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Position Effect at S. cerevisiae Telomeres: Reversible Repression of Pol II Transcription

Daniel E. Gottschling,* Oscar M. Aparicio,* Barbara L. Billington,* and Virginia A. Zakian†
* Department of Molecular Genetics and Cell Biology
The University of Chicago
Chicago, Illinois 60637
† Fred Hutchinson Cancer Research Center
1124 Columbia Street
Seattle, Washington 98104

Summary

S. cerevisiae chromosomes end with the telomeric repeat $(TG_{1-3})_n$. When any of four Pol II genes was placed immediately adjacent to the telomeric repeats, expression of the gene was reversibly repressed as demonstrated by phenotype and mRNA analyses. For example, cells bearing a telomere-linked copy of *ADE2* produced predominantly red colonies (a phenotype characteristic of *ade2*$^-$ cells) containing white sectors (characteristic of *ADE2*$^+$ cells). Repression was due to proximity to the telomere itself since an 81 bp tract of $(TG_{1-3})_n$ positioned downstream of *URA3* when *URA3* was ~20 kb from the end of chromosome VII did not alter expression of the gene. However, this internal tract of $(TG_{1-3})_n$ could spontaneously become telomeric, in which case expression of the *URA3* gene was repressed. These data demonstrate that yeast telomeres exert a position effect on the transcription of nearby genes, an effect that is under epigenetic control.

Introduction

Position effect is a term used to describe phenomena in which a gene's behavior is affected by its location on the chromosome (for a review see Lima-de-Faria, 1983b). The change in behavior can be manifested in a variety of ways, such as a difference in phenotype, transcription level, recombination frequency, or replication timing. Although position effects have been reported in insects, plants, and mice, most studies have been carried out in Drosophila, where euchromatic genes translocated near or within centromeric heterochromatin come under a position effect, typically exhibiting phenotypic repression (Spofford, 1976). More recently, in Saccharomyces cerevisiae the silent mating type loci, *HML* and *HMR*, have been shown to exert a position effect on the transcription of nearby genes (Brand et al., 1985; Mahoney and Broach, 1989; Schnell and Rine, 1986).

Telomeres, the ends of eukaryotic chromosomes, are believed to be essential for the stable maintenance and replication of linear chromosomes (reviewed in Zakian, 1989). Telomeric DNA in ciliates, humans, and probably other eukaryotes facilitates the complete replication of linear DNA molecules by serving as a substrate for telomerase (reviewed by Blackburn, 1990). Telomeres act as chromosome "caps"; in contrast to ends generated by chromosome breakage, telomeres are protected from exonucleolytic degradation and end-to-end fusions. Telomeres are also implicated in establishing nuclear organization by engaging in associations with other telomeres and with the nuclear envelope (Agard and Sedat, 1983; Lima-de-Faria, 1983a, and references within).

In S. cerevisiae, the simple DNA repeat (TG_{1-3}) is found at the ends of all linear chromosomes (Shampay et al., 1984; Walmsley et al., 1984). The repeated sequence is necessary and sufficient in *cis* to provide telomere function in vivo (Wellinger and Zakian, 1989): telomeric repeats are required at each end of a DNA molecule for it to be maintained in a linear form in yeast (Lundblad and Szostak, 1989; Pluta and Zakian, 1989). Examination of chromosomal ends reveals a heterogeneity in the number of (TG_{1-3}) repeats at individual telomeres both within and between strains, with an average of ~300 bp (Shampay and Blackburn, 1988; Walmsley and Petes, 1985). In addition to the (TG_{1-3}) repeats at the ends of chromosomes, most yeast telomeres bear middle repetitive elements called telomere-associated sequences (Chan and Tye, 1983a, 1983b). In S. cerevisiae there are two types of telomere-associated sequences: Y′ is a highly conserved sequence that exists in a long (~6.7 kb) and short (5.2 kb) form, whereas X is less well conserved and ranges in size from 0.3–3.8 kb. The sequences can occur in tandem arrays near the ends of the chromosome, where they are separated from one another by tracts of (TG_{1-3}) 50–130 bp in length (Walmsley et al., 1984). It is unclear whether the X and Y′ sequences serve a particular function, since they are absent from some telomeres (Jager and Philippsen, 1989; Zakian and Blanton, 1988); however, in humans and Drosophila telomere-associated sequences have been implicated in meiotic chromosome pairing and the establishment of heterochromatin (Ellis et al., 1989; Young et al., 1983).

