1 Mobility in *Saccharomyces cerevisiae*

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ABSTRACT

Although most Ty1 elements in *Saccharomyces cerevisiae* are competent for retrotransposition, host defense genes can inhibit different steps of the Ty1 life cycle. Here, we demonstrate that Rad27, a structure-specific nuclease that plays an important role in DNA replication and genome stability, inhibits Ty1 at a post-translational level. We have examined the effects of various *rad27* mutations on Ty1 element retrotransposition and cDNA recombination, termed Ty1 mobility. The point mutations *rad27-G67S*, *rad27-G240D*, and *rad27-E158D* that cause defects in certain enzymatic activities *in vitro* result in variable increases in Ty1 mobility, ranging from 4- to 22-fold. The C-terminal frameshift mutation *rad27-324* confers the maximum increase in Ty1 mobility (198-fold), unincorporated cDNA, and insertion at preferred target sites. The null mutation differs from the other *rad27* alleles by increasing the frequency of multimeric Ty1 insertions and cDNA recombination with a genomic element. The *rad27* mutants do not markedly alter the levels of Ty1 RNA or the TyA1-gag protein. However, there is an increase in the stability of unincorporated Ty1 cDNA in *rad27-324* and the null mutant. Our results suggest that Rad27 inhibits Ty1 mobility by destabilizing unincorporated Ty1 cDNA and preventing the formation of Ty1 multimers.

THE retrotransposon Ty1 is the most abundant mobile genetic element in *Saccharomyces cerevisiae*. These elements are present in ~30 copies per haploid genome and are structurally and functionally related to retroviruses (reviewed by Voytas and Boeke 2002; Figure 1A). Ty1 elements are flanked by long terminal repeats (LTRs) and are transcribed from end to end, resulting in RNA that serves as template for both translation and reverse transcription. Translation results in synthesis of TyA1, which encodes a gag-like capsid protein, and TyB1, which encodes the enzymatic proteins protease, reverse transcriptase/ribonuclease H, and integrase. The structural proteins assemble into a virus-like particle (VLP) within which reverse transcription takes place. The resulting linear double-stranded cDNA enters the genome through integrase-mediated integration at a new chromosomal site or, to a lesser degree, by homologous recombination with genomic elements. The cDNA recombination pathway is more active when the integrase-mediated pathway is blocked. Introduction of the Ty1 cDNA into the genome by integration or by homologous recombination is termed Ty1 mobility.

Ty1 retrotransposition is potentially mutagenic since these elements can transpose and mutate essentially any gene (Smith *et al.* 1995). Further, homologous recombination between Ty elements can lead to chromosomal rearrangements, thereby affecting the integrity of the genome (Voytas and Boeke 2002). However, yeast cells minimize such events by modulating several steps in the Ty1 life cycle. Host genes have been identified that affect Ty1 transcription (Winston *et al.* 1984), programmed +1 frameshifting that is required to synthesize the TyA1-TyB1 fusion protein (reviewed by Farabaugh 1995), Ty1 protein processing and VLP maturation (Curcio and Garfinkel 1992; Conte *et al.* 1998), target site preference (Ji *et al.* 1993; Devine and Boeke 1996; reviewed by Boeke and Devine 1998), and cDNA stability (Lee *et al.* 1998, 2000). Recently, *SGS1* has been shown to inhibit Ty1 mobility by preventing transposition of multimeric Ty1 elements (Bryk *et al.* 2001).

In this work we examine the effects of the host gene *RAD27* and other members of the RAD2 nuclease family on Ty1 element mobility. *RAD27*, the yeast homolog of the mammalian gene *FEN1*, is related to the RAD2 family of structure-specific nucleases that are involved in DNA metabolism and repair (reviewed by Friedberg 1991 and Prakash *et al.* 1993; Reagan *et al.* 1995). The members of the RAD2 nuclease family, *EXO1*, *DIN7*, *RAD27*, *YEN1*, and *RAD2* are conserved from humans to bacteriophages (reviewed by Lieber 1997) and mutations in these genes can result in increased risk of disease (Tishkoff *et al.* 1997). For example, mutations in human *XP-G* (*RAD2* homolog) cause xeroderma pigmentosum (reviewed by de Boer and Hoeijmakers 2000), and variants in *EXO1* may be associated with hereditary nonpolyposis colorectal cancer (Wu *et al.* 2001).

Rad27/Fen1 is a 5′-3′ exonuclease and a 5′-flap endonuclease that plays an important role in DNA replication, repair, and recombination (Lieber 1997). The nuclease activity of Rad27/Fen1 removes RNA primers made during lagging strand DNA synthesis (reviewed

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by Bambara *et al.* 1997). Rad27/Fen1 participates in DNA repair through removal of damaged DNA by the base-excision repair pathway (Klungland and Lindahl 1997; Johnson *et al.* 1998; Kim *et al.* 1998) and also prevents short sequence recombination (SSR), a process that can lead to genome rearrangements (Negritto *et al.* 2001). Consistent with its involvement in DNA metabolism, *rad27* deletion mutants are sensitive to DNA damaging agents (Reagan *et al.* 1995; Xie *et al.* 2001). Mutations in *RAD27* result in a potent mutator phenotype and display a variety of genomic rearrangements (Chen and Kolodner 1999; Greene *et al.* 1999; Xie *et al.* 2001). Deletion of *FEN1/RAD27* also results in expansion of di- and trinucleotide repeats that have been shown to be the underlying cause of several human disorders (Schweitzer and Livingston 1998; White *et al.* 1999). Our results indicate a novel role for *RAD27* in inhibiting Ty1 mobility, which is important for maintaining genome stability in yeast.

MATERIALS AND METHODS

Construction of mutants: The *exo1* mutant BLY187 was constructed by one-step gene disruption of the *EXO1* gene in strain DG1657 (*MAT***a** *ura3-167 his3*Δ*-200 trp1-hisG leu2-hisG* Ty1-270*his3-AI* Ty1-588*neo Ty1-146[tyb1::lacZ]*) using plasmid pSH164 (kindly provided by S. Holbeck) digested with *Nde*I. The *din7* mutant BLY210, an isogenic *din7::TRP1* derivative of the *DIN7* strain DG1657, was constructed by one-step gene replacement with a 2.3-kb *Nde*I/*Sph*I fragment from plasmid pBL26 (a pUC19 derivative that contains the *din7::TRP1* allele). These gene disruptions were verified by Southern analysis. The *rad27* deletion strain BLY184 was constructed in DG1657 by single-step gene disruption using plasmid pBL22 (a pUC19 derivative carrying *rad27::LEU2*) digested with *Hin*dIII and *Nde*I. The wild-type and *yen1* mutant strains W303-1A and LSY485-2C (kindly provided by L. Symington) were transformed with pBDG954, a centromere-plasmid containing Ty1-*neo-AI* (the original plasmid, pBJC546, was kindly provided by M. J. Curcio) to generate strains DG2250 and DG2248, respectively. The *rad27* mutants DG2102 (*rad27-G67S*), DG2103 (*rad27-G240D*), ANU115 (*rad27-324*), and the wild-type strain DG2101 were constructed by digesting plasmids pEAI143, pEAI144, pEAI142, and pEAI141 (kindly provided by E. Alani) with *Bgl*II (Xie *et al.* 2001), followed by transformation of the parental strain DG1657. Leu^+ transformants were selected and *rad27* mutants were identified by their mutator phenotype at the *CAN1* locus and by temperature sensitivity. The *rad27-E158D* mutant was made in two steps. First, the *Cla*I/*Bst*XI fragment carrying *rad27-E158D* mutation from plasmid pLAY362 (Negritto *et al.* 2001; kindly provided by A. Bailis) replaced the wild-type *RAD27* fragment present in pEAI141 to give rise to pANU101. Second, plasmid pANU101 was digested with *Bgl*II and introduced into strain DG1657. The correct Leu^+ transformant containing *rad27-E158D*, ANU105, was identified by its mutator phenotype at *CAN1* and then sequenced to confirm the presence of the *E158D* mutation. The *rad27* mutant strains used in the cDNA recombination assay (ANU116, ANU117, ANU118, ANU119, ANU120, and DG2180) were constructed essentially in the same manner as above except that the starting strain was DG2179 (*MAT*α *his3-*Δ*200 ura3-167 leu2-hisG trp1-hisG*).

