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Mutations in *RAD6*, a Yeast Gene Encoding a Ubiquitin-Conjugating Enzyme, Stimulate Retrotransposition

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The *Saccharomyces cerevisiae* DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme which polyubiquitinates histones in vitro. Here we show that mutations in *rad6* increase the frequency of transposition of the retrotransposon Ty into the *CAN1* and *URA3* loci. Using isogenic *RAD6* and *rad6* strains, we measured a more than 100-fold increase in the spontaneous rate of retrotransposition due to *rad6*, although there was no increase in the Ty message level. This is the first time that a mutation in a host gene has been shown to result in an increased rate of retrotransposition.

Retrotransposons such as Ty are common in eucaryotes. While they are not infectious, they otherwise resemble retroviruses in their structural organization and replication in viruslike particles via an RNA intermediate (for a review, see reference 1). The finding that two- to fourfold increases in the transcription of Ty message caused disproportionately large increases in Ty transposition led to speculation that the cell may exert controls on transposition at a posttranscriptional level (7). Possibly the extra Ty mRNA leads to a complete saturation of this hypothetical posttranscriptional regulatory system. Other evidence that the rate of Ty transposition is not simply proportional to the Ty message level comes from the observation that growth at a low temperature (17 versus 30°C) causes a 50- to 100-fold increase in the rate of Ty transposition without changing the level of Ty mRNA (24, 25). One explanation of this observation is that the reverse transcriptase activity is optimal at low temperatures (10).

Mutations in the *Saccharomyces cerevisiae RAD6* gene may result in several phenotypes, including extreme sensitivity to DNA-damaging agents, lack of induced mutagenesis, defective sporulation, increased mitotic recombination, and an increased rate of spontaneous mutation (for a review, see reference 13). *RAD6* encodes an $E2_{20k}$ enzyme which polyubiquitinates histones H2A and H2B in vitro (16, 35) and which may influence chromatin structure (16, 29, 35). Here we show that among the spontaneous mutations stimulated by *rad6* are mutations due to the transposition of Ty elements. Since the yeast RAD6 protein may be analogous to a mammalian 20-kilodalton E2 ubiquitin-conjugating enzyme (16, 26), these studies may be relevant to studies of the cellular mechanisms used to control the movements of retrotransposons and retroviruses in other eucaryotes.

MATERIALS AND METHODS

Strains. Strain LP2752-4B*rad6*Δ was derived from LP2752-4B (α *his4-260,39* pBR313 *his4-864,1176 lys1-1 ura3-52*) and contains a *URA3* disruption of the *rad6* coding region (22). L-1249 is a *ura3* derivative of LP2752-4B*rad6*Δ selected on medium containing 5-fluoroorotic acid (3). It was transformed (15) to Ura^+ with pR67 (22), a YCp50-based plasmid with a *Hin*dIII-*Bam*HI fragment containing the yeast *RAD6* gene. All Ura^+ transformants were UV resistant. Strains LP2752-4B and LP2752-4B*rad6*Δ and plasmid pR67 were kindly provided by Louise Prakash. N-152, also from Louise Prakash, is a *rad6-3* mutant (28) and was derived from B-635, a *cyc1-115* mutant derived from D311-3A (**a** *lys2 his1 trp2*), both kindly provided by Fred Sherman.

Mutant isolation. A large number of independent colonies from each strain were used to inoculate individual slants of complete medium, YPD (32), which were grown at 30°C. Cells suspended from these slants were plated either to synthetic complete medium lacking arginine and containing 60 μg of canavanine per ml (−Arg +Can) (32) or to medium containing 0.75 mg of 5-fluoroorotic acid per ml (3). To ensure independence, only a single resistant mutant from plates descending from the same independent colony was used in further analyses. All mutants resistant to 60 μg of canavanine per ml have been shown to carry mutations in the *CAN1* locus (37). The 5-fluoroorotic acid-resistant mutants were crossed to a *ura3* strain of the opposite mating type for complementation testing. Only the *ura3* mutants were studied further. The independent *can^r1* and *ura3* mutants were analyzed by Southern blot (33).

Mutation and transposition rates. Mutation rates were calculated by the method of the median (17) and by fluctuation tests (20). Twenty-four cultures inoculated with 100 to 160 cells were grown at 30°C for 22 to 28 h. Four were plated for viable cell counts. Each of the remaining cultures was plated in entirety to a −Arg +Can plate. For strain LP2752-4B (*RAD6*), there were $(3.1 \pm 0.9) \times 10^7$ cells per plate, 19 of the 20 plates had can^r1 colonies, and the median culture had six can^r1 colonies. For strain LP2752-4B*rad6*Δ, there were $(2.7 \pm 0.6) \times 10^5$ cells per plate, and 5 plates of the 20 contained can^r1 colonies. Reconstruction experiments showed that the plating densities used did not affect the detection of can^r1 cells.

