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Transcription of a yeast telomere alleviates telomere position effect without affecting chromosome stability

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ABSTRACT **Telomeres are required for the stable maintenance of chromosomes in the yeast *Saccharomyces cerevisiae*. Telomeres also repress the expression of genes in their vicinity, a phenomenon known as telomere position effect. In an attempt to construct a conditional telomere, an inducible promoter was introduced adjacent to a single telomere of a chromosome such that transcription could be induced toward the end of the chromosome. Transcription toward two other essential chromosomal elements, centromeres and origins of replication, eliminates their function. In contrast, transcription toward a telomere did not affect the stability function of the telomere as measured by the loss rate of the transcribed chromosome. Transcription proceeded through the entire length of the telomeric tract and caused a modest reduction in the average length of the transcribed telomere. Transcription of the telomere substantially reduced the frequency of cells in which an adjacent *URA3* gene was subject to telomere position effect. These results indicate that telomere position effect can be alleviated without compromising chromosome stability.**

Telomeres are the physical ends of eukaryotic chromosomes. In virtually all organisms they are composed of simple repeated sequences (reviewed in ref. 1). In yeast, telomeres are composed of 300 ± 75 (mean ± range) bp of the variable repeat $TG_{2-3}(TG)_{1-6}$, commonly abbreviated TG_{1-3} (2, 3). Telomeric DNA is essential for chromosome stability in the yeast *Saccharomyces cerevisae*. A chromosome that loses a telomere is dramatically destabilized and is lost from many cells (4).

Yeast telomeres have been shown to exert a position effect on the expression of nearby genes (5). Transcription of a gene near a telomere is repressed relative to its transcription elsewhere in the genome, a phenomenon known as telomere position effect (TPE). Although genes near telomeres can be expressed normally if induced, their constitutive expression is repressed. In the absence of induction, genes near telomeres switch back and forth between transcriptionally active and transcriptionally repressed states, each state being mitotically stable for many generations.

Transcription toward origins of replication (*ARS*) or centromeres (*CEN*) perturbs the function of these elements (6–8). Specifically, when a galactose-inducible promoter is introduced adjacent to an *ARS* on a plasmid in which that *ARS* is the only origin of replication, the plasmid is destabilized on galactose medium, where the promoter is induced, relative to its stability on glucose, where the promoter is repressed (8). Similarly, when a galactose-inducible promoter is placed adjacent to a *CEN* on a plasmid or chromosome, the plasmid or chromosome is unstable on galactose (7). In both cases, transcription does not proceed through the elements themselves but is instead blocked by the *ARS* or *CEN* (8, 9).

For the purpose of genetic dissection of telomere properties, we designed a system in which a subtle, reversible perturbation of a telomere could be induced. Since transcription reversibly perturbs the function of *CEN* and *ARS* elements, we speculated that transcription could be used to perturb a telomere as well. To that end, we constructed yeast strains in which a galactose-inducible promoter was positioned directly adjacent to a telomere. Growth of these strains on galactose medium induced transcription that proceeded through the telomeric DNA toward the end of the chromosome. Telomere transcription did not reduce the stability of the affected chromosome but did alleviate the TPE exerted by that telomere in most cells. These results suggest that the mechanism by which a telomere exerts transcriptional repression can be separated from the structure or function of a telomere that is essential for chromosome stability.