Ty1 mobility: To detect spontaneous Ty1 insertion events in strains bearing the Ty1*his3-AI* reporter, cells were streaked for single colonies on YPD plates and incubated at 20° for 5 days. The cells were then replica plated onto synthetic complete medium lacking histidine (SC-His) and incubated at 30° for 3–4 days. Quantitative Ty1*his3-AI* insertion rates were determined as described previously (Curcio and Garfinkel 1991).

Ty1 insertions at *SUF16*: Spontaneous Ty1 insertions upstream of the *SUF16* (glycine tRNA) locus on chromosome III were detected after growing individual colonies from wild-type and *rad27* mutant strains on YPD plates at 20° for 7 days. Three colonies from each strain were then inoculated into 10 ml of YPD broth and grown for 2 days at 20°. Total genomic DNA was isolated from each culture as described by Hoffman and Winston (1987) and analyzed by PCR. The primers SNR33 OUT (5′-TTTTAGAGTGACACCATCGTAC-3′, specific to the *SNR33* gene adjacent to the 3′ end of *SUF16* on chromosome III), and TYB OUT (5′-GAACATTGCTGATGT GATGACA-3′, specific to Ty1) were used in PCR reactions to amplify Ty1 insertions, as described previously (Lee *et al.* 2000). A sample of the PCR reaction was analyzed by agarose gel electrophoresis, transferred to Hybond-N membrane, and subjected to Southern hybridization using a ^{32}P-labeled Ty1 LTR probe. To ensure that the genomic DNA was PCR competent, DNA preparations were analyzed by PCR using primers that bracket *SUF16* (SNR33 OUT and YCR016W OUT 5′-GAT CATCATCTATTAGATTGGA-3′).

Northern analysis: Total RNA was isolated as described by Schmitt *et al.* (1990) from wild-type and *rad27* mutant strains grown to late log phase in 10 ml of YPD broth at 20°. The RNA samples were run on a 1% agarose/formaldehyde gel and transferred to nitrocellulose membrane. The ^{32}P-labeled DNA probes were made by randomly primed DNA synthesis (Amersham, Piscataway, NJ). The Ty1*his3-AI* probe was made by digesting plasmid pOY1 (Lee *et al.* 1998) with *Pst*I and gel purifying a 0.5-kb fragment. A 3.6-kb *Pvu*II fragment from pOY1 was used to make the Ty1 probe. The *PYK1* probe was made from a 1.4-kb *Eco*RI-*Xba*I fragment from pBDG502. Hybridization analysis was performed as described previously (Lee and Culbertson 1995; Lee *et al.* 1998), and the signals were quantified using a Typhoon 8600 phosphorimager (Molecular Dynamics, Sunnyvale, CA) and ImageQuant 1.2 software.

Western analysis: Total protein was isolated from wild-type and *rad27* mutant strains after growth in YPD broth at 20°, as described by Lee *et al.* (1998). Protein concentrations were determined using the BCA protein assay reagent kit (Pierce, Rockford, IL). Proteins were separated on a 10% SDS-polyacrylamide gel (Invitrogen, San Diego) and transferred to Immobilon-P membrane (Millipore, Bedford, MA) using a semidry electroblotter. The membrane was incubated with polyclonal antibodies to Ty1-VLPs for 2 hr. Detection was performed using an ECF Western blotting kit (Amersham), and signals were quantified by phosphorimaging according to the supplier's recommendations. The membrane was then stripped of the Ty1-VLP antibody and incubated with a polyclonal antibody to Hts1 (histidinyl tRNA synthetase, kindly provided by T. Mason) and processed as described above.

Southern analysis of Ty1 cDNA: A single colony from each strain was inoculated in 5 ml of YPD broth and grown to late log phase at 20°. A 5-μl aliquot of the culture was inoculated into 25 ml of fresh YPD and grown for an additional 2 days at 20°. Total genomic DNA was isolated from these cultures. The DNA samples were digested with *Pvu*II, separated on a 0.8% agarose gel, and transferred to Hybond-N membrane. Southern hybridization was performed using a ^{32}P-labeled DNA probe derived from the Ty1 *Pvu*II-*Sna*BI fragment of Ty1-H3. The intensity of the cDNA fragments was determined

by phosphorimage analysis and normalized to four conserved chromosomal Ty1 junction fragments, as described by Lee *et al.* (1998).

Detection of Ty1 multimeric arrays by PCR analysis: An assay developed by Bryk *et al.* (2001) was used essentially as described. Wild-type and *rad27* mutant strains carrying a Ty1*his3-AI* element were streaked for single colonies on YPD plates and grown at 20° for 2–3 days. The cells were then printed to SC-His plates and incubated for 2–3 days at 30° to isolate His^+ prototrophs that sustained a Ty1*HIS3* insertion. Ten independent His^+ prototrophs from each strain were clonally purified and grown in 5 ml YPD broth at 30° overnight. Total genomic DNA was isolated and the DNA samples were analyzed by PCR using HIS3 OUT (5′-GTACTAGAGGAGGC CAAGAG-3′) and TYA OUT (5′-TCTCTGGAACAGCTGAT GAAG-3′) primers. The genomic DNA was also subjected to PCR analysis using primers flanking the *FPR1* gene to ensure that the DNA was PCR competent.

cDNA recombination: A cDNA recombination assay developed by Bryk *et al.* (2001) was used essentially as described. The wild-type strain ANU116 and the *rad27* mutant strains ANU117, ANU118, ANU119, ANU120, and DG2180 were transformed with the integrating plasmid pBJC573 linearized wih *Pac*I, which results in the insertion of Ty1*his3-AI* and the *URA3* gene flanked by a 1.2-kb direct repeat from the *BIK1-HIS4* region on chromosome III. Ura^+ transformants were grown as patches on SC-Ura plates at 30°, and the resulting patches were replica plated to two YPD plates, one of which was incubated at 20° and the other at 30° for 3 days. The plates were then printed to SC-Ura-His plates and grown at 30° for 2 days. No His^+ Ura^+ colonies were observed from YPD plates grown at 30°, suggesting that the His^+ Ura^+ colonies that arose following incubation at 20° were independent. The Ura^+ His^+ colonies were grown as small patches on YPD plates, printed to 5-fluoroorotic acid (5-FOA)-His plates and incubated at 30° for 3 days. The fraction of Ura^+ His^+ patches that failed to grow on 5-FOA-His plates was then determined.

Ty1 cDNA stability: The Ty1 cDNA stability assay developed by Lee *et al.* (2000) was slightly modified for this study. A single colony from each strain was inoculated in 80 ml of YPD broth and grown for 2 days at 20°. A 40-ml sample of the culture was pelleted, washed, and resuspended in 80 ml of fresh YPD. Further dilution of the above culture was made by inoculating 40 ml of the culture in 1 liter of fresh YPD broth containing 600 μg/ml of the reverse transcriptase inhibitor phosphonoformic acid (PFA; Sigma, St. Louis). This concentration of PFA was found to inhibit cDNA synthesis without affecting cell growth. Immediately after addition of PFA, 100 ml of the culture was pelleted, washed, and stored at −70°. Subsequently, 100 ml aliquots were removed after 1, 2, 4, 6, and 8 hr of growth at 20°. The cells taken at each time point were quickly pelleted and stored at −70°. Total genomic DNA was isolated from each sample, digested with *Pvu*II, and processed for Southern analysis as described above. Hybridization signals were not heavily influenced by outgrowth of cells because all cells required at least 5 hr to double in density (DG1657, 5 hr; BLY184, 6.7 hr; ANU115, 5.4 hr). Signals were quantitated by phosphorimage analysis as described by Lee *et al.* (2000). A plot of the percentage of Ty1 cDNA remaining after each elapsed time point relative to the amount at zero time was plotted on a log scale. The half-life of cDNA was calculated from the slope of the best-fit line using Cricket Graph software.

RESULTS

***RAD27* inhibits Ty1 mobility:** Our previous work suggested that the DNA helicases Rad3 and Ssl2 inhibit Ty1 transposition by destabilizing Ty1 cDNA (Lee *et al.* 1998, 2000). Since Rad3 and Ssl2 probably do not degrade Ty1 cDNA directly, we tested the RAD2 family of structure-specific nucleases for their effects on Ty1 mobility. These genes were chosen because of their role in DNA replication and repair (Lieber 1997) and short sequence recombination (Negritto *et al.* 2001). We examined the effects of the null alleles of the five members of the RAD2 family on Ty1 mobility using the Ty1 transposition reporter gene, *his3-AI*, described in Figure 1B. We previously showed that disruption of *RAD2* had no effect on Ty1 mobility (Rattray *et al.* 2000). Deletion of *EXO1*, *DIN7*, and *YEN1* did not result in an increase in Ty1 mobility (Figure 2). However, deleting *RAD27* significantly increased Ty1 mobility, as monitored by the increase in the number of His^+ papillae appearing on SC-His plates.