To understand better the properties of telomeres, we began an investigation to map in vivo protein–DNA interactions at chromosomal termini in S. cerevisiae. We chose to examine a single telomere by introducing a unique marker adjacent to the tract of (TG_{1-3}) DNA at the end of a chromosome. However, early in the course of these investigations we realized that the transcription of the gene used to mark the telomere was altered. In this report we demonstrate that in S. cerevisiae, telomeres without an X or Y′ exert a position effect on the expression of genes located nearby. When *URA3*, *TRP1*, *HIS3*, or *ADE2* was located near a telomere, the gene's transcription was repressed. However, the expression of each gene was reversible between states of repressed and active transcription. Both transcriptional states were inherited mitotically in a semistable manner. Switching between the states appears to be under epigenetic control. At a locus ~20 kb from the telomere, transcription of *URA3* was not repressed, even when an 81 bp tract of (TG_{1-3}) sequence was located adjacent to the gene. However, the internal 81

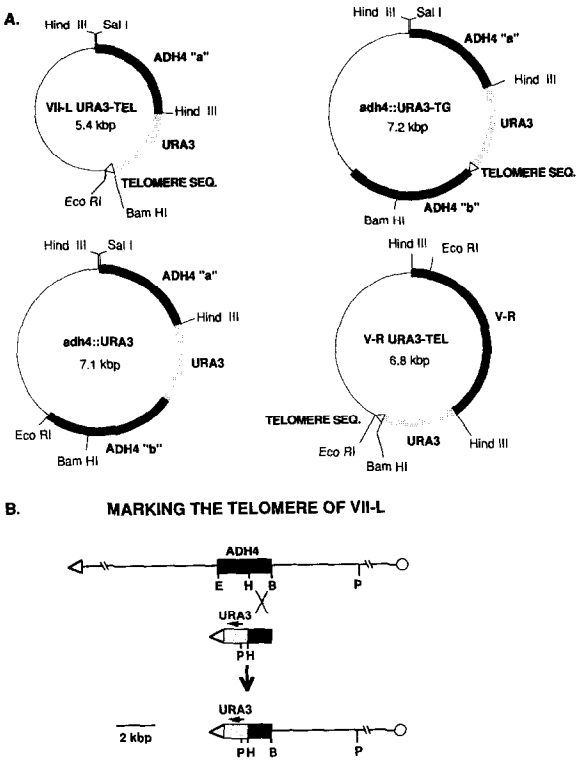

Figure 1. Marking the End of Chromosome VII-L with *URA3*

(A) Plasmids used to create S. cerevisiae strains shown in Figure 2 and described in the text. See Experimental Procedures for details on plasmid construction.

(B) A portion of the left arm of chromosome VII is presented. The *ADH4* locus (blackened area) is ~20 kb from the chromosomal terminus on VII-L (Walton et al., 1986). Strain DG20 was transformed with the SalI–EcoRI fragment of plasmid pVII-L URA3-TEL (see [A]). As a result, the *URA3* gene was placed immediately adjacent to a newly formed chromosomal end on the left arm of chromosome VII. The circle represents the centromere, and the open arrowhead represents the telomeric repeat sequence (TG_{1-3}). Arrows correspond to the transcription unit of *URA3* (stippled box). The restriction sites BglII (B), EcoRI (E), HindIII (H), and PstI (P) are indicated.

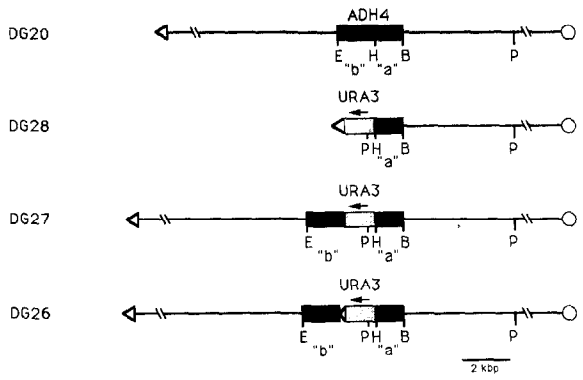

Figure 2. The Structure of Chromosome VII-L in Strains Used to Study Position Effects on *URA3* Expression

The construction of each strain is described in Experimental Procedures. For convenience of description, the *ADH4* locus is divided into two parts, the "a" fragment (BglII–HindIII) and "b" fragment (HindIII–EcoRI).

bp tract spontaneously became a telomere at a frequency of $\sim 10^{-6}$ and, in so doing, repressed the expression of the *URA3* gene. These results provide a molecular analysis of telomeric position effect, a phenomenon that may be generally characteristic of telomeres. In addition, the results provide a genetic method for analyzing telomere structure and formation of new telomeres from internal telomeric DNA sequences.

Results

Marking a Telomere with *URA3*

URA3, which is required for uracil biosynthesis, is normally found near the centromere on chromosome V. The entire gene, including the *cis* elements required for its normal regulation, is located on a 1.1 kb HindIII–SmaI fragment (Rose et al., 1984). This fragment was used in all of the *URA3* constructs described in this study. Experiments were carried out in haploid yeast strains that contained either of two nonreverting *ura3*$^-$ alleles: *ura3–52*, which contains a Ty transposon insertion within the *URA3* coding sequence (Rose and Winston, 1984) (UCC series), or *ura3*Δ*::LEU2*, in which the entire coding region of *URA3* on chromosome V has been replaced by the *LEU2* gene (DG series).