MATERIALS AND METHODS

UT modifications were made by transformation with a plasmid similar to pADH4UCA4 (ref. 4; see below for description of UT, UGPT, and UGXT designations). UGPT modifications were made using a plasmid identical except for the addition of a 618-bp *Sau*3a–*Bam*HI fragment from pLGSD5 (10) that contained the *GAL1,10 UAS* and the TATA element from the *CYC1* promoter. The *GAL UAS* and *TATA* element were inserted between *URA3* and the telomeric sequence oriented such that galactose-induced transcription was toward the telomeric DNA. UGXT modifications were similar but contained the *GAL1,10 UAS* without the *CYC1* TATA as a 371-bp *Sau*3a–*Xho* I fragment from pLGSD5. Each of the modifications to chromosome VII-L was introduced by fragment-mediated transformation into three haploid yeast strains: DG20 (*MATα ade2 ade5 lys5 cyh2^r can1 ura3::LEU2*) (5); DG21 (*MATα kar1-1 ura3 ade3 aro2*), a *ura3* derivative of 3920-1-2 (11); and LS15a (*MATα kar1-1 ura3 aro2 leu2*), which was derived from DG21. The growth rate and haploid viability of each of the modified strains were indistinguishable from the unmodified version of the same strain. The haploid strain IGA2 (*MATα ade2 ade5, lys5, cyh2^r, can1*) is isogenic to DG20 except that it contains *URA3* at its normal location on chromosome V. Disome strains for measuring the stability of chromosome VII were formed by passing the modified versions of chromosome VII from the karyogamy-deficient LS15a strains into the haploid strain LS11 (*MATα his3 ade2 can1 trp1 ura3 leu2 lys5 cyh2^r ade3*). In addition, a disome strain was used in which a telomere can be eliminated from the end of VII-L by a galactose-inducible double-strand break mediated by HO endonuclease, a nuclease normally involved in homothallic mating type switching. This strain,

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Abbreviations: TPE, telomere position effect; FOA, 5-fluoroorotic acid; FOA^r, FOA-resistant.

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referred to as the wild-type test chromosome strain in ref. 4, was isogenic to the disome strains described above except that the recognition sequence for HO endonuclease was deleted from the *MAT* locus, a galactose-inducible HO gene was introduced into the *ade3* locus, and a recognition site for the HO endonuclease was placed adjacent to the telomere of chromosome VII-L. Yeast complete synthetic medium (YC) and rich medium (YEP) were described previously (12).

For galactose-grown cells, 3% galactose was substituted for glucose. FOA medium and cycloheximide medium contained, respectively, 1 mg of 5-fluoroorotic acid (FOA) per ml and 10 mg of cycloheximide per ml. To monitor chromosome loss, disomes were streaked onto YC plates containing either glucose or galactose. Colonies were suspended in water and plated onto YEP plates and YEP cycloheximide plates. For each disome strain, 10 colonies were assayed from both glucose and galactose media. Rates of chromosome loss per cell division were calculated by the method of the median (13). To monitor TPE in haploid strains, colonies were picked from cells grown on YEP plates containing either glucose or galactose. Dilutions of these colonies were plated to YC plates, to YC plates lacking uracil, and to YC plates containing FOA. For each strain, at least five colonies were assayed from each test medium. The frequency of FOA-resistant (FOA^r) cells in the disome strains was assayed similarly except that all media lacked lysine and tyrosine. Northern blot analysis was performed as described (5). Blots were hybridized sequentially to a *HIS3/DED1* DNA probe, which hybridized to an ≈700-base *HIS3* transcript and to an ≈2300-base *DED1* transcript and then to either $C_{1-3}A$ or UG_{1-3} RNA probes (14).