The LP2752-4B transposition rate was estimated as described in Results. The measurement of transposition in LP2752-4B*rad6*Δ involved a fluctuation test (20) followed by Southern blot analysis (33) of the Can^r colonies from the mutation rate experiment. Ty transpositions occurred in can^r1 mutants found on 1 of the 20 plates.

DNA and RNA analysis. Yeast DNA was extracted from 5-ml cultures (39). Methods for restriction enzyme digestion, gel electrophoresis, transfer of separated DNA fragments to Hybond-N (Amersham Corp.), nick translation, and prehybridization, hybridization, and posthybridization washes

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have been described previously (27). Plasmid pSH2 (37a), derived from a partial *Eco*RI digest of TLC-1 (5; kindly supplied by Jim Broach) which removed 2μm sequences, was used as a probe to hybridize to Southern blots when *can*ʳ*1* mutants were scanned. The *ura3* mutants were scanned with the plasmid probe YIp5 (34). The Ty1 element probe used was the 3′, 1.6-kilobase (kb) *Eco*RI-*Bgl*II epsilon fragment from TyIX (19) cloned into the *Bam*HI-*Eco*RI sites of pUC9. The Ty2 probe contained the 1.7-kb Ty2-specific *Cla*I fragment cloned into pBR325 (kindly supplied by J. Boeke).

Yeast RNA was extracted from 10-ml cultures (9). Aurintricarboxylic acid rather than diethyl pyrocarbonate was used as a nuclease inhibitor (11). Total yeast RNA was separated on a 6.3% formaldehyde–1% agarose gel in 1× MOPS buffer [0.02 M 3-(*N*-morpholino)-propanesulfonic acid, 5 mM sodium acetate, 1 mM disodium EDTA, pH 7] containing 0.02% formaldehyde. The gel was transferred to GeneScreen (Du Pont Co.) in 1× SSC (0.15 M sodium chloride plus 0.015 M sodium citrate). Prehybridization, hybridization, and posthybridization washes were performed by the protocol without dextran sulfate as described in the GeneScreen manual.

RESULTS

Independent, spontaneous canavanine-resistant (*can*ʳ*1*) mutations were isolated at 30°C in isogenic *RAD6* and *rad6*::*URA3* disruption strains. Southern blot analyses (33) were used to distinguish point mutations in *CAN1* from deletions or insertions. Blots of *Eco*RI-cut genomic DNA from the parental *CAN1* and independent *can*ʳ*1* mutants were hybridized with a *CAN1* probe. The hybridizing bands from all 32 *can*ʳ*1* mutants isolated from the *RAD6* parent were unchanged, suggesting that these are point mutations. In contrast, 20 of 30 *can*ʳ*1* mutants isolated from the *rad6* strain had altered bands (Fig. 1 and Table 1). Four of these were deleted for all or part of the *CAN1* gene. In the other 16 cases, the altered bands were consistent with the insertion of a Ty1 element within the *CAN1* gene. These altered bands could be explained by a 6-kb insertion containing an *Eco*RI site at the position characteristic for a Ty1 element (38).

Confirmation that these inserts were Ty elements was first obtained by more extensive restriction maps of five representative inserts (Fig. 2). Each of these had Ty1-characteristic patterns for *Bgl*II, *Hin*dIII, *Xho*I, and *Bam*HI sites within the 6-kb insert (38). The data showing the positions of insertion of the other Ty elements in the *CAN1* gene isolated from this *rad6*::*URA3* strain and those from a *rad6-3* strain (see below and Table 1) are reported elsewhere (37a). Further confirmation that the inserts were Ty elements came from the rehybridization of washed blots with the Ty1 probe. In this way, we detected new Ty1-hybridizing bands corresponding to altered *CAN1*-hybridizing bands in seven of the mutants (data not shown). Since there were about 35 copies of Ty1 elements in our strains, the new Ty1 bands could not be distinguished from overlapping Ty1 bands in the remaining mutants.

