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Incorporation of Y′-Ty1 cDNA destabilizes telomeres in *Saccharomyces cerevisiae* telomerase-negative mutants.

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ABSTRACT

Ty1 retrotransposons in *Saccharomyces cerevisiae* are activated by telomere erosion. Ty1-dependent reverse transcription of mRNA from subtelomeric Y′ repeats generates chimeric Y′-Ty1 cDNA. Here, we show that Y′-Ty1 cDNA is incorporated at eroding telomeres in the absence of telomerase. Telomeric incorporation of Y′-Ty1 cDNA promotes genome rearrangements.

Telomerase, a ribonucleoprotein complex consisting of a reverse transcriptase enzyme and an RNA template, extends telomeric DNA repeats at the ends of linear chromosomes. In the absence of telomerase, *Saccharomyces cerevisiae* strains undergo replicative senescence as telomeric DNA shortens over 50 to 100 generations. A small fraction of cells escape senescence, and these "survivors" have altered telomere structures that are maintained by homologous recombination (LUNDBLAD and BLACKBURN 1993; TENG and ZAKIAN 1999). Mobility of the Ty1 long terminal repeat (LTR) retrotransposon in *Saccharomyces cerevisiae* is progressively induced by erosion of telomeres in the absence of telomerase, and mobility remains variably induced in survivors (SCHOLES *et al.* 2003). One consequence of the induction of Ty1 reverse transcriptase activity is the mobilization of subtelomeric Y′ elements in trans (MAXWELL *et al.* 2004). Chimeric Y′-Ty1 cDNA molecules are incorporated into the genome at frequencies as high as 7×10^{-6} in telomerase-negative survivors (MAXWELL *et al.* 2004). Y′-Ty1 cDNA is synthesized by reverse transcription initiating at the 3′ poly(A) tail of Y′ mRNA, using the end of either Ty1 LTR or, less frequently, internal regions of Ty1 cDNA in either orientation as a primer (MAXWELL *et al.* 2004). Other chimeric retrosequences consisting of cDNA derived from a variety of cellular mRNAs fused to Ty1 cDNA have also been detected, and they are incorporated into the genome at high frequencies in the absence of telomerase (DERR *et al.* 1991; SCHACHERER *et al.* 2004; MAXWELL and CURCIO 2007). Here, we determine whether incorporation of Y′-Ty1 cDNA extends telomeres by recombining with subtelomeric Y′ elements, as previously proposed (MAXWELL *et al.* 2004), and whether the presence of Y′-Ty1 cDNA at chromosome ends affects the stability of eroding telomeres.

To detect incorporation of Y′-Ty1 retrosequences into the genome, we used a strain harboring a single chromosomal Y′ element marked in the 3′ untranslated region with the

retrotranscript indicator gene, *his3AI* (MAXWELL *et al.* 2004). Splicing of the AI intron from the Y′*his3AI* transcript, followed by reverse transcription, results in the formation of Y′*HIS3* cDNA. Incorporation of Y′*HIS3* cDNA into the genome allows cells to become His^+ prototrophs. The majority of Y′*HIS3* events in *tlc1*Δ mutants, which lack the telomerase RNA template, have Ty1 sequences 3′ of the oligo(A) tract of the Y′*HIS3* cDNA (MAXWELL *et al.* 2004) (Figure 1A).