RESULTS

Transcription of a Telomere. To construct strains in which transcription could be induced toward the telomere of a chromosome, a galactose-inducible promoter was introduced directly adjacent to the left telomere of chromosome VII (VII-L) in three different strains (Fig. 1). The inducible promoter was introduced on a fragment that contained a short tract of TG_{1-3} DNA, *URA3* as a selectable marker, and a portion of the *ADH4* gene. Transformation with this fragment resulted in deletion of the ≈20 kb of DNA distal to the *ADH4* locus and formation of a new telomere by elongation of the short tract of TG_{1-3} on the fragment. The inducible promoter consisted of the *GAL1,10 UAS* and a TATA element, placed so that transcription would proceed toward the end of the chromosome. To distinguish effects due to activation of transcriptional activator GAL4 protein from those due to transcription itself, a version of the chromosome VII-L telomere was constructed that contained the *GAL UAS* without the TATA sequence. In this construct, GAL4 protein is expected to bind to the *UAS* and to be activated by phosphorylation when cells are grown in galactose (15–17) but no transcription of the telomere is expected. As an additional control, a version of the chromosome VII-L telomere that lacked both the *GAL UAS* and the TATA was constructed. Strains bearing the *GAL UAS* with TATA at the VII-L telomere are denoted UGPT for URA-GAL UAS-promoter-telomere. Strains bearing the *GAL UAS* without the TATA are denoted UGXT for URA-GAL UAS-no TATA (X)-telomere and those without the *GAL UAS* or the TATA are denoted UT for URA3-telomere. Telomeres formed by such transformations are functionally and structurally indistinguishable from natural telomeres as measured by chromosome stability, telomere length, and telomere chromatin structure (this study; ref. 18). Each of the three modified versions of chromosome VII-L was individually introduced into three strains. The three strains include two unrelated haploid strains, DG20 and DG21, and a disome strain.

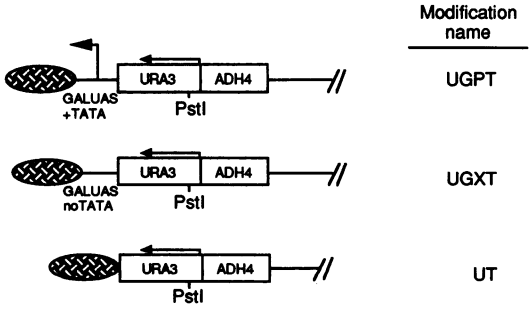

FIG. 1. Modification of the left arm of chromosome VII. The left arm of chromosome VII was modified by integration of fragments containing the *URA3* gene and telomeric sequence at the *ADH4* locus. UGPT-modified telomere contains the *GAL UAS* and a TATA sequence directly adjacent to the VII-L telomere, UGXT contains the *GAL UAS* but lacks the TATA sequence, and UT contains only *URA3* adjacent to the telomere. Telomeres are represented by hatched ovals; arrows indicate direction of transcription. Each of the three constructs was introduced into three different strains.

To establish that growth on galactose medium induced transcription toward the telomere of the UGPT-modified chromosome, Northern blot analysis was performed on the DG20 strains. Total RNA was isolated from the UGPT and control strains each grown in three different media. Strains were grown in complete glucose medium to assess uninduced telomere transcription, in glucose medium lacking uracil to assess the effect of *URA3* transcription on telomere transcription, and in complete galactose medium to determine the level of galactose-induced telomere transcription. Northern blots of the DG20 strains were probed with strand-specific probes for telomeric sequence.

Telomere transcription was detected in the UGPT strain grown on complete galactose medium (Fig. 2). The length (200–400 bases) and heterogeneity of the transcript were consistent with transcription of the full length of the telomeric tract. Transcripts hybridized to a $C_{1-3}A$ probe (Fig. 2) but not a UG_{1-3} probe (data not shown), indicating that transcription proceeded from the interior toward the end of the chromosome. Telomere transcription was not detected with either strand of telomeric probe in strains lacking the UGPT-modified telomere or in glucose-grown cells, whether the medium contained or lacked uracil (Fig. 2). Similar blots were performed on RNA isolated from DG21 strains with similar