The blots in Fig. 1 are also consistent with a previous report that *rad6* strains have an increased rate of mitotic recombination (21). The *RAD6* and *rad6*::*URA3* strains have pBR313 integrated between duplicated *his4* genes (see Materials and Methods). None of the 55 *can*ʳ*1* colonies from the *RAD6* strains (LP2752-4B and L-1249 plus pR67) were missing the pBR band (Fig. 1c), while 3 of the 30 *can*ʳ*1* colonies tested from the *rad6*::*URA3* disruption strain lacked this band (Fig. 1b, lanes h, j, and n). Thus, in the absence of selection at the *his4* locus, there appears to be a high rate of recombination between the duplicated *his4* genes in the *rad6*::*URA3* strain. However, it is possible that the colonies from which the *can*ʳ*1* mutants were isolated were derived from a culture in which approximately 10% of the cells had previously lost the integrated pBR313 sequences.

To confirm that the increased level of Ty transposition observed in the *rad6*::*URA3* disruption strain was in fact due to the *rad6* mutation, a *ura3* derivative of the *rad6* disruption strain was transformed with a plasmid containing *RAD6*. Southern blot analysis of *Eco*RI-cut DNA from 23 independent *can*ʳ*1* mutants isolated from this transformed strain (L-1249 plus pR67) showed no alterations in the *CAN1* hybridizing bands (Table 1). Thus, the increased transposition was due to the *rad6* mutation.

In addition, we also examined independent *can*ʳ*1* and *ura3* mutants isolated from an unrelated strain containing the *rad6-3* point mutation and its isogenic parents. The results show that *rad6-3* stimulates Ty transposition at both the *CAN1* and *URA3* loci (Table 1). Mutations due to the insertion of Ty elements were identified by Southern blot mapping and hybridization to Ty probes as described above. No Ty insertions were detected among the 34 *can*ʳ*1* mutants scanned from the *RAD6* parent strains. In contrast, 13 of 40 *can*ʳ*1* mutants scanned from the *rad6-3* strain were due to Ty insertions (Table 1). Eleven had 6-kb inserts with *Eco*RI sites characteristic for Ty1 elements. One of these was mapped more extensively with *Eco*RI, *Bgl*II, *Hin*dIII, *Xho*I, and *Bam*HI and was found to have sites characteristic for Ty1. Four others had new Ty1-hybridizing bands corresponding to altered *can*ʳ*1* bands. One of the two 6-kb inserts that did not contain an *Eco*RI site had a new Ty2-hybridizing band corresponding to an altered *can*ʳ*1* band.

Similar analyses were done to identify Ty insertions among the *ura3* mutants. In the *rad6-3* strain and its isogenic B-635 parent, the *URA3* gene is located within *Eco*RI, *Bgl*II, and *Hin*dIII fragments of 17, 4.8, and 1.1 kb, respectively. Mutations were considered to be due to Ty transpositions if they caused alterations in these bands that could be explained by the insertion of a Ty element with characteristic *Eco*RI, *Bgl*II, and sometimes *Hin*dIII sites within a 6-kb insert. No Ty insertions were detected in 25 *ura3* mutants scanned from the *RAD6* parent strain. In contrast, 4 of 26 *ura3* mutants scanned from the *rad6-3* strain were characterized as due to Ty insertions.

The spontaneous rate of mutation to canavanine resistance per cell per generation was found to be $(6.6 \pm 1.9) \times 10^{-8}$ in the *RAD6* strain LP2752-4B and $(7.8 \pm 1.7) \times 10^{-7}$ in the isogenic *rad6*::*URA3* disruption strain (a 7- to 20-fold increase), confirming previous reports (12, 18) that *rad6* strains show an increased rate of spontaneous mutation. Since no Ty insertions were obtained among the 55 previously characterized independent *can*ʳ*1* mutants isolated in the isogenic *RAD6* strains, we estimate that a maximum of about 2% of the spontaneous mutations could be due to Ty1 insertions in this strain. This estimate is in agreement with the 2% frequency of Ty insertions found among spontaneous *lys2* mutants isolated at 30°C (8). Since the mutation rate in the *RAD6* strain is $(6.6 \pm 1.9) \times 10^{-8}$, we estimate the maximum transposition rate to be about 1.3×10^{-9}. The rate of Ty transposition into the *CAN1* gene was $(1.4 \pm 0.3) \times 10^{-7}$ in the *rad6*::*URA3* disruption strain. Thus, there is a more than 100-fold increase in Ty transposition in the *rad6*::*URA3* disruption strain.