As an initial test of the hypothesis that Y′*HIS3*-Ty1 cDNA is incorporated into the genome by recombination with subtelomeric Y′ elements at eroding telomeres, we determined if Y′*HIS3* retrosequence formation is dependent on homologous recombination proteins Rad51p or Rad52p. Following segregation of a *TLC1* plasmid in *tlc1*Δ Y′*his3AI* derivatives of strain BY4742 (MAXWELL *et al.* 2004) with or without a deletion of *RAD52* or *RAD51*, the frequency of His^+ prototroph formation was measured. Rad52p is required to form telomerase-negative survivors (TENG and ZAKIAN 1999), so we measured His^+ frequencies after subculturing once in the absence of telomerase (~25 generations, Table 1). The median His^+ frequency of *tlc1*Δ *rad52*Δ isolates was at least 10-fold lower and significantly different ($P = 0.001$) from the median His^+ frequency of *tlc1*Δ isolates (Table 1). Rad51p is not required for recovery from senescence (CHEN *et al.* 2001; TENG *et al.* 2000), so we assayed His^+ frequencies after subculturing twice (~50 generations) when cell populations were senescent. The median His^+ frequency of *tcl1*Δ *rad51*Δ populations was 16-fold lower and significantly different ($0.005 < P < 0.01$) than that of *tlc1*Δ populations (Table 1). There was no difference in the population doubling times that could account for the difference in Y′*HIS3* retrosequence formation between *tlc1*Δ and *tlc1*Δ *rad51*Δ strains (data not shown). Furthermore, there was no substantial difference in the quantity of unmarked Y′-Ty1 cDNA detected using a competitive PCR assay (MAXWELL *et al.* 2004). The *tlc1*Δ and *tlc1*Δ *rad51*Δ strains had 4.8×10^{-3} ($\pm 0.76 \times 10^{-3}$) copies/genome and 6.9×10^{-3} ($\pm 1.7 \times 10^{-3}$) copies/genome,

respectively. Using PCR analysis, we determined that sequences from the first 50 bp of Y′ are almost always detected 5' of the *HIS3* marker in His^+ isolates (data not shown). This is also consistent with incorporation of Y′*HIS3-Ty1* cDNA through homologous recombination rather than integration, since integrated Y′*HIS3-Ty1* cDNA would lack sequences from untranscribed regions of Y′. We conclude from these data that incorporation of Y′*HIS3* cDNA occurs predominantly through homologous recombination.

A second prediction of our hypothesis is that Y′*HIS3*-Ty1 retrosequences are located centromere-distal to X elements and in the same orientation as native Y′ elements. X elements are subtelomeric repeats found centromere-proximal to Y′ elements or telomeric DNA at all *S. cerevisiae* telomeres (ZAKIAN 1996) (Figure 1B). Genomic DNA from 29 His^+ survivors harboring Y′*HIS3*-Ty1 cDNA and 2 His^- survivors was digested with *Nsi*I or *Pvu*II, neither of which have cleavage sites in Y′ or *HIS3* DNA. Diluted DNA was ligated and used as a template for inverse PCR with an X primer and a primer to the *HIS3* splice junction (Figure 1B). Products were obtained from 22 of the 29 samples prepared from survivors harboring Y′*HIS3*-Ty1 cDNA, but no specific products were obtained from His^- survivors. The 22 products obtained each contained Ty1 sequences ligated to sequences adjacent to X DNA (Figure 1B and 1C). Therefore, Y′*HIS3*-Ty1 cDNA was centromere-distal to X, and in the orientation expected if Y′*HIS3*-Ty1 cDNA molecules recombined with subtelomeric Y′ elements, in at least 76% of His^+ strains. Twelve inverse PCR products contained unique subtelomeric sequences, which allowed us to identify eight different telomeres that acted as recipients in recombination with Y′*HIS3*-Ty1 cDNA (Figure 1C and data not shown). The absence of a product for seven samples could be due to the presence of long Y′ arrays between X and Y′*HIS3*-Ty1 retrosequences, which would reduce the efficiency of intramolecular

ligation, or to the incorporation of Y′*HIS3*-Ty1 cDNA at non-telomeric sites. Regardless, our results indicate that Y′*HIS3*-Ty1 cDNA is frequently incorporated at telomeres.