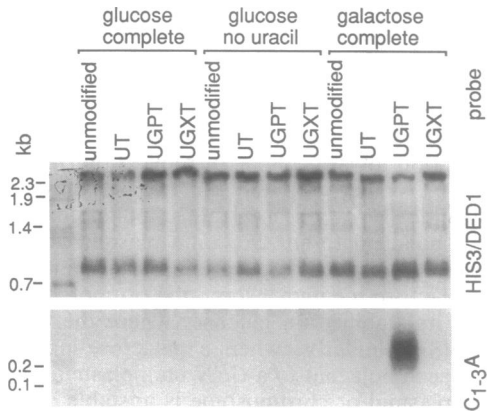

FIG. 2. Transcription is induced by galactose through the UGPT telomere. Northern blot analysis was performed on RNA from each of the DG20 modified VII-L strains and from an unmodified isogenic strain, IGA2. (*Upper*) Total RNA probed with the *HIS3/DED1* DNA probe to indicate the relative amount of total RNA loaded in each lane. (*Lower*) Same blot probed with a single strand $C_{1-3}A$ RNA probe. Strains and media are indicated at the top of autoradiogram.

results (data not shown). Thus, growth on galactose medium specifically induced transcription of the UGPT telomere and the induced transcription proceeded through the telomere to the end or very near the end of the chromosome.

Transcription of a Telomere Decreases Telomere Length. To determine if transcription of a telomere affected its length, the effect of galactose on the length of the telomeric DNA at the VII-L telomere and at other telomeres was examined by Southern blot analysis. The average length of telomeric DNA varies from strain to strain and from telomere to telomere within a strain (19, 20). To determine the length of telomeres in the strains used in this study, the lengths of Y′ telomeres were assessed. Y′ elements are telomere-associated sequences present on a majority of chromosomal telomeres (reviewed in ref. 1). In DG21 and DG20 cells grown on complete glucose medium, TG_{1-3} tract lengths at Y′ telomeres were 400 ± 100 bp and 260 ± 75 bp, respectively (data not shown).

Telomere length is subject to clonal variation, and the average length of telomeres from any given colony can vary by as much as 100 bp from other colonies of the same strain (21). To minimize the effect of clonal variation, the telomeric DNA from three independent isolates of each of the DG21-derived strains was examined on both glucose and galactose. On complete glucose medium all of the modified telomeres exhibited normal telomere length, indistinguishable from the average length of Y′ telomeres in that strain. On complete galactose medium, the UGXT- and UT-modified telomeres exhibited a very modest ≈25-bp reduction in telomere length (Fig. 3) as did Y′ telomeres (data not shown). In contrast, the UGPT telomere was reduced in length by ≈90 bp by growth on complete galactose medium (Fig. 3). The lengths of the UGPT telomeres on glucose and galactose media were 370 ± 85 bp and 280 ± 85 bp, respectively. Thus, when the telomere was transcribed, there was a small but reproducible ≈90-bp reduction in telomere length.

The transcription-mediated reduction of telomere length occurred slowly, reaching a new equilibrium after 40 generations of growth on galactose, and was reversible (data not shown). As with many telomere-related phenomena, the transcription-mediated reduction in telomere length was subject to strain variation. In a strain unrelated to those used in this study, telomere transcription caused an ≈120-bp reduction in telomere length (data not shown). In the disome strain used in the chromosome loss assays described below, an ≈90-bp reduction in telomere length due to transcription was observed (data not shown). In the DG20 strain used in this study, a transcription-mediated reduction in telomere length was not seen, perhaps because that strain had relatively short telomeres on glucose.

Telomere Transcription Does Not Affect Chromosome Stability. To examine the effect of telomere transcription on chromosome stability, chromosome VII disome strains were constructed. Such strains are formed by introduction of an extra copy of chromosome VII into an otherwise haploid strain. Four disome strains were constructed that contained the UGPT-, UT-, and UGXT-modified chromosomes and an unmodified chromosome VII. In addition, as a positive control for perturbation of telomere function, a disome strain in which growth on galactose caused elimination of the VII-L telomere (4) was used. Owing to the presence of *ADE3* and *CYH2*^{2} on the introduced chromosome, the disome strains produced red, cycloheximide-sensitive colonies (Fig. 4). Cells that had lost the introduced chromosome VII produced white, cycloheximide-resistant colonies. The frequency of white, cycloheximide-resistant colonies was used to monitor loss of the introduced chromosome (4).