ADH4 is the most distal gene on the left arm of chromosome VII (Walton et al., 1986). Fragment-mediated transformation (Rothstein, 1983) was used to introduce *URA3* onto the left arm of chromosome VII, to create the haploid strain DG28 (Figures 1 and 2). In DG28, a portion of *ADH4* and the DNA distal to it are deleted and replaced with *URA3* and an 81 bp stretch of (TG_{1-3}). After transformation into yeast, the 81 bp stretch is extended to ~300 bp of (TG_{1-3}), a length typical of all other telomeres in this strain. Transcription of the *URA3* gene is toward the telomere, with its promoter ~1.3 kb from the end of the chromosome (see Figure 7).

Position Effect at Yeast Telomeres

The chemical 5-fluoro-orotic acid (5-FOA) can be used in the negative selection of *URA3* expression; 5-FOA is converted into a toxic substance by the *URA3* gene product (Boeke et al., 1987). The constitutive level of *URA3* expression in a cell is normally sufficient to yield cells sensitive to 5-FOA (5-FOA^S). Resistance to 5-FOA (5-FOA^R) can be used as a method to select for cells that have lost or mutated the wild-type *URA3* gene. Therefore, sensitivity to 5-FOA was used as a means to determine *URA3* expression as a function of chromosomal location.

The frequency of a spontaneous 5-FOA^R allele arising at the normal *URA3* locus is $\sim 10^{-7}$ (Table 1, 1GA2; Boeke et al., 1984). Since 5-FOA^R cells derived in this way have mutations in the *URA3* gene, they are Ura$^-$ (i.e., unable to grow in the absence of uracil). In contrast, when cells with *URA3* at the telomere (DG28; Figure 2) were pregrown in media containing uracil (no selection for *URA3* expression) and then plated for single colonies onto 5-FOA, 33% of the cells gave rise to 5-FOA^R colonies (DG28; Table 1

Table 1. Resistance of Cells to 5-FOA as a Function of the Chromosomal Location of *URA3*

Strain	Location of *URA3*	Fraction of FOA^R Colonies	
		Complete	– Uracil
1GA2	Wild-type locus (V)	1.5×10^{-7} (1.0–3.0)	$<2 \times 10^{-7}$
DG20	Deleted	1.0 (0.94–1.1)	N.A.
DG28	VII-L telomere	3.3×10^{-1} (2.0–6.0)	2.0×10^{-5} (0.5–4.3)
DG27	Within *ADH4* locus	$<6 \times 10^{-7}$	$<2 \times 10^{-7}$
DG26	Within *ADH4* locus and adjacent (TG_{1-3})	2.4×10^{-6} (0.8–4.5)	$<10^{-7}$
DG33	V-R telomere	3.7×10^{-2} (0.4–9.4)	$<2 \times 10^{-6}$

See Figure 2 for the structure of chromosome VII-L in strains DG20, DG28, DG27, and DG26.

Cells were pregrown into colonies on plates with complete medium (COMPLETE), or plates lacking uracil (– URACIL). Colonies were picked from each medium, and cells were plated for single colonies onto both 5-FOA and complete plates. The average fraction, based on at least five samples, of 5-FOA^R colonies for each strain is presented; the range of values for individual colonies is indicated in parentheses. DG33 is similar to DG28 except that the *URA3* gene is located adjacent to the telomere on the right arm of chromosome V. FOA^R colonies from strain DG28, DG26, or DG33 were tested for their ability to grow in the absence of uracil by replica plating. In all cases, 100% of the colonies were Ura$^+$.

and Figure 3B). Moreover, when these 5-FOA^R colonies were replica plated to media that lacked uracil, cells were able to grow. That is, the cells were still *URA3*$^+$. These results suggested that the 5-FOA^R exhibited by these cells was not due to an inordinately high mutation rate in or loss of the *URA3* gene, but rather that *URA3* expression at the telomere was reduced below the killing threshold of 5-FOA. Nonetheless, cells in a 5-FOA^R colony still had the ability to produce sufficient *URA3* gene product to overcome a lack of uracil in the medium.

When DG28 cells were pregrown in medium lacking uracil (selecting for *URA3* expression), one out of 10^5 cells produced a colony on plates containing 5-FOA (Table 1). Once again, each of these 5-FOA^R colonies was still *URA3*$^+$. Taken together, these results suggested that under nonselective growth conditions expression of the *URA3* gene in about one-third of the DG28 cells was sufficiently repressed to allow growth on 5-FOA, but that under selection, expression of the telomere-linked *URA3* gene is still possible in many or all of the cells. The resistance to 5-FOA of cells with *URA3* at the VII-L telomere has been observed in a number of strains. While there have been strain-specific differences in the fraction of 5-FOA^R cells when cells were pregrown under nonselective conditions, these values (0.10–0.90) were all within an order of magnitude of one another (UCC74 and UCC81, Table 3; D. E. G., O. M. A., and B. L. B., unpublished data) and indicate that repression of a telomere-linked *URA3* gene on VII-L is a general phenomenon.