Northern (RNA) blot analysis showed no increase in the

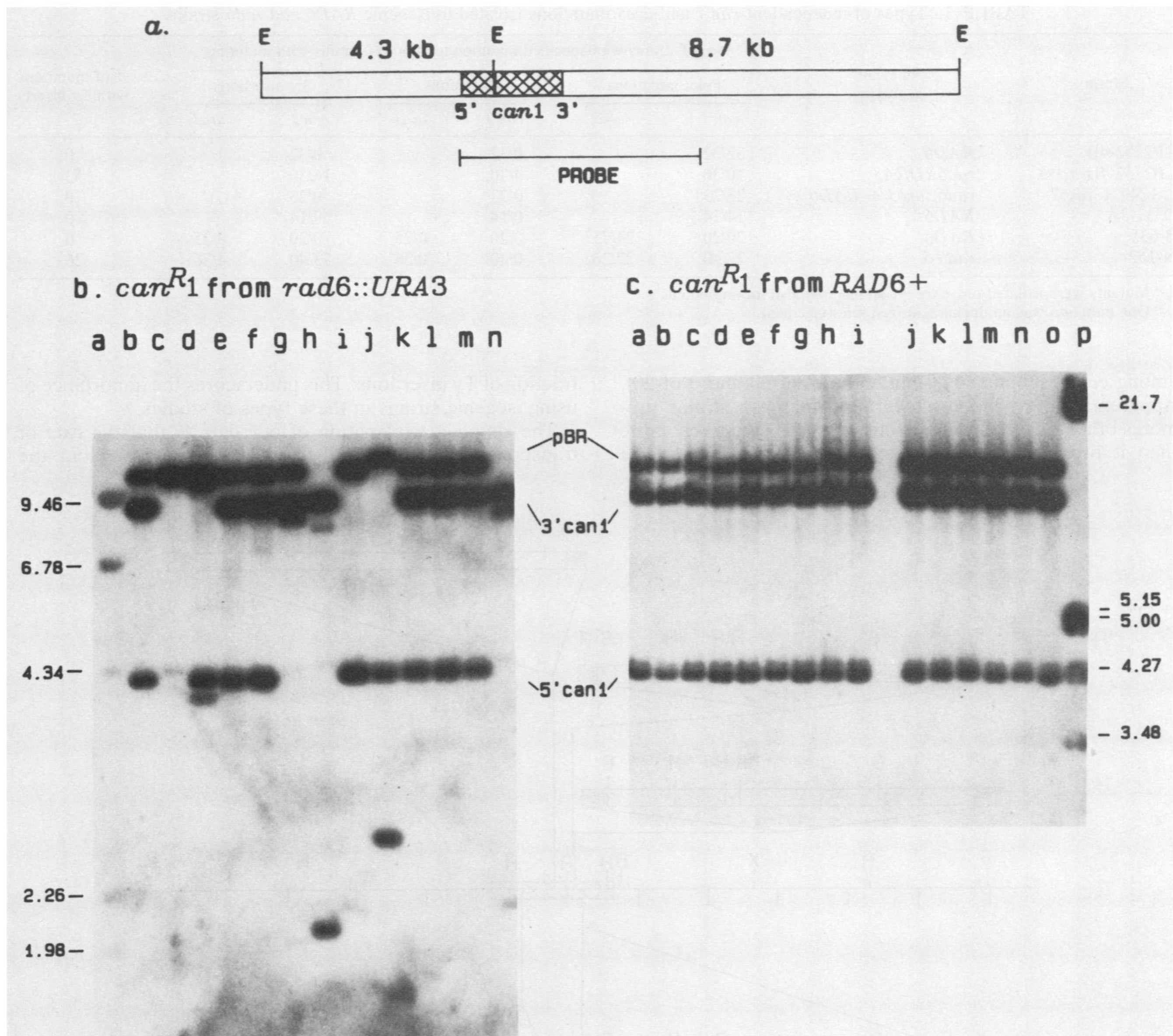

FIG. 1. (a) *Eco*RI (E) restriction map of genomic *CAN1* region in strain LP2752-4B (*RAD6*). The cross-hatched box represents the *CAN1* gene (14). (b) Southern hybridization of 13 representative independent *can*^{r}*1* mutants isolated from strain LP2752-4B*rad6*Δ (*rad6*::*URA3*) to the *CAN1* probe shown in panel a. (c) Southern hybridization of 15 representative independent *can*^{r}*1* mutants isolated from strain LP2752-4B (*RAD6*) to the *CAN1* probe shown in panel a. For both blots, total genomic DNA was digested with *Eco*RI. Lambda size markers (b, lane a; c, lane p) are indicated on each side (in kilobases). pBR denotes the hybridization of vector sequences on the probe to pBR313 sequences integrated at the duplicated *his4* locus of these strains.

level of the Ty message in the *rad6*::*URA3* disruption strain compared with that of its isogenic *RAD6* parent (Fig. 3). Apparently the *rad6* effect on transposition is not mediated at the level of Ty transcription.