Each of the five strains was assayed for the frequency of cells that lacked the introduced chromosome after growth on complete glucose medium or complete galactose medium (Fig. 5). The rates of chromosome loss, calculated from the frequency values, were between 1.3×10^{-5} and 2.7×10^{-5}. For each of the disome strains on glucose medium the frequency of cells lacking the chromosome was between 0.03% and 0.1%, frequencies indistinguishable from that of the strain carrying the unmodified chromosome VII. Growth on galactose medium dramatically destabilized chromosome VII in the strain in which the galactose-inducible HO endonuclease eliminated the VII-L telomere, confirming previous results (4). In the HO-cut strain, the frequency of cells lacking the chromosome was 17% when the cells were grown on galactose medium compared to 0.1% when the cells were grown on glucose medium. In contrast, for the UGPT-, UGXT-, and UT-modified chromosome VII strains, the frequency of cells that lacked the chromosome on galactose medium was indistinguishable from cells grown on glucose medium and, on both media, the frequencies were indistinguishable from values for the unmodified control strain. Thus, transcription through a telomere had no detectable effect on chromosome stability.

Telomere Transcription Substantially Reduces the Frequency of Cells Exhibiting TPE. To determine whether transcription of a telomere perturbed TPE, the effect of galactose on the repression of *URA3* at the VII-L telomere was

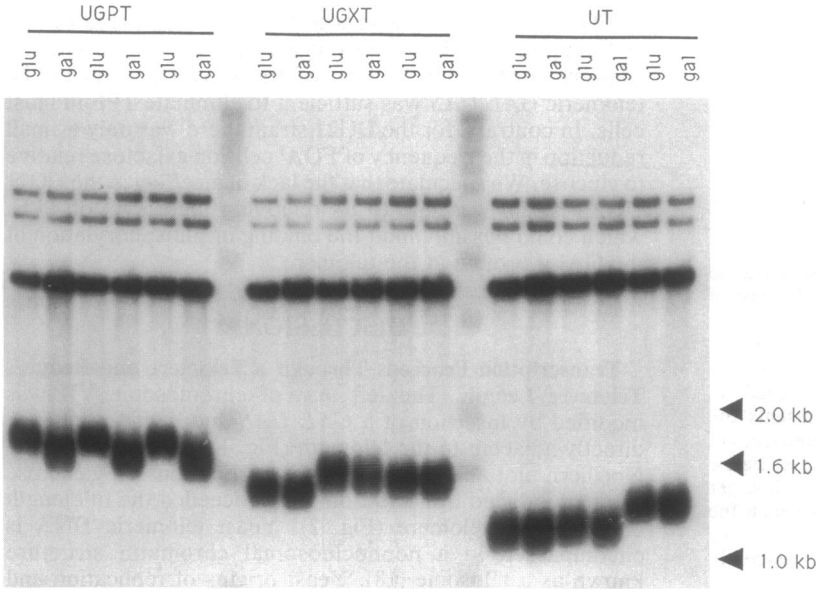

FIG. 3. Transcription of a telomere causes a reduction in telomere length. Southern blots of *Pst* I-digested DNA from DG21 strains grown in either glucose or galactose media were hybridized to a 1.1-kb fragment of *URA3*. Three subclones from each strain were grown on complete glucose or complete galactose medium. The diffuse bands between 1.2 and 2.0 kb are the terminal telomeric fragments from chromosome VII-L. The three distinct bands larger than 2 kb are the *Pst* I proximal segment of the telomeric *URA3* gene and the two halves of the mutant *ura3* gene at its normal locus on chromosome V. The telomeric fragment of the UGPT strain contains 895 bp of the *URA3* gene, 618 bp of *GAL UAS CYC1* promoter sequence, plus ≈400 bp of telomeric sequence. The telomeric *Pst* I fragment of the UGXT strain contains the 895 bp of *URA3*, 371 bp of the *GAL UAS*, plus the ≈400 bp of telomeric sequence. The terminal fragment of the UT strain contains the 895 bp of *URA3* plus ≈400 bp of telomeric sequence.