Effect of *rad27* mutants on Ty1 mobility: Since deletion of *RAD27* increased Ty1 mobility (Figure 2), we analyzed several *RAD27* point mutants that have been examined previously for their roles in mutation avoidance, flap cleavage, repeat-tract instability (Xie *et al.* 2001), or short sequence recombination (Negritto *et al.* 2001). The *rad27* point mutations, *G67S* and *G240D*, fall within the nuclease domains that are highly conserved in both prokaryotes and eukaryotes (Shen *et al.* 1997; Hosfield *et al.* 1998), and their biochemical activities have been studied (Xie *et al.* 2001; Kao *et al.* 2002). The *G67S* mutant has a weak exonuclease but near wild-type levels of single- and double-flap endonuclease activities, while the *G240D* mutant is devoid of exonuclease activity but has significant double-flap endonuclease activity. The *rad27-324* mutant has a frameshift mutation at codon 324 that truncates the C-terminal 58 amino acids. This mutant has DNA repair defects and mutator phenotype at the *CAN1* locus similar to those of the null mutant (Xie *et al.* 2001), but has not been biochemically characterized. The *rad27-E158D* mutant has a conserved glutamate residue changed to an aspartate and retains partial flap endonuclease activity, but no exonuclease activity (Frank *et al.* 1998; Negritto *et al.* 2001), and partially complements the elevated SSR phenotype of a *rad27* null mutant (Negritto *et al.* 2001).

We studied the effects of these *rad27* mutants on Ty1 mobility by monitoring the rate of His^+ colony formation using a chromosomally marked Ty1 element carrying *his3-AI*. All *rad27* mutants increased Ty1 mobility relative to the wild-type strain DG2101 (Table 1). The point mutations *rad27-G67S*, *rad27-G240D*, and *rad27-E158D* increased Ty1 mobility from 4- to 22-fold relative to the wild-type strain. The null mutant *rad27::LEU2* showed an increase in mobility similar to that of the point mutant *rad27-G67S*. The *rad27-324* frameshift mutant had the maximum increase in Ty1 mobility (198-fold) compared to the point mutants and also the null mutant. Since *rad27-324* appeared to be more severe than the null mutant in terms of increased Ty1 mobility,

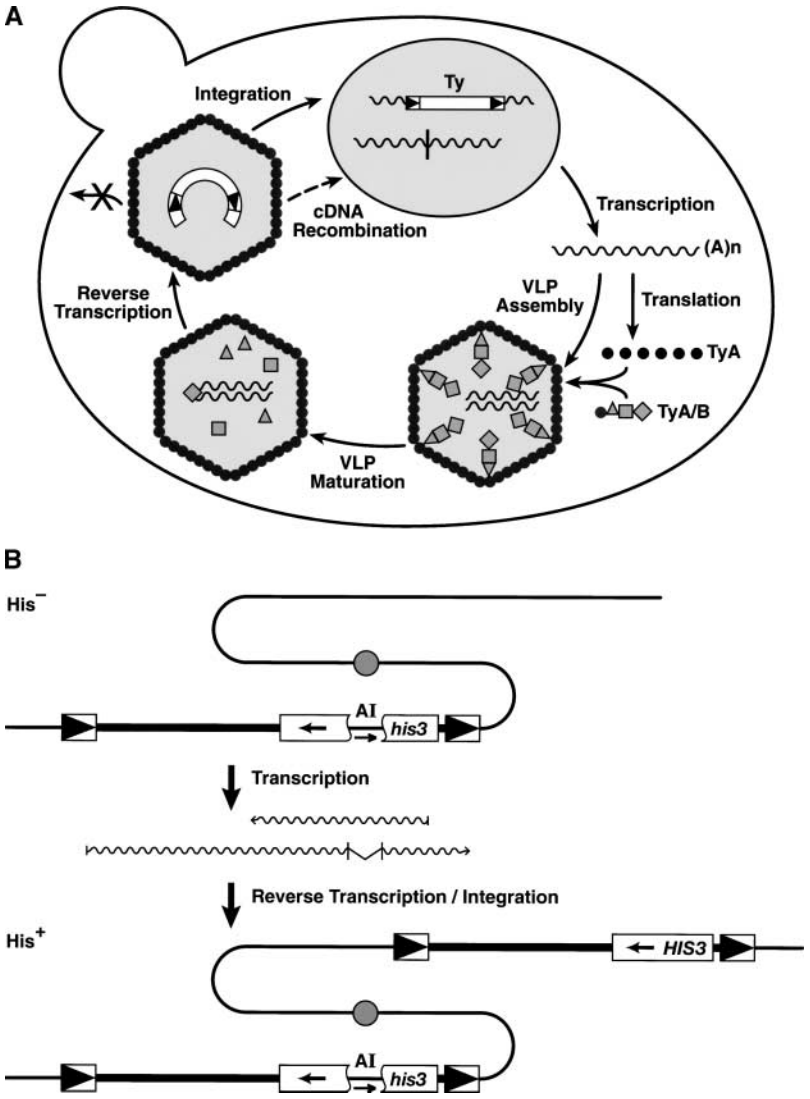


FIGURE 1.—Life cycle of Ty1. (A) Ty1 elements in the genome are normally transcribed and a full-length Ty1 RNA is made. The Ty1 RNA serves as template for both translation and reverse transcription. Translation of Ty1 RNA results in synthesis of proteins that are required for transposition. These include the TyA1-gag protein, which forms the structural component of the virus-like particle (VLP) and the enzymatic proteins protease, integrase, and reverse transcriptase/ribonuclease H that are required for cDNA synthesis and integration into the genome. Reverse transcription takes place within the VLPs and a full-length cDNA is made. The cDNA enters the genome predominantly through integrase-mediated integration into a new chromosomal site or, to a lesser degree, by recombination with preexisting genomic Ty1 elements. (B) A phenotypic assay for Ty1 mobility. The tagged element consists of Ty1 containing the reporter gene, *his3-AI*, where the *HIS3* gene (boxed) is interrupted by an artificial intron (AI). The arrows indicate the direction of transcription of *his3-AI*. The transcript is shown as a wavy line and vertical lines in the transcript indicate the splicing of the AI. When this spliced transcript undergoes reverse transcription and integration, a functional *HIS3* is recreated and the cells become His^+. This assay provides a phenotypic selection of a single Ty1 element undergoing retrotransposition or cDNA recombination, termed cDNA mobility.

we determined if this mutant was dominant to *RAD27*. To perform the dominance test, we constructed diploid strains bearing the wild type and mutant alleles and determined whether the wild-type *RAD27* could complement the phenotypes of the mutant *rad27* alleles when compared to a set of homozygous control strains. Our results indicate that the mutator, temperature sensitivity, and Ty1 mobility phenotypes exhibited by all five *rad27* mutants are recessive, since they are effectively complemented by *RAD27* in the diploid strains (data not shown). Finally, we constructed double mutants containing the *rad3-G595R*, which has been previously shown to increase SSR and Ty1 retrotransposition (LEE *et al.* 2000), and the *rad27* alleles for epistasis analysis. As is true of many double mutants containing a mutation in *RAD27* and a mutation in another gene involved in DNA repair (SYMINGTON 1998; TONG *et al.* 2001), all of the *rad27 rad3-G595R* double mutants grew very poorly and were not pursued further.