This unexpected behavior of the *URA3* phenotype (colonies that were 5-FOA^R yet still Ura$^+$) caused us to examine the steady-state levels of *URA3* mRNA in cells with the gene either at its normal chromosomal position or at the telomere of VII-L. RNA was isolated from cells grown under either selective or nonselective conditions for *URA3* expression. Consistent with earlier studies, cells with *URA3* at its normal chromosomal locus had a modest in-

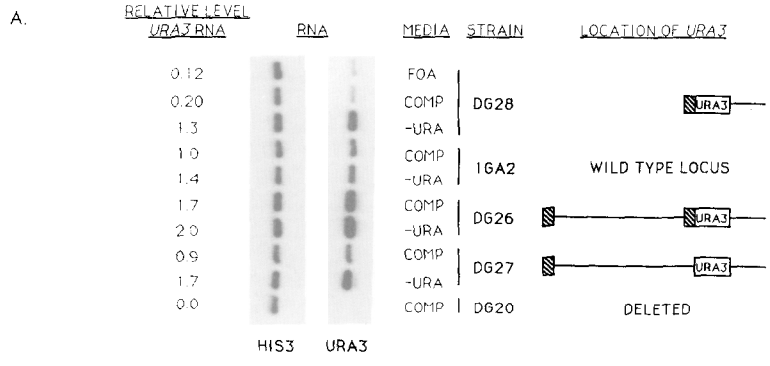

Figure 3. Constitutive Expression of the *URA3* Gene Is Repressed When It Is Located Adjacent to a Yeast Telomere

(A) Total RNA was isolated from cells at a mid-logarithmic stage of growth and subjected to Northern blot analysis. The blot was hybridized to a ^{32}P-labeled *URA3* RNA strand-specific probe. Following autoradiography, the *URA3* probe was removed, and the Northern blot was hybridized to a *HIS3*-specific RNA probe. Both autoradiograms are shown. The level of *URA3* RNA in each sample was standardized relative to the *HIS3* RNA level; the values given are an average of two measurements that were within 20% of each other. For each sample the relative *URA3* RNA level normalized to the "1GA2 COMP" sample is given in the diagram. Cells were grown in complete liquid medium (COMP) or liquid medium that lacked uracil (–URA). DG28 cells were also grown in medium containing 5-FOA (1 g/liter) and uracil (35 mg/liter) (FOA).

The location of *URA3* in the genome is denoted for each strain in a manner similar to that shown in Figure 2. The striped boxes represent the (TG_{1-3}) sequence. Strain 1GA2 is the parent of DG20 with *URA3* at its normal locus on chromosome V.

(B) Cells were streaked onto a plate with complete medium and grown for 2 days at 30°C. The cells were then replica plated to a 5-FOA plate. A photograph of the 5-FOA plate after 4 days of growth at 30°C is shown. The strain PT1 is unrelated to 1GA2 (see legend above), but it also has *URA3* at the wild-type locus. All other strains are described in Figure 2.

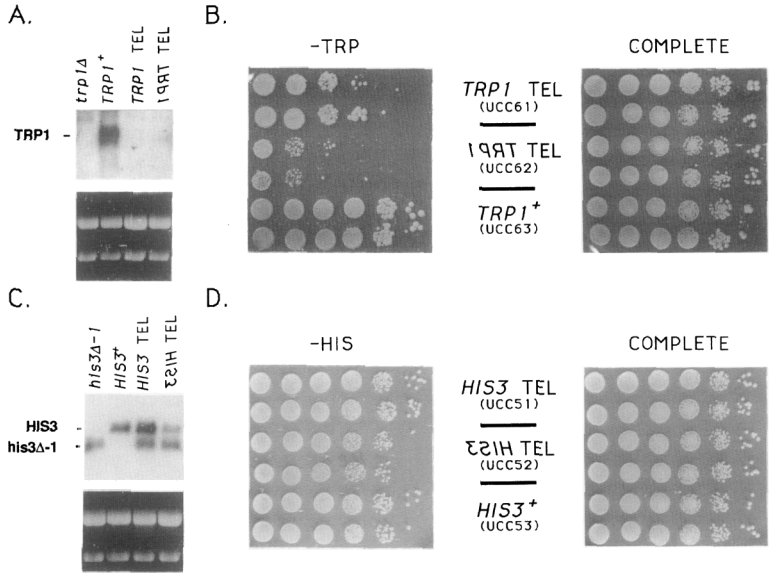

Figure 4. Telomeric Position Effect on the *TRP1* and *HIS3* Genes

(A) Northern blot analysis of total RNA with a *TRP1* probe from strains having *TRP1* at telomeric and nontelomeric loci. *trp1Δ* = the parent strain 4–1, which bears a deletion of the *TRP1* gene; *TRP1*$^+$ = strain UCC63, in which the *TRP1* gene fragment has been inserted into the *URA3* locus on chromosome V; *TRP1* TEL = strain UCC61, which has the *TRP1* gene located adjacent to telomere VII-L with transcription toward the telomere. *TRP1* (reverse) TEL = strain UCC62, in which the *TRP1* gene is located adjacent to telomere VII-L with transcription away from the telomere. Ethidium bromide staining of rRNA in the gel prior to transfer is shown below the autoradiogram.