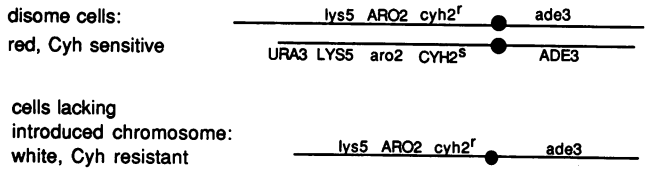

FIG. 4. Disome system used to assess the stability of chromosome VII. The two copies of chromosome VII in the otherwise haploid disome strains are depicted schematically. The introduced copy of chromosome VII carries wild-type *URA3*, *LYS5*, *CYH2*^{s}, and *ADE3*. The disome strains are *Ade2*$^-$. The disome strains produce red, cycloheximide-sensitive colonies. Loss of the introduced chromosome VII generates cells that produce white, cycloheximide-resistant colonies.

examined in strains containing the modified chromosomes (Fig. 6). To assay TPE on the *URA3* gene, cells were grown on medium containing either glucose or galactose then plated to determine the frequency of cells that could form colonies on FOA medium. Cells expressing *URA3* at wild-type levels are sensitive to FOA, whereas cells that do not express *URA3* are resistant to FOA (FOA^r) (22). Thus, the frequency of FOA^r cells can be used to monitor the frequency of cells in which the *URA3* gene is repressed. In all cases the frequency of cells that could grow on plates lacking uracil was ≈100% (data not shown), indicating that the *URA3* gene was present and could be expressed in most cells.

For all strains containing modified chromosome VII-L telomeres, the frequency of FOA^r cells was high in cells that had been grown on complete glucose medium, indicating the *URA3* gene was repressed in a large proportion of the cells (Fig. 6). For comparison, a control strain with *URA3* at its normal location near the centromere of chromosome V produced FOA^r cells at a frequency of $<10^{-6}$ (data not shown). Growth on galactose medium caused little or no reduction in the frequency of FOA^r cells in the UT strains, demonstrating that growth on galactose medium had little or no nonspecific effect on TPE. In contrast, growth on galactose medium caused a dramatic reduction in the frequency of

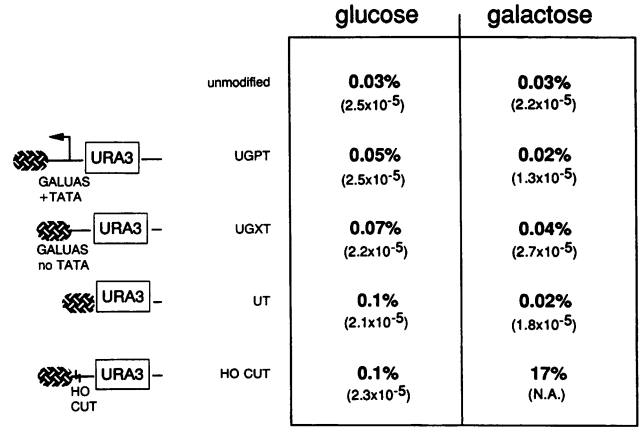

FIG. 5. Transcription of a telomere does not affect chromosome stability. The frequency of cells lacking the introduced copy of chromosome VII was monitored by assaying the frequency of white, cycloheximide-resistant colonies. The five different disome strains—unmodified VII, UGPT, UGXT, UT, and the strain with the HO endonuclease recognition site at VII-L—were each assayed on glucose and galactose. Each value shown in bold represents the average frequency from 10 different colonies. The calculated rates of chromosome loss per cell division are shown in parentheses below the frequency values. Calculation of the rate of chromosome loss per cell division was not applicable (N.A.) for the strain in which the telomere was eliminated by HO endonuclease on galactose since telomere elimination caused the chromosome to be lost frequently and the loss did not occur at a constant rate (4).