Ty1 insertion at the *SUF16* locus increases in *rad27* mutants: Ty1 elements preferentially target genomic regions upstream of tRNA genes (JI *et al.* 1993; DEVINE and BOEKE 1996). To determine if the *rad27* mutations affected target site selection, we monitored spontaneous Ty1 insertion events using a qualitative PCR assay (LEE *et al.* 1998) at one such tRNA (*SUF16*) on chromosome III (Figure 3A). Our results showed an elevated level of insertion at the target site (*SUF16*) in all the *rad27* mutants relative to wild type as observed by the intensity of the PCR products that hybridized with a radiolabeled Ty1 LTR probe (Figure 3B). The maximum level of Ty1 transposition was observed with the *rad27-324* mutant. The highly intense signal observed with *rad27-G240D* suggests that a Ty1 insertion occurred early during cell growth and propagated through subsequent generations, creating a "jackpot" event. As a negative control, genomic DNA from an isogenic *spt3-101* strain, in which transcription of Ty1 is severely reduced (WINSTON *et al.* 1984), was analyzed for insertions near *SUF16*. The hybridization patterns reflect the window of insertion of the Ty elements ranging from 500 to 1800 bp upstream of *SUF16*, which has been observed previously (LEE *et al.*

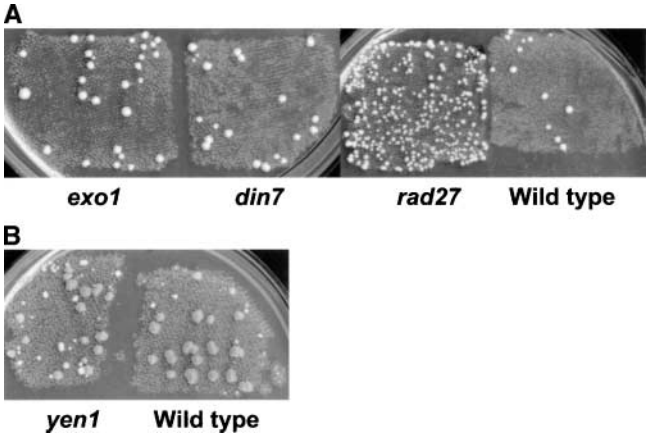


FIGURE 2.—Effect of the RAD2 gene family on Ty1 mobility. (A) Null mutants of *exo1* (BLY187), *din7* (BLY210), *rad27* (BLY184), and the wild-type parent (DG1657) were monitored for Ty1 mobility using Ty1*his3-AI*. (B) A null mutant of *yen1* (LSY485-2C) and the wild type (W303-1A) were assayed using Ty1*neo-AI* Cell patches were grown on YPD (A) or SC-Ura (B) plates at 20° for 4 days and then replica plated to SC-His (A) or YPD + G418 (B). These plates were incubated at 30° for 3 days and then photographed.

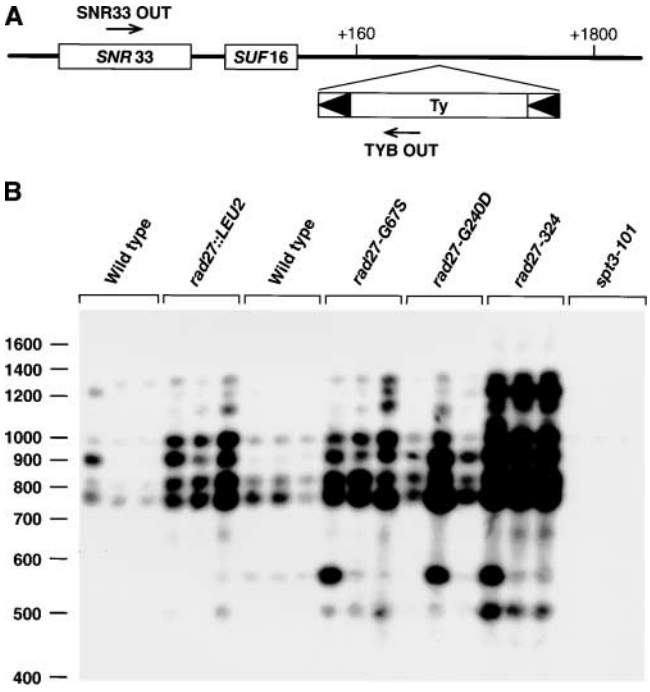


FIGURE 3.—Spontaneous Ty1 insertions upstream of *SUF16*. (A) Schematic representation of the *SUF16* locus on chromosome III. Ty elements are known to preferentially insert upstream of the glycine tRNA gene *SUF16*. The arrows indicate the PCR primers, SNR33 OUT and TYB OUT, with homologies to the *SNR33* gene and *TYB1*, respectively. The insertion patterns for various strains reflect the hotspots for Ty1 integration upstream of *SUF16*. (B) PCR analysis of DNA from three colonies of each strain. The PCR fragments were resolved on a 1.2% agarose gel, transferred to Hybond-N membrane and subjected to a Southern hybridization using a ^{32}P-labeled LTR probe. The genotype of each strain is indicated. Size markers are alongside the figure.

1998). These results suggest that the normal target site preferences are maintained in *rad27* mutants and that the level of insertion (as monitored by the intensity of the hybridization fragments) parallels the transposition rate observed in these mutants (Table 1).

Ty1 transcript level in *rad27* mutants: Northern analysis was performed to determine if the *rad27* mutations increased Ty1 transposition by altering the level of Ty1 or Ty1*his3-AI* transcripts. Total RNA was isolated from wild-type, *rad27::LEU2*, *rad27-G67S*, *rad27-G240D*, *rad27-E158D*, and *rad27-324* strains and subjected to Northern blot hybridization using ^{32}P-labeled probes specific to Ty1 or *his3-AI* (Figure 4). The Ty1 probe detects all Ty1 element transcripts while the *his3-AI* probe detects only the marked Ty1 element transcript. The level of Ty1 or Ty1*his3-AI* RNA was normalized to that of the *PYK1* transcript and ratios were determined by phosphorimage analysis. The *rad27-G67S*, *rad27-G240D*, *rad27-E158D*, and *rad27-324* mutants showed no significant increase in the levels of Ty1 or Ty1*his3-AI* transcripts relative to the wild-type strain. The *rad27::LEU2* null mutant, however, had an increase in the level of Ty1 RNA (3-fold) and Ty1*his3-AI* RNA (1.5-fold). Taken together, these results suggest that *RAD27* inhibits Ty1 mobility predominantly at a post-transcriptional step.

***RAD27* inhibits Ty1 transposition at a post-translational step:** We next determined whether *RAD27* inhibited Ty1 transposition by altering the level of endogenous TyA1-gag protein, which can be detected in unfractionated cell extracts. Total protein was extracted from wild-type, *rad27::LEU2*, *rad27-G67S*, *rad27-G240D*, *rad27-E158D*, and *rad27-324* strains and subjected to quantitative Western analysis using anti-Ty1 VLP antibodies to detect TyA1 protein (Figure 5). The blot was then stripped of Ty1 VLP antibodies and reprobed with antibodies to the Hts1 protein (cytoplasmic and mitochondrial histidinyl tRNA synthetase). The amount of

TABLE 1

Effect of *rad27* mutants on Ty1 mobility

Strain	Genotype	*N*	Median frequency His$^+$ ($\times 10^{-8}$)[a]	Fold induction
DG2101	Wild type	4	4 ± 2.1	1
DG2102	*rad27-G67S*	2	90.5 ± 2.1	22.5
DG2103	*rad27-G240D*	1	22.7	5.6
ANU105	*rad27-E158D*	2	18.9 ± 1.4	4.7
ANU115	*rad27-324*	2	800 ± 41	198.5
DG1657	Wild type	2	2.7 ± 0.16	1
BLY184	*rad27::LEU2*	2	59.8 ± 0.5	22

N, number of different tests performed.
[a] Average ±SD of median frequencies from different tests.

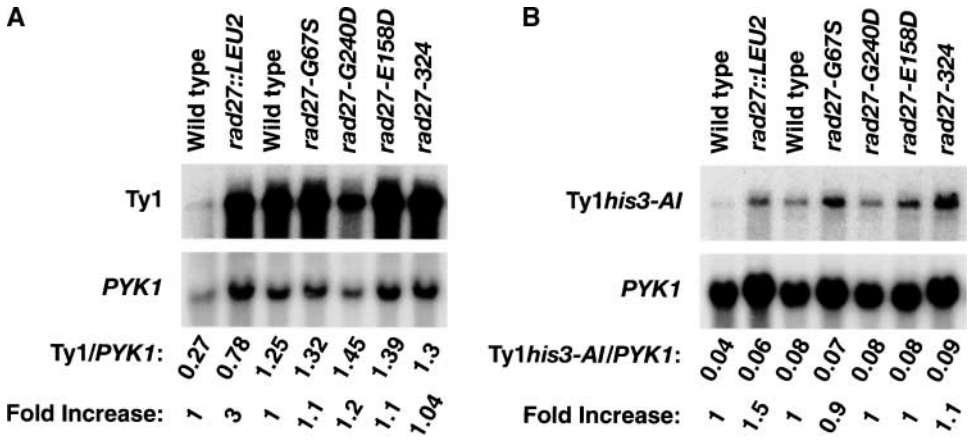


FIGURE 4.—Level of Ty1 RNA in the *rad27* mutants. Northern analysis of total RNA isolated from various *rad27* strains with Ty1 and *PYK1* (A) or *his3-AI* and *PYK1* radiolabeled probes (B). The Ty1 probe detects all Ty1 transcripts while the *his3-AI* probe hybridizes only with the marked Ty1*his3-AI* RNA. The signals were quantitated by phosphorimage analysis and normalized to the *PYK1* transcript signal. The numbers below each lane indicate the fold increase in the total Ty1 RNA (A) or Ty1*his3-AI* RNA (B) relative to the wild-type strain.