(B) Viability of strains having *TRP1* at telomeric and nontelomeric loci when starved for tryptophan. Colonies grown 2–3 days on complete synthetic medium were resuspended in H_2O, and 10-fold serial dilutions were plated on complete synthetic medium or synthetic medium lacking tryptophan. Two independent colonies from each strain were plated. The strains are described in (A).

(C) Northern blot analysis of total RNA from strains having *HIS3* at telomeric and nontelomeric loci. Total RNA was isolated and hybridized with a *HIS3* probe. *his3Δ-1* = the parent strain 3482–16–2, which bears a small deletion in the *HIS3* gene; *HIS3*$^+$ = strain UCC53, which has the wild-type *HIS3* gene at its normal chromosomal locus; *HIS3* TEL = strain UCC51, which contains the *HIS3* gene located adjacent to telomere VII-L with transcription toward the telomere; *HIS3* (reverse) TEL = strain UCC52, which has the *HIS3* gene located adjacent to telomere VII-L with transcription away from the telomere.

(D) Viability of strains having *HIS3* at telomeric and nontelomeric loci when starved for histidine. Serial dilutions were as in (B), except cells were plated to synthetic medium lacking histidine.

crease in *URA3* mRNA levels (~1.4-fold) when grown under selective conditions compared with growth under nonselective conditions (Figure 3A; strain 1GA2) (Bach et al., 1979; Lacroute, 1968; Rose and Botstein, 1983). However, a major difference in *URA3* mRNA levels was observed in cells with the *URA3* gene at the telomere. RNA levels in DG28 cells grown under nonselective conditions were one-fifth that of cells with *URA3* at its normal locus (Figure 3A; strains DG28 and 1GA2, COMP). In contrast, under selective conditions, RNA levels in cells with *URA3* at the telomere were equivalent to the level in cells with *URA3* at its normal locus (Figure 3A; strains DG28 and 1GA2, −URA). Thus consistent with the 5-FOA^R phenotype, the constitutive level of *URA3* RNA is significantly reduced when the gene is located next to the telomere at VII-L compared with when it is at its normal chromosomal locus. Yet under selection, the level of *URA3* RNA at the telomeric locus in DG28 cells is virtually the same as when *URA3* is at its normal chromosomal locus.

To determine whether repression occurs at telomeres other than VII-L, strain DG33 was constructed. This strain has the *URA3* gene inserted near the telomere on the right arm of chromosome V (V-R), in a manner similar to that for *URA3* on VII-L in strain DG28 (Figure 2). Determination of the fraction of 5-FOA^R colonies (Table 1) and analysis of mRNA levels (data not shown) in strain DG33 indicate that constitutive expression of *URA3* is also repressed at this telomere. The difference in the fraction of 5-FOA^R cells between the two strains (0.33 for DG28, 0.04 for DG33; Table 1) presumably reflects differences between individual telomeres in terms of their specific chromosomal environments. *URA3* was also repressed when positioned near the telomere of a telocentric version of chromosome IV or of a 60 kb artificial linear chromosome (D. E. G., R. Wellinger, and V. A. Z., unpublished data). Thus the ability to repress the expression of a nearby *URA3* gene appears to be a general property of S. cerevisiae telomeres.

Repression by Proximity to a Telomere Occurs for Other Genes

In general, a region of the chromosome that exerts a position effect does so in a gene nonspecific manner. Therefore, we examined whether genes other than *URA3* were repressed by proximity to a telomere. The *TRP1*, *HIS3*, or *ADE2* gene was inserted between *URA3* and the telomere DNA sequence at the BamHI site of plasmid pVII-L URA3-TEL (Figure 1A). Each gene was inserted in both orientations. These constructs were then used to introduce *TRP1*, *HIS3*, or *ADE2* adjacent to the VII-L telomere, by selecting for *URA3* expression. In selecting only for *URA3* expression during strain construction, no selective pressure was placed upon the telomeric *TRP1*, *HIS3*, or *ADE2* gene.

In strains bearing a telomere-linked copy of *TRP1*, and grown under nonselective conditions, *TRP1* RNA was undetectable by Northern analysis, regardless of the gene's orientation at the telomere (Figure 4A, UCC61 and UCC62). By examining very long exposures of the autoradiograms in Figure 4A, we estimated that the RNA level from the telomeric *TRP1* was ≤1% of the RNA level

when the same *TRP1* fragment was located at an internal chromosomal site within the normal *URA3* locus on chromosome V (data not shown).