The incorporation of Y′-Ty1 cDNA at telomeres could compensate for the loss of DNA from chromosome termini in telomerase mutants (MAXWELL *et al.* 2004), thereby contributing to the formation of stable alternative telomere structures. On the other hand, the introduction of Ty1 sequences at chromosome termini could destabilize telomeres if telomeric Ty1 sequences recombine with Ty1 elements at other genomic locations. To determine if telomeres harboring Y′-Ty1 retrosequences are stable, we examined isolates of telomerase-negative survivors that harbored or lacked Y′*HIS3*-Ty1 cDNA for evidence of chromosomal rearrangements using pulsed-field gel electrophoresis. Only 5% of His^- isolates lacking Y′*HIS3*-Ty1 cDNA had a missing or new chromosome band relative to the original His^- survivors from which they were derived. By comparison, 76% of the His^+ isolates had new or missing chromosome bands compared to the original His^- survivors (Figure 2 and Table 2). Of the His^+ isolates that had one or more new chromosome bands, 60% had *HIS3* sequences on a new chromosome band (Figure 2 and Table 2), consistent with the involvement of the Y′*HIS3*-Ty1 cDNA in the rearrangement. The strong correlation between incorporation of Y′*HIS3*-Ty1 cDNA and the presence of chromosomal rearrangements supports the hypothesis that incorporation of Y′-Ty1 cDNA destabilizes telomeres. The observations strengthen the argument that Y′*HIS3*-Ty1 retrosequences destabilize telomeres and promote genome rearrangements.

In summary, this work indicates that Ty1's role in mobilizing Y′ elements can result in the incorporation of Ty1 sequences at a specific genomic site, telomeres, at which Ty1 sequences are not normally found. Telomeres that terminate with Ty1 sequences are likely unstable and undergo secondary recombination events that produce chromosomal rearrangements. Based on our previous

characterization of chimeric retrosequences at chromosomal breakpoint junctions (MAXWELL and CURCIO 2007), the secondary events are likely to involve recombination between Ty1 cDNA at telomeres and Ty1 elements at internal positions on other chromosomes to produce translocations. Potentially, translocations formed by Y'-Ty1 cDNA retrosequence incorporation could form dicentric chromosomes, which would lead to the generation of additional rearrangements through the chromosome breakage-fusion-bridge cycle (BAILEY and MURNANE 2006). This phenomenon could explain the existence of multiple rearrangements in some strains harboring Y′*HIS3* retrosequences (Figure 2).

The frequency of Y′-Ty1 retrosequence formation in telomerase-negative survivors is high enough to compensate for the loss of telomeric DNA in the absence of telomerase (MAXWELL *et al.* 2004). However, the results presented here are not consistent with the idea that incorporation of Y′-Ty1 cDNA contributes to telomere maintenance. Instead, incorporation of Y′-Ty1 retrosequences at telomeres, similar to the incorporation of single copy gene retrosequences at chromosomal breakpoints (MAXWELL and CURCIO 2007), promotes restructuring of the genome during telomere crisis. Our work also supports the hypothesis that retrotransposition of mammalian L1 elements to telomeres triggers the formation of chromosome rearrangements (MORRISH *et al.* 2007).

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LITERATURE CITED

BAILEY, S. M., and J. P. MURNANE, 2006 Telomeres, chromosome instability and cancer. Nucleic Acids Res. **34:** 2408-2417.

CHEN, Q., A. IJPMA and C. W. GREIDER, 2001 Two survivor pathways that allow growth in the absence of telomerase are generated by distinct telomere recombination events. Mol. Cell. Biol. **21:** 1819-1827.

DERR, L. K., J. N. STRATHERN and D. J. GARFINKEL, 1991 RNA-mediated recombination in *S. cerevisiae*. Cell **67:** 355-364.

FOSTER, P. L., 2006 Methods for determining spontaneous mutation rates. Methods Enzymol. **409:** 195-213.

LUNDBLAD, V., and E. H. BLACKBURN, 1993 An alternative pathway for yeast telomere maintenance rescues *est1-* senescence. Cell **73:** 347-360.

MAXWELL, P. H., C. COOMBES, A. E. KENNY, J. F. LAWLER, J. D. BOEKE *et al.*, 2004 Ty1 mobilizes subtelomeric Y' elements in telomerase-negative *Saccharomyces cerevisiae* survivors. Mol. Cell. Biol. **24:** 9887-9898.

MAXWELL, P. H., and M. J. CURCIO, 2007 Retrosequence formation restructures the yeast genome. Genes Dev. **21:** 3308-3318.

MORRISH, T. A., J. L. GARCIA-PEREZ, T. D. STAMATO, G. E. TACCIOLI, J. SEKIGUCHI *et al.*, 2007 Endonuclease-independent LINE-1 retrotransposition at mammalian telomeres. Nature **446:** 208-212.