TyA1 protein in the wild-type and mutant *rad27* strains was determined by normalizing to the level of Hts1 protein by phosphorimage analysis. As a positive control, purified Ty1-VLPs (kindly provided by S. Moore) were probed with similar antibodies. Protein samples from an isogenic *spt3-101* strain served as a negative control and had undetectable levels of TyA1 protein. The amount of endogenous TyA1 remained essentially unaltered in the *rad27* mutants as compared to the wild-type strain. These results suggest that *RAD27* inhibits Ty1 mobility at a post-translational step.

Ty1 cDNA increases in *rad27* mutants: To determine if *RAD27* inhibited Ty1 transposition by affecting the level of unincorporated Ty1 cDNA, total genomic DNA was isolated from wild-type, *rad27-G67S*, *rad27-G240D*, *rad27-E158D*, *rad27-324*, and *rad27::LEU2* strains and digested with *Pvu*II. *Pvu*II cleavage produces a 2-kb Ty1 fragment that is indicative of unintegrated Ty1 cDNA (Figure 6, top). The *Pvu*II-digested samples were then subjected to Southern hybridization using a ^{32}P-labeled *Pvu*II-*Sna*BI fragment of Ty1. This probe hybridizes with unincorporated Ty1 cDNA fragments of 2 kb and also with chromosomal Ty1 elements, thereby generating a variety of larger fragments consisting of Ty1 elements joined to genomic DNA. The amount of unincorporated Ty1 cDNA was determined by phosphorimage analysis of the blot and normalized to four conserved chromosomal Ty1 junction fragments (Figure 6, bottom).

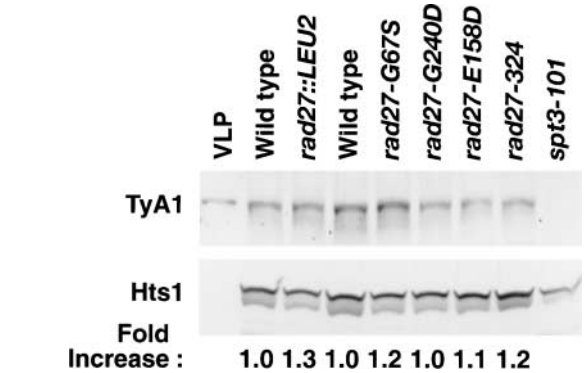


FIGURE 5.—Level of TyA1 protein in the *rad27* mutants. Total protein extracted from wild-type or *rad27* mutant strains was separated on a 10% SDS-polyacrylamide gel, immunoblotted to Immobilon-P membrane, and probed with antibodies against VLP to detect TyA1 or the histidinyl tRNA synthetase, Hts1. Immunodetection was performed using the ECF Western blotting kit and the signals were quantitated by phosphorimage analysis. The numbers below each lane indicate the ratio of TyA1 to Hts1 signal for each mutant over that of the wild type.

All *rad27* mutants showed an increase in the levels of Ty1 cDNA that ranged from a modest 1.3-fold up to 5-fold relative to the wild-type strain. As expected, we could not detect any Ty1 cDNA in the *spt3-101* strain. The *rad27-324* mutant showed the maximum increase (5-fold) in Ty1 cDNA followed by the *rad27::LEU2* mutant. We also observed that the increase in the cDNA levels roughly parallels the increased Ty1 mobility in these mutants.

Ty1 multimeric arrays in *rad27* mutants: We consistently observed an intense 2.2-kb *Pvu*II-generated fragment above the unincorporated Ty1 cDNA fragment in the *rad27::LEU2* strain (Figure 6). The size of the 2.2-kb fragment suggests two possible mechanisms by which it could be formed. First, the fragment could have resulted from a multimeric Ty1 element insertion upon digestion with *Pvu*II. Such multimeric Ty1 elements have previously been observed at *HML*α in a wild-type yeast strain (WEINSTOCK *et al.* 1990) and at an elevated level in an *sgs1* null mutant (BRYK *et al.* 2001). Second, the 2.2-kb *Pvu*II fragment could have been be formed by digestion of a circular Ty1 element containing a single LTR. Although circular forms of unintegrated retroviral DNA are routinely observed in infected cells, a circular form of Ty1 cDNA does not accumulate to an appreciable amount within VLPs isolated from yeast cells undergoing a high level of Ty1 transposition (EICHINGER and BOEKE 1988).

To determine if increased multimeric Ty1 elements

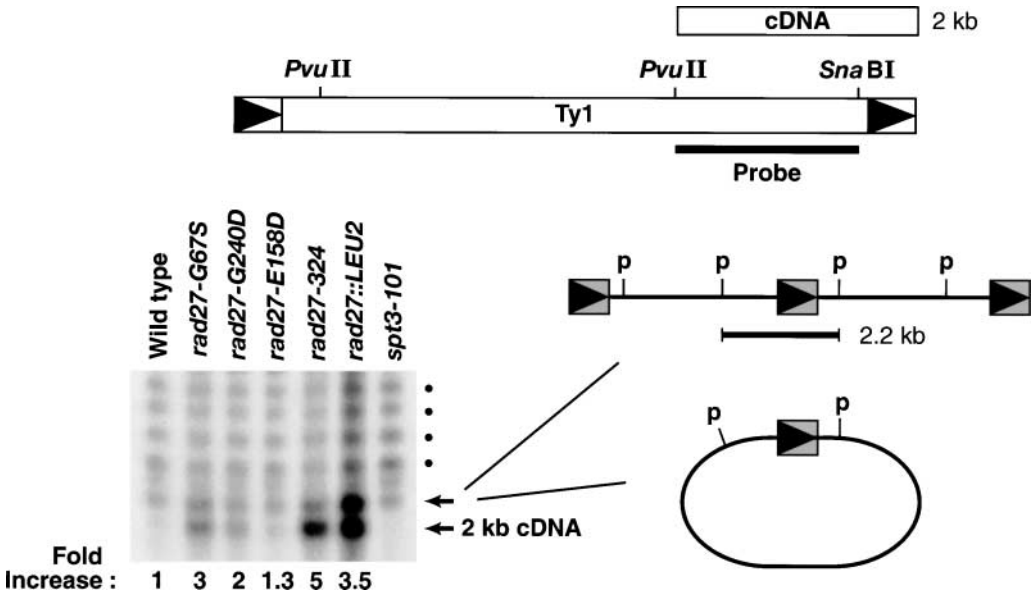
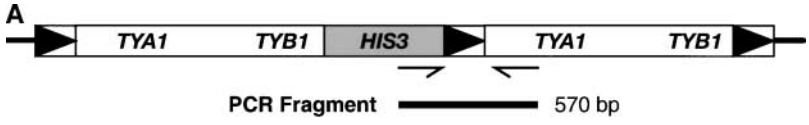


FIGURE 6.—Ty1 cDNA increases in the *rad27* mutants. The figure at the top depicts the 2-kb cDNA fragment of Ty1 that is released after *Pvu*II digestion of total yeast DNA. The solid box represents the *Pvu*II/*Sna*BI restriction fragment of Ty1 that was used as probe in the Southern hybridization. Total DNA was prepared from wild-type and *rad27* mutant strains, digested with *Pvu*II, and subjected to Southern hybridization using a ^{32}P-labeled probe derived from *TyB1* described above. The position of the 2-kb cDNA fragment is shown by the lower arrow. The four conserved chromosomal junction fragments used for normalization of the cDNA fragment are indicated by the dots alongside the figure. The top arrow on the right refers to the 2.2-kb fragment that was intensified in the *rad27::LEU2* mutant. Two possible mechanisms by which the 2.2-kb *Pvu*II fragment could have arisen are indicated. In the first case (top), the 2.2-kb fragment released after *Pvu*II (p) digestion is from a multimeric Ty1 element, while in the second case the 2.2-kb *Pvu*II fragment is from a single Ty1 LTR circle.