DISCUSSION

The results described demonstrate that *rad6* mutations greatly increase the rate of Ty transposition. Isogenic *RAD6* and *rad6* strains showed a dramatic difference in the fraction of *can*^{r}*1* and *ura3* mutations due to Ty insertions. Furthermore, the increased fraction of Ty insertions in one of the *rad6* strains was eliminated by transformation with a *RAD6*-containing plasmid (Table 1). Since we and others (12, 18) have shown that *rad6* increases the rate of spontaneous mutations, it is clear that the enlarged fraction of spontaneous mutations due to Ty insertions represents an absolute increase in the Ty transposition rate. If the 7- to 20-fold increase in mutation rate we observed were due only to increases in transposition, then the overwhelming majority of mutants recovered in *rad6* strains would be due to Ty insertions (6 of 7 to 19 of 20). However, we found that only one in two (53%) of the *can*^{r}*1* mutations was due to Ty insertions. Thus, point mutations and other types of rearrangements (such as deletions) are also increased in these *rad6* strains. In other nonisogenic strains carrying the *rad6*::*URA3* disruption, Ty insertions were not detected as often

TABLE 1. Types of independent *can*^{r}*1* and *ura3* mutations isolated in isogenic *RAD6* and *rad6* strains

Strain	Relevant genotype	No. of strains with specified mutation/total no. of strains with mutation						% of mutations with Ty inserts
		Point mutations		Deletions		Ty insertions		
		can^r*1*	*ura3*	*can*^{r}*1*	*ura3*	*can*^{r}*1*	*ura3*	
LP2752-4B	*RAD6*	32/32		0/32		0/32		0
LP2752-4B*rad6*Δ	*rad6*::*URA3*	10/30		4/30		16/30		53
L-1249 + pR67	*rad6*::*ura3* + *RAD6*	23/23[a]		0/23		0/23		0
D311-3A	*RAD6*	14/14		0/14		0/14		0
B-635	*RAD6*	20/20	24/25[b]	0/20	0/25	0/20	0/25	0
N-152	*rad6-3*	27/40	22/26	0/40	0/26	13/40	4/26	26

[a] Mutants were isolated on −Arg +Can medium also lacking uracil.
[b] One mutation was an uncharacterized rearrangement.

among *can*^{r}*1* spontaneous mutations (2 of 20 and 0 of 20). Apparently, the genetic background in these strains promotes other types of spontaneous mutations relatively more than it promotes Ty transposition, thereby reducing the fraction of Ty insertions. This underscores the importance of using isogenic strains in these types of studies.

The simplest explanation of our data is that the rate of transposition is stimulated about 100-fold throughout the