SCHACHERER, J., Y. TOURRETTE, J. L. SOUCIET, S. POTIER and J. DE MONTIGNY, 2004 Recovery of a function involving gene duplication by retroposition in *Saccharomyces cerevisiae*. Genome Res. **14:** 1291-1297.

SCHOLES, D. T., A. E. KENNY, E. R. GAMACHE, Z. MOU and M. J. CURCIO, 2003 Activation of a LTR-retrotransposon by telomere erosion. Proc. Natl. Acad. Sci. USA **100:** 15736-15741.

TENG, S. C., J. CHANG, B. MCCOWAN and V. A. ZAKIAN, 2000 Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. Mol. Cell. Biol. **6:** 947-952.

TENG, S. C., and V. A. ZAKIAN, 1999 Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 8083-8093.

ZAKIAN, V. A., 1996 Structure, function, and replication of *Saccharomyces cerevisiae* telomeres. Annu. Rev. Genet. **30:** 141-172.

FIGURE LEGENDS

FIGURE 1. Y′*HIS3* cDNA is incorporated at telomeres. (A) A schematic representation of Y′*HIS3*-Ty1 cDNA. "A_n" represents the oligo(A/T) tract that is derived from the polyadenylated tail of the Y′*HIS3* RNA. (B) A drawing depicts Y′*HIS3*-Ty1 cDNA centromere-distal to a subtelomeric X element. To test for the presence of this structure, independent strains harboring Y′*HIS3*-Ty1 cDNA were obtained by selecting for His^+ prototrophs from *tlc1*Δ survivors of strains JC3840 (MAXWELL *et al.* 2004) and JC4153 (*MATα ade2::hisG his3*Δ*1 leu2*Δ*0 lys2*Δ*0 tlc1::LEU2 ura3*Δ*0 Y′his3AI* pRS317TLC1), as previously described (MAXWELL *et al.* 2004). To perform inverse PCR, genomic DNA was digested with *Nsi*I or *Pvu*II and ligated in a total volume of 120 or 150 µl overnight. Aliquots were amplified with primer H3HOPA2 (TCTCCTACTTTCTCCCTTTGCAAACC), a primer that anneals across the *HIS3* splice junction, which is indicated by a dotted line, and Xcore2 (ACATGCCATACTCACCTTCAC), a primer that anneals to all core X element sequences present adjacent to *S. cerevisiae* telomeres. Arrows in the diagram indicate the position of these primers. When ligated *Pvu*II fragments were used as templates, a second semi-nested PCR analysis was performed using primers PJ160 (CTTCGTTTATCTTGCCTGCTC), which anneals to the *HIS3* promoter, and Xcore2 (not illustrated). The major product obtained from each reaction was gel extracted and directly sequenced or cloned and sequenced. The expected structure of inverse PCR products is illustrated. (C) The specific structures of four representative inverse PCR products are shown. Corresponding positions in Ty1 element YPLWTy1-1 or in the indicated chromosome (www.yeastgenome.org) are given for the inverse PCR product junction. Drawings are not to scale.

FIGURE 2. Incorporation of Y′*HIS3*-Ty1 cDNA is correlated with the appearance of chromosome rearrangements in telomerase-negative survivors. Intact yeast chromosome preparations were separated by clamped homogeneous electric fields (CHEF) gel electrophoresis

and used for Southern blots as described (MAXWELL and CURCIO 2007). (A) A representative CHEF gel of intact chromosomes obtained from the telomerase-positive strain (P, lane 1), a His^- survivor (S, lane 2), and isolates of the His^- survivor, three of which remained His^- (lanes 3-5), and six of which were selected for the His^+ phenotype (lanes 6-11). Asterisks indicate new chromosome bands harboring *HIS3* sequences (see below). The yeast chromosome or pair of chromosomes corresponding to each band is listed on the right of the gel image. (*B*) A Southern blot of the gel shown in (*A*) probed for *HIS3* sequences. The *his3Δ1* allele on chromosome XV was detected in all lanes. Y′*his3AI* is present on chromosome V or VIII, and this band was also detected in all lanes. At least one additional band was detected in each sample from His^+ clones (lanes 6-11). Asterisks indicate the same bands indicated on the gel in panel (*A*).