are present in *rad27* mutants, especially in the null mutant, we isolated 10 independent Ty1*HIS3* events from wild-type and *rad27* mutant cells containing a genomic Ty1*his3-AI* element. Total genomic DNA was analyzed by PCR to determine if the Ty1*HIS3* element was present as part of a multimer (BRYK *et al.* 2001). Note that the initial Ty1*his3-AI* element is not multimeric. Primers specific to *HIS3* and *TYA1* were used to detect a Ty1 multimer consisting of a tagged element joined to another Ty1 element (Figure 7A). A PCR fragment of 570 bp is indicative of a single LTR Ty1 multimer while a 905-bp fragment suggests a 2-LTR Ty1 multimer. Our results indicate that only the null mutant has an increased fraction of Ty1 multimers (9/10) and all contain a single LTR (Figure 7B). The *rad27-G67S*, *rad27-G240D*, *rad27-E158D*, and *rad27-324* mutants did not show an increase in the number of Ty1 multimers relative to the wild-type strain. This result was confirmed by subjecting the same 10 His$^+$ strains to Southern analysis after digestion by *Pvu*II and hybridization with a *HIS3* probe (data not shown).

Deletion of *RAD27* leads to increased recombination between Ty1 cDNA and genomic Ty1 elements: To test the possibility that the Ty1 multimers observed in the *rad27* deletion strain were correlated with an increase in Ty1 cDNA recombination, we determined the frequency of recombination between Ty1*HIS3* cDNA and an introduced genomic Ty1*his3-AI* element (BRYK *et al.* 2001). A plasmid consisting of a Ty1*his3-AI* element, the *URA3* gene, and sequences upstream of *HIS4* was integrated into the wild-type and *rad27* mutant strains at *HIS4* on chromosome III. Transcription, splicing, and reverse transcription of Ty1*his3-AI* results in Ty1*HIS3* cDNA with a functional *HIS3* gene. Recombination between the Ty1*HIS3* cDNA and genomic Ty1*his3-AI* results in conversion of the genomic Ty1*his3-AI* to Ty1*HIS3* adja-

A



B

Strain	Genotype	No. of Multimers
DG2101	Wild type	1/10
DG2102	*rad27-G67S*	3/10
DG2103	*rad27-G240D*	1/10
ANU105	*rad27-E158D*	0/10
ANU115	*rad27-324*	2/10
BLY184	*rad27::LEU2*	9/10

FIGURE 7.—Ty1 multimer analysis in the *rad27* mutants. (A) A Ty1 multimer within a chromosome is depicted at the top. For simplicity, two Ty1 elements sharing a single LTR between the upstream Ty1*HIS3* element and another Ty1 element are shown. PCR primers homologous with the *HIS3* gene and to the *TyA1* region are indicated. The size of the PCR fragment amplified by these primers is 570 bp. (B) Total yeast DNA isolated from 10 independent His$^+$ cultures from each strain was analyzed by PCR and the number of multimers observed for each strain is shown.

TABLE 2

Increased levels of cDNA recombination in *rad27* deletion mutant

Strain	Genotype	No. of His^- Ura^-/ His^+ Ura^{+a} (%)
ANU116	Wild type	4/253 (1.8)
ANU117	*rad27-G67S*	0/112 (<0.09)
ANU118	*rad27-E158D*	2/115 (1.7)
ANU119	*rad27-G240D*	2/117 (1.7)
ANU120	*rad27-324*	5/257 (2)
DG2180	*rad27::LEU2*	18/266 (7)

[a] The numbers represent the fraction of His^+ Ura^+ cells that become His^- Ura^- as a result of selection against *URA3*.

cent to *URA3*. Selecting for loss of *URA3* by growing the cells in 5-FOA results in concomitant loss of the *HIS3* gene due to recombination between the flanking direct repeats of *HIS4* sequence. The fraction of the His^+ Ura^+ colonies that became His^- Ura^- as a result of selection for loss of *URA3* was determined to detect the cDNA recombinants (Table 2). The *rad27-G67S*, *rad27-G240D*, *rad27-E158D*, and *rad27-324* mutants did not show any significant increase in the number of His^- Ura^- colonies relative to the wild-type strain. The *rad27* deletion mutant, however, had a significant ($P \leq 0.005$) increase in the number of His^- Ura^- colonies relative to wild type (7 *vs.* 1.8%). These results suggest that *RAD27* inhibits recombination between Ty1 cDNA and genomic Ty1 elements and that Ty1 multimers may arise by homologous recombination.

Stability of Ty1 cDNA is increased in *rad27* mutants: The increased level of Ty1 cDNA observed in *rad27* mutants could be caused by increased synthesis or stability prior to integration into the genome. Either mechanism would lead to an increase in the level of Ty1 cDNA and transposition, since Ty1 cDNA is a limiting component required for high levels of transposition *in vitro* (Eichinger and Boeke 1990). Therefore, we measured the decay rates of unincorporated Ty1 cDNA in wild type and *rad27* mutants after inhibiting reverse transcription with the nonnucleotide reverse transcriptase inhibitor, phosphonoformic acid (PFA; Bergdahl *et al.* 1998; Lee *et al.* 2000). We chose *rad27-324* and *rad27::LEU2* for this experiment because these mutants had the maximum increase in Ty1 cDNA relative to the other *rad27* mutants. The wild-type, *rad27-324*, and *rad27::LEU2* strains were initially grown in different concentrations of PFA (100 μg/ml to 1 mg/ml) to determine the drug concentration that fully inhibited Ty1 cDNA accumulation without affecting cell growth (data not shown). When the wild type and the *rad27* mutants were grown in 600 μg/ml of PFA for 48 hr, Ty1 cDNA was nearly undetectable (Figure 8A, lanes 1–4). The *spt3-101* mutant was used as a negative control and had, as expected, an undetectable level of Ty1 cDNA. We next measured the cDNA decay rates of wild type and the *rad27* mutants by treating them with 600 μg/ml PFA. An aliquot of cells from each strain was removed at different time points (0–8 hr) after addition of PFA and total genomic DNA was isolated. After digestion with *Pvu*II, the genomic DNA samples were subjected to Southern analysis using a ^{32}P-labeled Ty1 probe (Figure 8A). The cDNA fragments were analyzed as described earlier (Figure 6) and the half-lives were determined (see materials and methods; Figure 8B). The half-lives of *rad27* mutants were increased nearly fivefold (444 min for *rad27-324* and 451 min for *rad27::LEU2*) relative to the wild-type strain (93 min).

DISCUSSION

From our analysis of members of the RAD2 nuclease family, the results presented here indicate an important role for the structure-specific nuclease Rad27 in inhibiting Ty1 mobility. We determined whether members of the RAD2 nuclease family modulate Ty1 mobility for two reasons. The RAD2 nucleases recognize specific nucleic acid structures; therefore, we hypothesized that these nucleases may affect Ty1 transposition, possibly by recognizing Ty1 reverse transcription or gapped integration intermediates as substrates. In addition, Negritto *et al.* (2001) have shown that *RAD27* inhibits short sequence recombination, a process that is related to the stability of Ty1 cDNA through the action of the NER/TFIIH helicase genes *RAD3* and *SSL2* (Lee *et al.* 2000). Our results indicate that among the RAD2 nuclease family, only *RAD27* markedly affects Ty1 mobility by altering the stability of Ty1 cDNA.