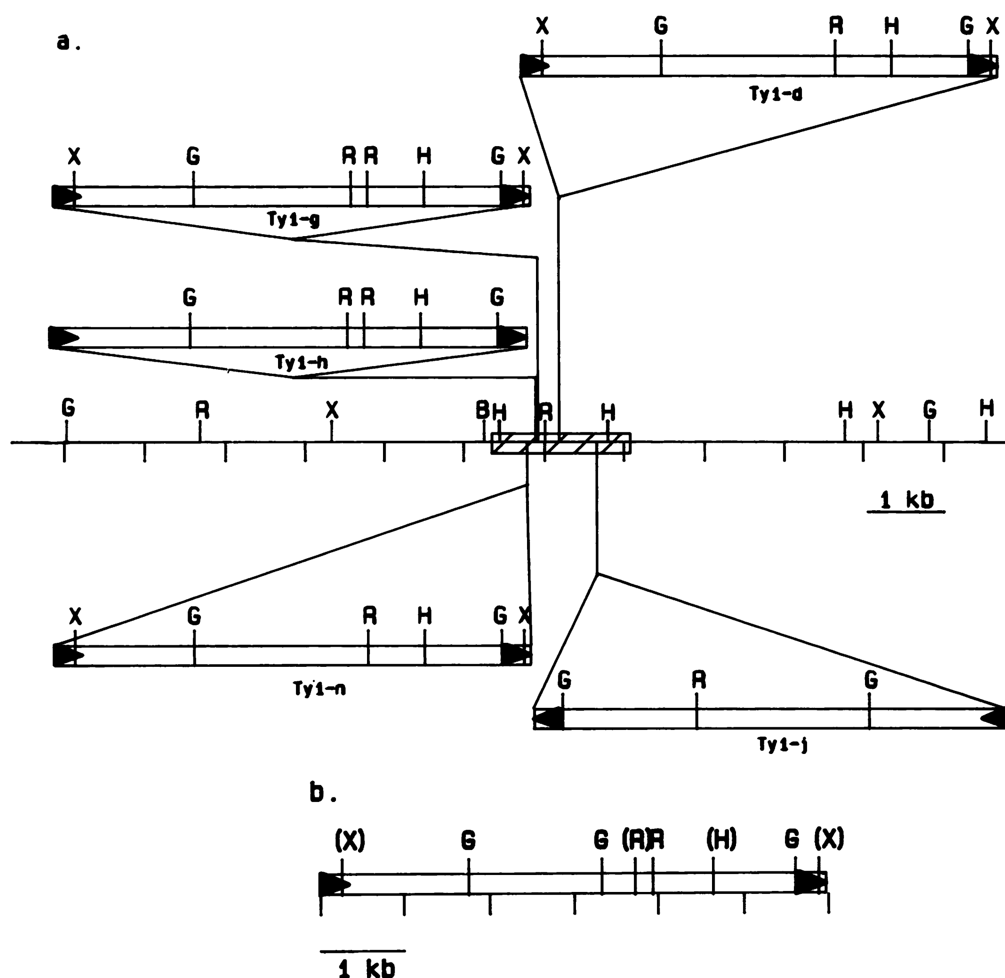

FIG. 2. (a) Restriction maps of five representative Ty1 insertions at *CAN1*. Insertion sites of the Ty1 elements were estimated by Southern analyses (33) of genomic digests of *can*^{r}*1* mutants from strain LP2752-4B*rad6*Δ (*rad6*::*URA3*) by using the *CAN1* probe shown in Fig. 1a. The hatched rectangle represents the *can1* gene (14). At the end of each Ty1 element are the long terminal repeats (delta elements) (▶, ◀). Each Ty1 element was given a letter designation corresponding to a lane on the Southern blot shown in Fig. 1b (e.g., the *Eco*RI hybridizing bands of the Ty1-d insertion at *can1* is shown in Fig. 1b, lane d). (Note that although all Ty1 elements have an internal *Bgl*II site, the internal *Bgl*II Ty fragments do not hybridize to the *CAN1* probe used to determine these maps, so this *Bgl*II site is not shown here). (b) Restriction map of a typical Ty1 element. Only sites for the five enzymes used in panel a are shown. Sites in parentheses are present in some Ty1 elements but not in others (38). Abbreviations: B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; R, *Eco*RI; and X, *Xho*I.

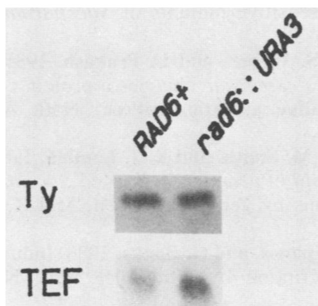

FIG. 3. Northern hybridization of yeast RNA from LP2752-4B (*RAD6*) and LP2752-4B*rad6*Δ (*rad6*::*URA3*) to a Ty-specific probe. Total yeast RNA (30 μg) was separated on a formaldehyde gel and hybridized to the *Xho*I-*Xho*I piece from Ty D15 (6; kindly supplied by Robert Elder). The blot was then washed and probed with the internal *Eco*RI-*Hin*dIII fragment of *TEF1* (23; kindly supplied by Jae Mahn Song) as a control.

genome in *rad6* strains. This increase is similar to the 50- to 100-fold stimulation of transposition caused by growth at low temperatures (24, 25). We do not yet know if the *rad6* and low-temperature effects on the transposition rate are epistatic, additive, or synergistic. The answer to that question will suggest whether *rad6* and cold temperatures are likely to control transposition in the same or different ways. Although the rate of transposition is increased 100-fold by *rad6*, it remains quite low [$(1.4 \pm 0.3) \times 10^{-7}$ per cell per generation into *CAN1*]. Indeed, this level of transposition is too low to allow detection at unselected sites. Similarly, the rate of transposition at low temperatures is not high enough to allow the frequent appearance of new bands hybridizing to a Ty probe in unselected, randomly screened colonies, unless the level of transposition is further enhanced by expression of a galactose-inducible Ty element which causes an increase in Ty mRNA (2). However, when DNA was extracted from 10 unselected subclones of the *rad6*::*URA3* strain and analyzed, 1 subclone did have an additional band which hybridized to the Ty-specific probe (data not shown). Since Ty elements are known to move much more frequently by gene conversion than by transposition (30) and since recombination is stimulated in *rad6* strains, this probably represents either the conversion of a solo delta element by a complete Ty element or recombination between two Ty elements. Either of these events would result in a new Ty-hybridizing band in the absence of transposition. Two of these subclones were also missing the pBR band as previously described (e.g., Fig. 1).