TABLE 1

***RAD52* and *RAD51* are required for efficient Y′*HIS3*-Ty1 retrosequence formation**

			His$^+$ Frequency $\times 10^{-9a}$		
Relevant Genotype	N[b]	Subculture[c]	Range	Median	Relative
Y′*his3AI tlc1*Δ	7	1	1.2-34[d]	5.7	100%
Y′*his3AI tlc1*Δ *rad52*Δ	9	1	<0.43-1.7	<0.56[e]	<9.8%[e]
Y′*his3AI tlc1*Δ	8	2	94-1600[d]	490	100%
Y′*his3AI tlc1*Δ *rad51*Δ	6	2	12-600	31	6.3%

[a] The frequency of His$^+$ prototroph formation per total viable cells plated was obtained by pooling the results from triplicate cultures, each initiated at a density of 1×10^5 cells/ml, as previously described (MAXWELL *et al.* 2004).

[b] Number of independent isolates examined for each genotype. Two or three independent *rad52*Δ (*rad52::URA3* or *rad52::KanMX*) or *rad51::KanMX* transformants were generated by one-step gene replacement.

[c] Number of times each *tlc1*Δ isolate was subcultured following segregation of the *TLC1* plasmid.

[d]The His$^+$ frequency in the Y′*his3AI tlc1*Δ strain is higher at subculture 2 because cell populations are senescent.

[e] Calculation of the median frequency using the p_0 method (FOSTER 2006) yielded a value of 6.6 $\times 10^{-11}$, corresponding to a relative frequency of 1.2%.

TABLE 2

Chromosome rearrangement frequencies in survivors

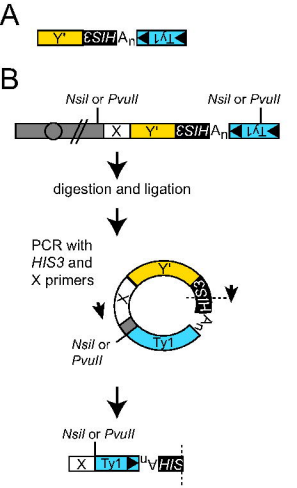
Survivor Phenotype	No.[a]	No. with Rearrangements[b]	Rearrangements per Isolate	Fraction of Isolates with *HIS3* on a New Chromosome Band[c]
His-	19	1	0.21±0.21*	-
His+	29	22	1.41±0.21*	9/15

[a] Total number of independent isolates from five independent His^- survivors that were examined.

[b] New bands or missing bands on CHEF gels were scored as rearrangements.

[c] Only His^+ isolates with new chromosome bands were used to obtain this fraction.

* Difference in means is statistically significant, $P = 0.0001$


A
Y'
εSIH
An
Ty1
B
NsiI or PvuII
NsiI or PvuII
X
Y'
εSIH
An
Ty1
digestion and ligation
PCR with
HIS3 and
X primers
Y'
εSIH
An
Ty1
NsiI or
PvuII
NsiI or PvuII
X
Ty1
uΨHIS
C
NsiI 4274 Ty1
X
ARS503
Ty1
uΨHIS
ChrV 6762
PvuII 478 Ty1
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Ty1
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X
PAU19
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Ty1
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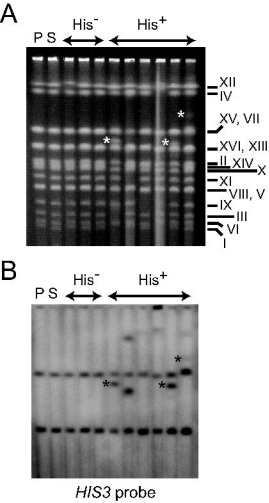
ChrIII 2649


A
P S
His-
His+
XII
IV
XV, VII
XVI, XIII
II, XIV
X
XI
VIII, V
IX
III
VI
I
B
P S
His-
His+
HIS3 probe