To understand how *RAD27* inhibits the Ty1 mobility (Figure 1), we have determined if several genetically and biochemically defined *rad27* point mutations, as well as a *rad27* null mutation, alter transpositional integration at the *SUF16* locus, Ty1 RNA and TyA1 protein level, cDNA accumulation and recombination, and Ty1 multimer formation. Our work indicates that there is not a simple correspondence between the severity of different *rad27*-mediated DNA repair and nuclease defects and Ty1 retrotransposition. For example, the *rad27-G240D* mutant has a more severe exonuclease and single-flap endonuclease defect than *rad27-G67S* (Xie *et al.* 2001), yet *rad27-G240D* has a more modest increase in Ty1 mobility (5-fold) than *rad27-G67S* (22-fold). Mutations that specifically destroy the endonuclease activity but do not affect the flap exonuclease activity of *RAD27* would help determine whether both nuclease activities are required to inhibit Ty1 mobility. The most striking mutant analyzed in our study is *rad27-324*, which has not been characterized biochemically and resembles a null mutant in phenotypic analyses (Xie *et al.* 2001). Surprisingly, *rad27-324* increases Ty1 mobility much more (198-fold) than the *rad27::LEU2* null mutation

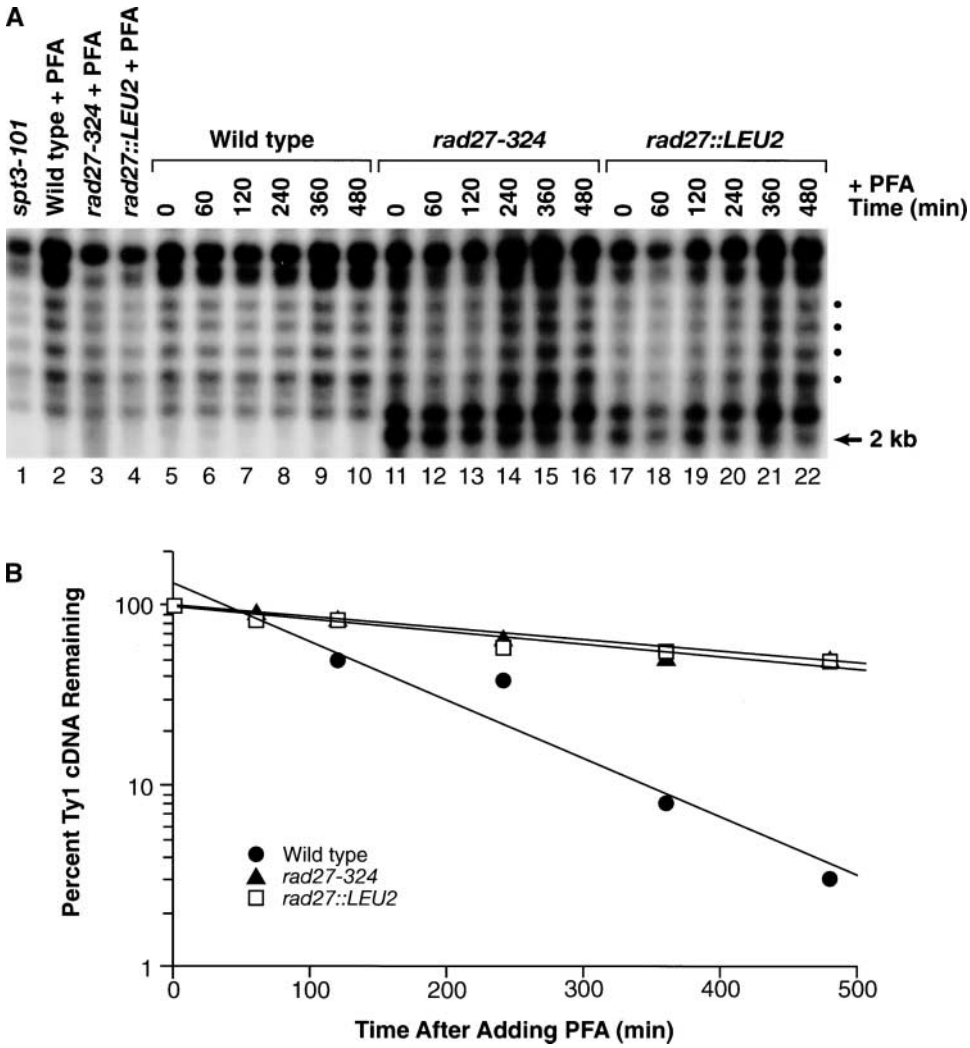


FIGURE 8.—Stability of Ty1 cDNA from *RAD27* wild-type, *rad27-324*, and *rad27::LEU2* strains after treatment with the reverse transcriptase inhibitor phosphonoformic acid (PFA) (A). Total genomic DNA isolated from wild-type, *rad27-324*, and *rad27::LEU2* strains grown in the presence of PFA (600 μg/ml) to midlog phase was digested with *Pvu*II, separated on a 0.8% agarose gel, and subjected to Southern hybridization with a ^{32}P-labeled Ty1 probe (lanes 2, 3, and 4). As a control, *Pvu*II-digested DNA from an *spt3-101* strain (lane 1) not treated with PFA is shown. Lanes 5–22 represent decay rates of Ty1 cDNA after treatment of the indicated strains with PFA for various time periods from 0 to 480 min. Lanes 5–10, 11–16, and 17–22 represent the decay rates for *RAD27* wild-type, *rad27-324*, and *rad27::LEU2* strains, respectively. The 2-kb cDNA fragment is indicated by the arrow and the Ty1 chromosomal junction fragments that were used for normalization of the cDNA level are noted by the dots alongside the figure. (B) A logarithmic plot of the cDNA decay rates from the above blot. The percentage of Ty1 cDNA remaining after growth in PFA for various time points relative to the cDNA amount at 0 time is plotted. The half-lives of the Ty1 cDNA in *RAD27* wild-type, *rad27-324*, and *rad27::LEU2* strains were determined from the best-fit slope of the line.

does (22-fold), yet *rad27-324* appears to be recessive to wild type with respect to Ty1 mobility and also its mutator phenotype at *CAN1*. A dominant *rad27* mutant with defects in both nuclease activities has been reported by GARY *et al.* (1999a). It would be interesting to analyze the effects of this mutation on Ty1 mobility.

Although the relationship between the biochemical and genetic phenotypes of various *rad27* point mutations appears complex, only the *rad27* null mutation significantly increases the level of cDNA recombination and multimer formation as well as retrotransposition. This result strongly suggests that the presence of any of the mutant Rad27 proteins suppresses cDNA recombination and multimer formation. How can this occur? One idea put forth by XIE *et al.* (2001) to explain the variable biochemical activities and genetic phenotypes observed for the *rad27-G67S* and *rad27-G240D* mutants is that Rad27 might play a structural role in maintaining genome stability in addition to its catalytic functions. In support of this idea, Rad27 has been shown to interact with DNA replication proteins (see below), and large complexes containing both recombination and DNA repair proteins are present in eukaryotic cells (WANG *et al.* 2000). Furthermore, a systematic genomic screen has shown strong genetic interactions between *RAD27* and many DNA repair and genome stability genes (TONG *et al.* 2001). The alteration of key components could affect these complexes, allowing Ty1 mobility to increase. We may also learn more about the relationship between *RAD27* and Ty1 mobility by determining the level of Rad27 protein in the point mutants. Alternatively, the *rad27* mutations could affect Ty1 mobility indirectly by altering the level or activity of other replication, repair, or recombination proteins, perhaps by disrupting the cell cycle (VALLEN and CROSS 1995).

Like *rad3-G595R* and *ssl2-rtt* (LEE *et al.* 1998, 2000), Ty1 integration at the Ty1 hotspot *SUF16* (JI *et al.* 1993; DEVINE and BOEKE 1996) increases to varying degrees

in the *rad27* mutants. However, since the Ty1 insertion pattern remains unchanged, target site specificity is not determined by *RAD27*. *RAD27* mutants have a variety of genome instability defects, including an increase in the rate of spontaneous mutation, DNA repeat-tract instability, and chromosome loss (Vallen and Cross 1995; Tishkoff *et al.* 1997; Parenteau and Wellinger 1999; Xie *et al.* 2001). Increased Ty1 transcription and transposition also occurs in response to DNA damage caused by chemical mutagens or UV radiation (McClanahan and McEntee 1984; Rolfe *et al.* 1986; Bradshaw and McEntee 1989; Staleva and Venkov 2001). Given the mutator phenotype of *rad27* mutants, an increase in Ty1 mobility may be the result from an increase in Ty1 RNA level. However, our results suggest that the Ty1 or Ty1*his3-AI* RNA level remains about the same in the *rad27* point mutants or the *rad27-324* frameshift mutant relative to the wild-type strain. There is an increase in the Ty1 RNA level (threefold) in the *rad27::LEU2* deletion mutant that could be a response to DNA damage. However, TyA1 protein level remains essentially unchanged in the *rad27* mutants. Taken together, our results suggest that *RAD27* inhibits Ty1 mobility at a post-translational step.