Retrotransposons such as Ty elements bear a striking resemblance to the integrated proviral form of retroviruses (for a review, see reference 1). It is important to identify any general cellular functions that may be involved in the expression, replication, or integration of these elements. Exposure to UV light has been shown to increase both the level of Ty RNA (31) and the expression of human immunodeficiency virus type 1 (36), suggesting that the expression of Ty elements and at least some retroviruses may be influenced by similar factors. Three yeast genes (*SPT3*, *SPT7*, and *SPT8*) which are required for proper initiation of the full-length Ty RNA transcript (40) have been identified. Mutations in *SPT3* have been shown to eliminate Ty transposition (4). It is also clear that tRNA encoded by the host is required as a primer during replication of the elements (1). Now we report that mutations in the yeast *RAD6* gene which encodes a polyubiquitin-conjugating enzyme can increase the frequency of Ty transposition more than 100-fold without increasing the level of Ty mRNA. The increase in Ty transposition in *rad6* strains could reflect the failure to properly ubiquitinate Ty proteins. According to this hypothesis, one of the functions of the RAD6 protein may be to prevent the processing of Ty precursor proteins or to enhance the degradation of mature Ty proteins, thereby limiting transposition. Alternatively, chromatin structure may be altered in *rad6* strains, perhaps by improper ubiquitination of histones, allowing greater access to both transposition and recombination complexes.

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LITERATURE CITED

1. **Boeke, J. D., and D. J. Garfinkel.** 1988. Yeast Ty elements as retroviruses, p. 15–39. *In* Y. Koltin and M. J. Leibowitz (ed.), Viruses of fungi and simple eukaryotes. Marcel Dekker, Inc., New York.
2. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40**:491–500.
3. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine 5′-phosphate decarboxylase activity in yeast: 5-fluoroorotic acid resistance. Mol. Gen. Genet. **197**:345–346.
4. **Boeke, J. D., C. A. Styles, and G. R. Fink.** 1986. *Saccharomyces cerevisiae SPT3* gene is required for transposition and transpositional recombination of chromosomal Ty elements. Mol. Cell. Biol. **6**:3575–3581.
5. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
6. **Cameron, J. R., E. Y. Loh, and R. W. Davis.** 1979. Evidence for transposition of dispersed repetitive DNA families in yeast. Cell **16**:739–751.
7. **Curcio, M. J., N. J. Sanders, and D. J. Garfinkel.** 1988. Transpositional competence and transcription of endogenous Ty elements in *Saccharomyces cerevisiae*: implications for regulation of transposition. Mol. Cell. Biol. **8**:3571–3581.
8. **Eibel, H., and P. Philippsen.** 1984. Preferential integration of yeast transposable element Ty into a promoter region. Nature (London) **307**:386–388.
9. **Elder, R. T., E. Y. Loh, and R. W. Davis.** 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. USA **80**:2432–2436.
10. **Garfinkel, D. J., J. D. Boeke, and G. R. Fink.** 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell **42**:507–517.
11. **Hallick, R. B., B. K. Chelm, P. W. Gray, and E. M. Orozco.** 1977. Use of aurintricarboxylic acid as an inhibitor of nucleases during nucleic acid isolation. Nucleic Acids Res. **4**:3055–3064.
12. **Hastings, P. J., S. K. Quah, and R. C. von Borstel.** 1976. Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA. Nature (London) **264**:719–722.
13. **Haynes, R. H., and B. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces cerevisiae*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Hoffmann, W.** 1985. Molecular characterization of the *CAN1* locus in *Saccharomyces cerevisiae*. J. Biol. Chem. **260**:11831–

11837.

15. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
16. **Jentsch, S., J. P. McGrath, and A. Varshavsky.** 1987. The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature (London) **329**:131–134.
17. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49**: 264–285.
18. **Lemontt, J. F., and S. V. Lair.** 1982. Plate assay for chemical- and radiation-induced mutagenesis of *CAN1* in yeast as a function of post-treatment DNA replication: effect of *rad6-1**. Mutat. Res. **93**:339–352.
19. **Liebman, S. W., P. Shalit, and S. Picologlou.** 1981. Ty elements are involved in the formation of deletions in *DEL1* strains of *Saccharomyces cerevisiae*. Cell **26**:401–409.
20. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
21. **Montelone, B. A., S. Prakash, and L. Prakash.** 1981. Recombination and mutagenesis in *rad6* mutants of *Saccharomyces cerevisiae*: evidence for multiple functions of the *RAD6* gene. Mol. Gen. Genet. **184**:410–415.
22. **Morrison, A., E. J. Miller, and L. Prakash.** 1988. Domain structure and functional analysis of the carboxyl-terminal polyacidic sequence of the RAD6 protein of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:1179–1185.
23. **Nagata, S., K. Nagashima, Y. Tsunetsugu-Yokota, K. Fujimura, M. Mijazaki, and Y. Kaziro.** 1984. Polypeptide chain elongation factor 1α (EF-1α) from yeast: nucleotide sequence of one of two genes for EF-1α from *Saccharomyces cerevisiae*. EMBO J. **3**:1825–1830.
24. **Paquin, C. E., and V. M. Williamson.** 1984. Temperature effects on the rate of Ty transposition. Science **226**:53–55.
25. **Paquin, C. E., and V. M. Williamson.** 1986. Ty insertions at two loci account for most of the spontaneous antimycin A resistance mutations during growth at 15°C of *Saccharomyces cerevisiae* strains lacking *ADH1*. Mol. Cell. Biol. **6**:70–79.
26. **Pickhart, C. M., and I. A. Rose.** 1985. Functional heterogeneity of ubiquitin carrier proteins. J. Biol. Chem. **260**:1573–1581.
27. **Picologlou, S., M. E. Dicig, P. Kovarik, and S. W. Liebman.** 1988. The same configuration of Ty elements promotes different types and frequencies of rearrangements in different yeast strains. Mol. Gen. Genet. **211**:272–281.
28. **Prakash, L., and S. Prakash.** 1977. Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. Genetics **86**:33–55.
29. **Reynolds, P., S. Weber, and L. Prakash.** 1985. *RAD6* gene of *Saccharomyces cerevisiae* encodes a protein containing a tract of 13 consecutive aspartates. Proc. Natl. Acad. Sci. USA **82**:168–172.
30. **Roeder, G. S., M. Smith, and E. J. Lambie.** 1984. Intrachromosomal movement of genetically marked *Saccharomyces cerevisiae* transposons by gene conversion. Mol. Cell. Biol. **4**:703–711.
31. **Rolfe, M., A. Spanos, and G. Banks.** 1986. Induction of yeast Ty element transcription by ultraviolet light. Nature (London) **319**:339–340.
32. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1979. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Southern, E. M.** 1975. Detection of specific sequence among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
34. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
35. **Sung, P., S. Prakash, and L. Prakash.** 1988. The RAD6 protein of *Saccharomyces cerevisiae* polyubiquitinates histones, and its acidic domain mediates this activity. Genes Dev. **2**:1476–1485.
36. **Valerie, K., A. Delers, C. Bruck, C. Thiriart, H. Rosenberg, C. Debouck, and M. Rosenberg.** 1988. Activation of human immunodeficiency virus type I by DNA damage in human cells. Nature (London) **333**:78–81.
37. **Whelan, W. L., E. Gocke, and T. R. Manney.** 1979. The *CAN1* locus of *Saccharomyces cerevisiae*: fine structure analysis and forward mutation rates. Genetics **91**:35–51.

37a. **Wilke, C. M., S. H. Heidler, N. Brown, and S. W. Liebman.** 1989. Analysis of yeast retrotransposon Ty insertions at the *CAN1* locus. Genetics **123**:655–665.

38. **Williamson, V. M.** 1983. Transposable elements in yeast. Int. Rev. Cytol. **83**:1–25.
39. **Winston, F., F. Chumley, and G. R. Fink.** 1983. Eviction and transplacement of mutant genes in yeast. Methods Enzymol. **101**:211–228.
40. **Winston, F., C. Dollard, E. A. Malone, J. Clare, J. G. Kapakos, P. Farabough, and P. L. Minehart.** 1987. Three genes are required for *trans*-activation of Ty transcription in yeast. Genetics **115**:649–656.