Colonies of cells with *TRP1* at the telomere or at an internal locus were grown on nonselective medium and then plated in serial dilution to medium that lacked tryptophan. All of the cells with *TRP1* at an internal site on the chromosome (Figure 4B, UCC63) formed colonies on plates lacking tryptophan. However, those with *TRP1* at the telomere showed a reduction in colony forming ability on plates lacking tryptophan (Figure 4B, UCC61 and UCC62). Only 10^{-2} cells with *TRP1* oriented such that transcription was directed toward the telomere formed colonies in the absence of tryptophan (Figure 4B, UCC61). When *TRP1* transcription was away from the telomere, $\sim 10^{-3}$ cells formed colonies (Figure 4B, UCC62). In addition, the UCC61 cells formed robust colonies in 3 days, while the UCC62 colonies were smaller (Figure 4B).

The telomeric *TRP1* RNA levels and plating efficiency data indicate that under nonselective growth conditions the majority of cells with *TRP1* near the telomere had very low or no *TRP1* expression. In all three *TRP1* constructs described, a portion of the UAS/promoter elements found at the normal *TRP1* locus was missing (Kim et al., 1986). While these missing elements have no apparent effect on the ability of cells to grow without tryptophan when *TRP1* is at an internal locus, their absence may explain why *TRP1* expression was more severely repressed at the telomere compared with expression of *URA3* at the telomere.

When *HIS3* was placed at the telomere and its transcription was directed away from the telomere, there was a detectable reduction in RNA levels compared with when the gene was at its normal chromosomal locus (Figure 4C, UCC52). When the direction of transcription at the telomere was reversed, there was a slight increase in RNA levels (Figure 4C, UCC51). Phenotypically, there was a modest (less than 10-fold) reduction in plating efficiency on media lacking histidine for UCC52, but no effect on UCC51, a result consistent with the relative RNA levels (Figure 4D).

Transcriptional Repression at Telomeres Is Reversible and Inherited in a Semistable Fashion

As shown above, when the *URA3* gene was telomere linked (DG28), cells from colonies that were 5-FOA^R could still grow when placed on medium lacking uracil. Conversely, cells grown in the absence of uracil were able to form colonies when placed on medium containing 5-FOA. These results suggested that a telomere-linked *URA3* gene could switch between repressed and active transcriptional states. The *ADE2* gene provides a convenient color assay for determining whether the gene is expressed; *ADE2*$^+$ colonies are white, whereas *ade2*$^-$ colonies are red (Roman, 1956). Thus, expression of a telomere-linked *ADE2* gene can be monitored by determining the color of colonies produced by cells carrying this marked telomere.

When the *ADE2* gene was placed at the telomere such that *ADE2* transcription was directed toward the telomere (UCC41), all colonies contained red and white sectors. This sectored phenotype indicated a switch between the repressed and active transcriptional states of *ADE2* during colony development (Figure 5). The colonies displayed a wide range of sectoring phenotypes. Some colonies were primarily white (active) and gave rise to red (repressed) sectors near the periphery. An equal number of colonies were primarily red with white sectors near the periphery. Intermediate levels of sectoring between these two extremes were also readily visible (Figures 5 and 6). In some colonies multiple switches between transcriptional states can be inferred. For example, in Figure 5 a predominantly red colony has a large white sector. Within this white portion, red sectors are clearly visible. The reversibility was further demonstrated by isolating cells within a white (or red) sector and plating them for single colonies. Each new colony contained red and white sectors (D. E. G. and O. M. A., unpublished data). In contrast with the results in UCC41, when *ADE2* transcription was directed away from the telomere (UCC42), no red sectors were observed in a colony (data not shown).

These results demonstrate that the expression of a telomere-linked *ADE2* gene can switch between an active and repressed state and that the expression state is semistable during mitotic growth. Based on the results with *URA3*, *TRP1*, and *HIS3*, we infer that the control of *ADE2* expression is at the transcriptional level. However, it was not possible to determine the level of RNA produced at the telomere-linked *ADE2*, because a transcript of identical size was made by the *ade2* allele at its normal chromosomal locus.

The probability of a telomere-linked *ADE2* gene (UCC41) being in an active (repressed) transcriptional state was estimated from the fraction of predominantly white (red) colonies. Five colonies of UCC41 cells grown on nonselective medium were plated for single colonies onto nonselective medium. Approximately equal numbers of colonies were found that had primarily red centers giving rise to white sectors, and primarily white centers that gave rise to red sectors (Figure 6A). This result indicates that a telomere-linked *ADE2* gene on VII-L has approximately equal probability of being in an active or repressed transcriptional state.