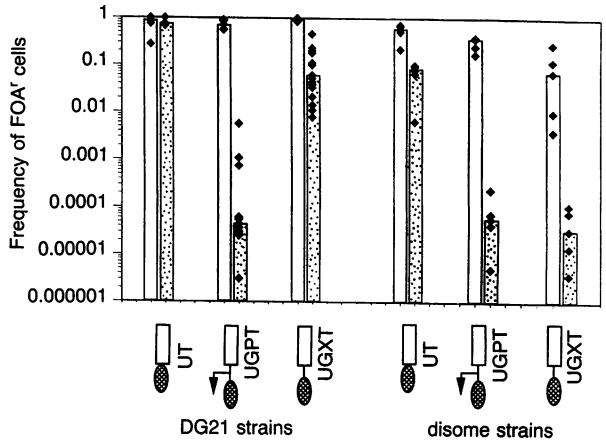

FIG. 6. Transcription of a telomere reduces the fraction of cells in which an adjacent gene is repressed. The frequency of FOA^r cells in individual colonies of the haploid DG21 strains and the disome strains was determined. Bars indicate the median frequency of FOA^r cells. Open bars indicate glucose-grown cells. Stippled bars indicate galactose-grown cells. Diamonds indicate the frequency of FOA^r cells from individual colonies. Five colonies were tested for each strain from each medium with the exception of DG21 UGPT and DG21 UGXT on galactose, where the number of colonies tested was 15 and 20, respectively. In cases where separate colonies produced identical frequencies of FOA^r cells, the number of diamonds appears fewer than the number of colonies tested.

FOA^r cells in the UGPT strains. In the DG21 UGPT strain, the median frequency of FOA^r cells was reduced four orders of magnitude, from 0.7 for glucose grown cells to 4×10^{-5} for galactose-grown cells. Similarly, in the disome UGPT strain, the median frequency of FOA^r cells was reduced from a median frequency of 0.4 for glucose grown cells to 6×10^{-5} for galactose-grown cells. Thus, transcription through a telomere alleviated TPE in most cells. Northern analysis indicated that the reduction in TPE was correlated with expression of *URA3* at levels equal to or slightly higher than the level of expression of the gene at its normal location on chromosome V (data not shown).

To determine if activation of GAL4 protein bound at the telomeric *UAS* was sufficient to eliminate TPE or if transcription *per se* was required for the effect, the level of TPE was examined in the UGXT strains on both glucose and galactose media. For the disome UGXT strain, the frequency of FOA^r cells was substantially reduced on galactose medium relative to the frequency on glucose. The median frequency of FOA^r cells on galactose was 3×10^{-5}, compared to 7×10^{-2} on glucose. This result suggests that the presence of the transcriptionally active form of the GAL4 protein at the telomeric *GAL UAS* was sufficient to eliminate TPE in most cells. In contrast, for the DG21 strain there was only a small reduction in the frequency of FOA^r cells on galactose relative to glucose. We speculate that the lack of an effect in the DG21 UGXT strain was due to its relatively high level of TPE, which could possibly limit the binding or phosphorylation of the GAL4 protein at the telomere.

DISCUSSION

Transcription Proceeds Through a Telomere and Reduces Telomere Length. The left arm of chromosome VII was modified by insertion of a *GAL UAS* and TATA sequence directly adjacent to the telomere (Fig. 1). As determined by Northern blot analysis, growth of such strains on galactose medium induced transcription that proceeded the full length of the VII-L telomere (Fig. 2). Yeast telomeric DNA is encompassed in a nonnucleosomal chromatin structure known as a telosome (18). Yeast origins of replication and

centromeres are also assembled into protein–DNA complexes (23, 24), known respectively as ORC and kinetochore. Transcription proceeds through a telosome without eliminating the chromosome stability function of the telomere. In contrast, although transcription is blocked by *ARS* and *CEN* chromatin, it disrupts the function of both elements. These data suggest that telosomes are more flexible chromatin structures than are ORCs or kinetochores.