Eichinger and Boeke (1990) made the interesting observation that Ty1 cDNA is limiting for integration in a cell free system using purified VLPs. Mutations in DNA repair and recombination genes, such as those in the *RAD52* epistasis group (Rattray *et al.* 2000; Scholes *et al.* 2001), *RAD3* and *SSL2* (Lee *et al.* 1998, 2000), and as shown here *RAD27*, have an increased level of unincorporated Ty1 cDNA. Therefore, Ty1 cDNA level may also be limiting for Ty1 mobility *in vivo*. We have shown that the *rad27::LEU2* and *rad27-324* mutations increase the steady-state level of Ty1 cDNA by about fivefold. We then used PFA inhibition of Ty1 reverse transcriptase (Lee *et al.* 2000) to show that the half-life of Ty1 cDNA also increases about fivefold in the *rad27::LEU2* or *rad27-324* mutants. These results suggest that Rad27 can influence the stability of Ty1 cDNA, perhaps by degrading partially reverse-transcribed molecules containing flaps or RNA/DNA hybrids when they enter the nucleus. A DNA flap created by an internal plus strand initiation site has been implicated in transport and integration of HIV-1 (Zennou *et al.* 2000). Ty1 elements also contain an internal priming site (Pochart *et al.* 1993; Heyman *et al.* 1995), but a similar flap structure has not been detected. Furthermore, it has been reported that a significant fraction of cDNA associated with VLPs isolated from cells expressing a specific Ty1 element is composed of incompletely reverse-transcribed molecules containing RNA/DNA hybrids (Muller *et al.* 1991), although this result is probably element specific (Eichinger and Boeke 1988). Since we are measuring the level of cDNA produced by all Ty1 elements in the genome, it is likely that a variety of reverse transcription intermediates could be present. Not all mutations that increase Ty1 transposition post-translationally, however, cause an increase in cDNA accumulation. Deletion of *SGS1* results in an elevated level of Ty1 mobility without a concomitant increase in cDNA (Bryk *et al.* 2001). Epistasis tests between *rad27* and *sgs1* null mutations suggest these genes function in different genetic pathways to modulate Ty1 mobility (data not shown).

Rad27 may also be involved in repair synthesis across the gap created after attachment of the 3′ ends of Ty1 cDNA to host DNA, since Ty1 integrase makes a 5-bp staggered cleavage at a target site (Voytas and Boeke 2002). Fen-1 is able to remove the 2-base 5′-flap that is generated during retroviral integration using model substrates *in vitro* (Brin *et al.* 2000; Yoder and Bushman 2000). However, since Ty1 integration incorporates a blunt-ended cDNA into the genome (Braiterman and Boeke 1994; Moore *et al.* 1995), a 5′-flap should not be present in the gapped integration product. It is also difficult to envision how a requirement for Rad27 in repairing a gapped integration intermediate would lead to an increase in Ty1 mobility in the absence of Rad27. Therefore, we do not favor the idea that Rad27 acts at this step in the process of Ty1 retrotransposition.

Our work has revealed interesting allele-specific phenotypes conferred by the *rad27* mutations. In particular, the *rad27* null mutant contains a Ty1 *Pvu*II fragment that is consistent with the presence of a multimeric array (Weinstock *et al.* 1990; Sharon *et al.* 1994). To detect the tail-to-head joint molecule characteristic of an integrated Ty1 dimer, we have performed PCR analysis of spontaneous Ty1*HIS3* insertions using one primer homologous with *HIS3* and a second from the 5′ end of *TYA1* (Bryk *et al.* 2001). Surprisingly, almost every Ty1*HIS3* insertion (9/10) analyzed is part of a multimer in the *rad27::LEU2* mutant, but multimeric arrays are not elevated in the other *rad27* mutants. Theoretically, we expect to recover only half the possible multimers if they are all dimeric and composed of one marked insertion. The observation that 90% of the Ty1*HIS3* insertions are multimeric suggests that these arrays contain only marked elements and/or they are larger than dimers. Southern analysis indicates that the Ty1 multimers obtained in the null mutant have not simply recombined with the preexisting Ty1*his3-AI* element, but are dispersed elsewhere in the genome (data not shown). Clearly, *RAD27* is essential for suppressing Ty1 multimer formation, a process that can add many Ty1 elements to the genome (Weinstock *et al.* 1990). Increased multimer formation is also observed when retroviral or Ty1 integration is blocked (Hagino-Yamagishi *et al.* 1987; Sharon *et al.* 1994) or, to a more limited degree, in an *sgs1* mutant (Bryk *et al.* 2001).

Multimeric elements likely arise by homologous recombination between LTRs of different cDNA molecules prior to integration into the genome or by recom-

bination of the cDNA molecules with genomic Ty1 elements (Weinstock *et al.* 1990; Sharon *et al.* 1994; Bryk *et al.* 2001). We have investigated the possibility that elevated Ty1 cDNA recombination in *rad27::LEU2* is correlated with increased multimer formation by measuring the frequency of recombination between Ty1 cDNA and a specific marked genomic Ty1 element. Our results show that the level of cDNA recombination increases significantly in the *rad27::LEU2* null mutant when compared to the wild-type strain or the other *rad27* mutants including the *rad27-324* mutant. This result is consistent with the notion that the multimers observed in the complete absence of Rad27 protein can result from recombination between Ty1 cDNA and a genomic element. Whether Ty1 multimers always arise from homologous recombination with a genomic element remains to be determined. The fact that *de novo* Ty1 insertions occur more frequently upstream of *SUF16* and maintain the wild-type pattern in a *rad27::LEU2* null mutant suggests that multimers may also form ectopically by homologous recombination and then insert via Ty1 integrase in the absence of *RAD27*. However, even the defective Rad27 proteins Rad27-G67S, -G240D, -E158D, and -324 apparently block Ty1 cDNA recombination as well as multimer formation. In contrast, Ty1 multimer formation in an *sgs1* mutant results from intermolecular recombination between unincorporated cDNA molecules (Bryk *et al.* 2001).

The mechanism underlying the allele-specific effects of *rad27-324* on Ty1 mobility is interesting to consider. One idea is that the Rad27-324 protein may not enter the nucleus, since the frameshift mutation truncates part of a putative nuclear localization signal (NLS) at the C terminus of the protein. The mammalian Rad27 homolog, Fen-1, has a functional bipartite NLS, KRKxxxxxxxxKKK located at residues 354–367 (Qiu *et al.* 2001), while Rad27 has a putative bipartite NLS, KKFxxxxxxxxxxxLKK at residues 317–333 (from PSORT II server, unpublished result). Our results suggest, however, that a Rad27-324/green fluorescent protein fusion protein remains nuclear localized (data not shown). Another idea is that Rad27-324 is altered in binding to additional proteins that depend upon Rad27 to gain access to Ty1 cDNA or that stimulate Rad27 activities. The C-terminal residues 337–350 of Rad27 are involved in binding to proliferating cell nuclear antigen (PCNA; Gary *et al.* 1999a,b). PCNA is a processivity factor that binds to and enhances the catalytic activities of Fen-1/Rad27 during DNA synthesis (Li *et al.* 1995) and DNA repair (Gary *et al.* 1999b). The *rad27-324* mutant might be defective in binding PCNA since the mutation truncates the PCNA-binding motif and this could affect its enzymatic functions that are required to inhibit Ty1 mobility. Interactions with other proteins such as the Dna2 helicase (Budd and Campbell 1997) might also be affected in *rad27-324*. It would be interesting to analyze the biochemical activities of Rad27-324, as well as to determine the effect that Rad27 interactors have on Ty1 mobility.

In summary, our results indicate an important and novel role for *RAD27* in inhibiting Ty1 mobility by affecting the fate of cDNA. Since *RAD27* functions in the nucleus, it may act once the Ty1 cDNA enters the nucleus as part of a preintegration complex, but prior to integration. The increased stability of Ty1 cDNA in *rad27* mutants would then allow the cDNA to enter the genome by integrase-mediated integration or homologous recombination with preexisting elements. In addition, intermolecular recombination events would generate multimeric Ty1 insertions if Rad27 is absent. Understanding how *RAD27* is integrated into the growing number of Ty1 host defense genes (Scholes *et al.* 2001) will be necessary to understand fully how Ty1 elements and their yeast host coexist.

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