However, when five colonies of UCC41 were pregrown in the absence of adenine (selecting for *ADE2* expression) and then plated onto nonselective plates, there were up to nine times as many colonies with white centers than with red centers (Figure 6B). Closer examination of colonies with white centers revealed that red sectors generally did not appear until very close to the periphery of the colony. This observation suggested that the active expression state of *ADE2* was stable for many generations. The distance from the center of these colonies to the points at which multiple red sectors appeared was measured. This value was used to compute the fraction of the total colony volume (assuming a half-sphere geometry for a colony) that comprised the nonsectored center of the colony. The number of cells within this region of the colony was calculated (assuming there are $\sim 10^8$ cells in a colony), and this value was used to derive the number of cell divisions

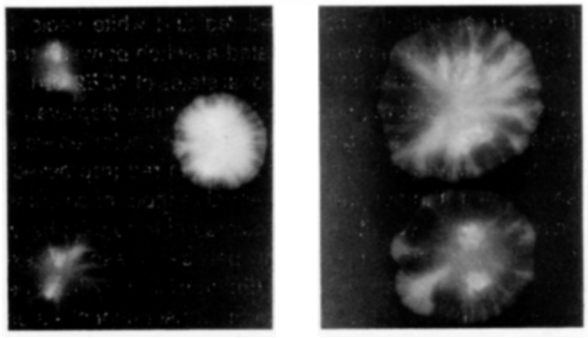

Figure 5. Telomeric Position Effect on *ADE2* Expression Results in Variegated Colonies

(A) Colonies of strain UCC41 grown on a nonselective medium that facilitates red color (dark in the photo) development in cells that are phenotypically *ade2*$^-$ (*ADE2*$^+$ cells are white). In UCC41 the *ADE2* gene is located near the left telomere of chromosome VII. The left panel shows extremes in colony phenotype; one colony in which the cells are primarily *ADE2*$^+$ (white) and give rise to *ade2*$^-$ sectors (dark), and two colonies that are primarily *ade2*$^-$ and yield *ADE2*$^+$ sectors. The panel on the right shows two heavily variegated colonies. Note, in the lower colony, the white sector in which red sectors appear near the periphery.

required to produce the quantity of white cells from a single progenitor. From these rough calculations we estimate that the active transcriptional state of *ADE2* is inherited for 15–20 generations in these colonies.

The phenotypic switching displayed by *ADE2* at a telomere was also observed with the *URA3* gene using a single-cell analysis. Freshly budded cells grown on medium containing 5-FOA were moved by micromanipulation

Table 2. The Ability of Individual DG28 Cells Grown on 5-FOA to Form Colonies

	Cell Transferred to 5-FOA Medium	Cell Transferred to Nonselective Medium
Full colony formed	81	57
Microcolony formed	9	0
No colony formed	29	33
Total cells	119	90

Strain DG28 cells were pregrown for 2 days on plates containing 5-FOA, then spread onto a fresh plate of 5-FOA. After one or two generations, newly budded cells were selected by microdissection and moved to medium either with or without 5-FOA. Microcolonies were composed of 4–8 cells.

to a region of the plate where they could develop into full colonies. The majority (81/119) of the cells formed colonies, but 8% (9/119) of the cells formed microcolonies consisting of 4–8 cells (Table 2). Microcolonies were not detected in a control experiment in which no 5-FOA was present in the medium (Table 2). Therefore the microcolonies presumably represent cells arrested in growth on the 5-FOA owing to the *URA3* gene being switched to an actively expressing state after budding. The cells that did not form colonies may have been progeny of cells that had switched to an actively expressing state prior to budding or were inviable as a result of the micromanipulation method. A rough value for the switching of *URA3* from a repressed to an active state in DG28 cells was calculated by dividing the number of cells that formed microcolonies (9) by the total number of colony forming cells (9 + 81), which yields an estimated switch rate of 10^{-1} per division.