Transcription of a telomere caused a reduction in the length of the telomere (Fig. 3). No specific reduction in telomere length was observed in a strain lacking the TATA element, suggesting the progression of the transcription complex through the telomere was required for the reduction in length. One possible explanation for the reduction in telomere length is that disruption of telomeric DNA–protein interactions by advancing transcription complexes perturbs the equilibrium between processes that serve to lengthen or protect telomeres and those that shorten or degrade telomeric DNA.

Telomere Transcription Does Not Affect Chromosome Stability. Telomeres, along with centromeres and origins of replication, are essential for chromosome stability (4). Given that transcription toward an *ARS* or *CEN* disrupts its function, it was surprising that transcription toward a telomere had no effect on chromosome stability (Fig. 5). Elimination of the function of a single telomere by endonucleolytic cleavage is sufficient to destabilize a chromosome (ref. 4; Fig. 5). Since a chromosome with a transcribed telomere was not destabilized, the stability function of a telomere must be resistant to perturbation by transcription. The resistance of the stability function of a telomere was manifest in spite of the fact that transcription proceeded through the entire length of the telomeric DNA. The resilience of telomere function to disruption by transcription could be due to the binding properties of telosomal proteins or to the repetitive nature of telomeric DNA. Proteins that mediate telomere function may remain associated with telomeric DNA during transcription, possibly "stepping around" an advancing transcription complex, as has been suggested for histone octamers (25). Alternatively, the aspect of a telomere that is required for chromosome stability could be mediated through multiple interactions that occur at many sites along the telomeric tract (discussed in ref. 26). For example, potential binding sites for RAP1 protein occur approximately every 35 bp in telomeric DNA (20, 27). Transcription might perturb a subset of these interactions but the residual unperturbed interactions could be sufficient for chromosome stability.

Transcription of a Telomere Reduces the Fraction of Cells in Which an Adjacent Gene Is Transcriptionally Repressed. Transcription across a telomere dramatically reduced the frequency of cells in which the affected telomere exerted TPE. One model for TPE is that telomeres initiate a specialized chromatin structure that spreads into adjacent regions and limits the access of transcription factors (28). In this model, transcription of the VII-L telomere might alleviate TPE by preventing the spread of the repressive chromatin structure from that telomere to the promoter of the adjacent *URA3* gene. In other models for TPE, associations between telomeres and a nuclear structure such as the nuclear envelope or nuclear matrix are correlated with repression (29, 30). In such a model of TPE, transcription of the VII-L telomere could alleviate repression of the adjacent *URA3* gene by promoting dissociation of the telomere from a nuclear structure such as the nuclear envelope.

Although the molecular basis of TPE is unknown, TPE can clearly be alleviated without compromising chromosome stability. In the disome UGPT strain on galactose medium, TPE at the VII-L telomere was eliminated in most cells, whereas the stability of the modified chromosome was normal (Figs. 5 and 6). The normal stability of a chromosome with a transcribed telomere was in striking contrast to the instability of a chromosome that had the function of a telomere completely eliminated by endonucleolytic removal (Fig. 5). Disruption of TPE by mutations in either the *SIR3* or *SIR4* gene is correlated with a very small decrease in chromosome stability (29). However, our results demonstrate that disruption of TPE at a single telomere had no effect on the stability of the affected chromosome. Thus, whether TPE is due to spreading of a repressive chromatin structure initiated from a telomere or to association of a telomere with a nuclear structure or to some other mechanism, that phenomenon can be separated from the chromosome stability function of telomeres.

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