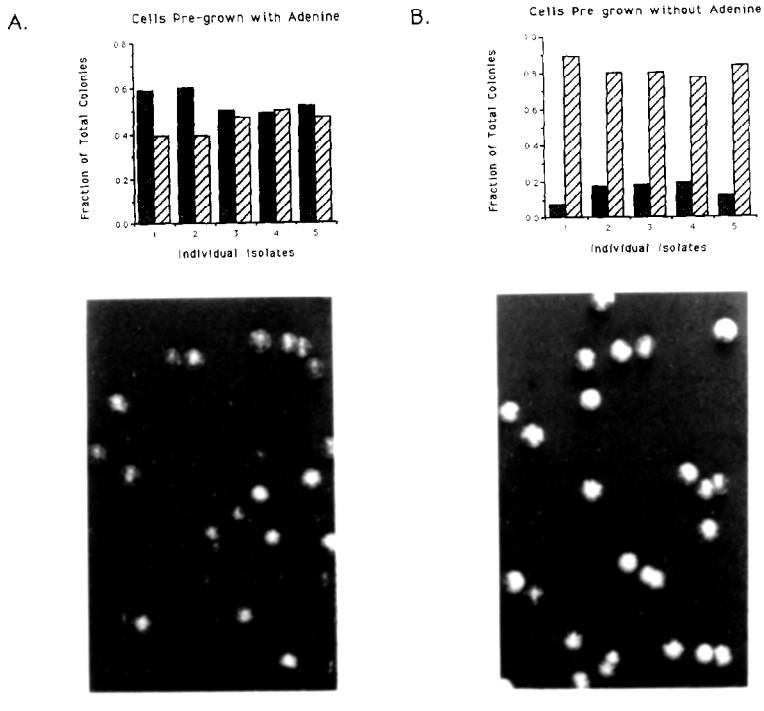

Figure 6. Telomeric *ADE2* Expression Is Inherited in a Stable Manner

(A) Five colonies of UCC41 were picked from nonselective medium after 2 days of growth and plated at a density of 50–200 cells per plate onto nonselective medium that allowed color development. The number of colonies that had primarily red centers and the number with white centers were determined. The percentage of each class is presented in the bar graph. The dark bars represent colonies with primarily red centers; the light bars represent the colonies that were primarily white. (Very highly sectored colonies, which represented 2%–5% of the total colonies counted, were not classified into either the red or white group and were not included in the graph.) At least 500 colonies were counted for each of the five isolates. A portion of a plate from isolate number 1 is shown below the graph.

(B) As in (A), except the five colonies were picked from medium that lacked adenine. A portion of a plate from isolate number 1 is shown below the graph.

Table 3. The Effect of DNA Sequences Inserted between *URA3* and the VII-L Telomere

Strain	VII-L Structure	Insert Size (kb)	Fraction FOA^R
UCC61	*URA3-TRP1*-TEL	0.85	0.021 (± 0.01)
UCC62	*URA3-1PRT*-TEL		0.14 (± 0.09)
UCC51	*URA3-HIS3*-TEL	1.8	3.0 (± 1.7) × 10^{-4}
UCC52	*URA3-3SIH*-TEL		0.26 (± 0.17)
UCC41	*URA3-ADE2*-TEL	3.6	3.5 (± 3.0) × 10^{-5}
UCC42	*URA3-2EDA*-TEL		$\leq 10^{-6}$
UCC74	*URA3*-TEL	0	0.80 (± 0.12)
UCC81	*URA3*-TEL		0.23 (± 0.10)

DNA fragments containing the *ADE2*, *HIS3*, or *TRP1* gene were inserted between the *URA3* gene and the telomere on VII-L. The fraction of 5-FOA^R colonies produced from cells pregrown as colonies under nonselective conditions is presented for each strain. The values are the average (and standard deviation) from at least five individual colonies. The order of the genes adjacent to the VII-L telomere is indicated. Transcription of a gene is toward the telomere unless the gene's name is spelled backward. Strains UCC41, 42, 61, 62, and 81 are derived from the same parent. Strains UCC 51, 52, and 74 are derived from a second parent.

The Distance over Which Telomeres Exert a Position Effect on *URA3* Expression

To obtain an estimate of the distance over which the telomere exerted a position effect, the *URA3* gene was placed ~20 kb from the end of the left arm of chromosome VII by insertion within the *ADH4* locus (DG27; Figure 2). Based on both RNA analysis and the frequency of 5-FOA^R colony formation, cells with *URA3* inserted within *ADH4* had levels of *URA3* expression comparable to cells with *URA3* at its normal locus, under either selective or nonselective growth conditions (Table 1 and Figure 3). Thus on the left arm of chromosome VII the telomeric repression was no longer detectable when *URA3* was ~20 kb from the telomere.

To determine whether repression occurred over distances less than 20 kb from the telomere, the constructs described above, in which *TRP1*, *HIS3*, or *ADE2* was inserted between *URA3* and the telomere DNA sequence, were analyzed for *URA3* expression. The inserted genes increased the distance between *URA3* and the telomere by 0.85, 1.8, or 3.6 kb, respectively.

Cells with each of the constructs were pregrown in complete synthetic medium; thus no selection for the expression of *URA3* or the inserted gene was introduced. The cells were then plated to medium containing 5-FOA, and the fraction of 5-FOA^R colonies was determined. The analysis revealed that as the distance between *URA3* and the telomere was increased, the level of repression decreased (Table 3). For instance, insertion of the 0.85 kb *TRP1* fragment yielded 5-FOA^R colonies at a frequency of 0.02–0.14 (UCC61 and UCC62), while insertion of the 3.6 kb *ADE2* fragment yielded $\leq 10^{-5}$ 5-FOA^R cells (UCC41 and UCC42). However, the level of *URA3* expression was also influenced by the orientation of the inserted DNA fragment. This conclusion was best demonstrated by the result with the *HIS3* fragment: when transcription of the *HIS3* gene was toward the telomere (UCC51), ~10^{-4} cells were 5-FOA^R; when *HIS3* transcription was away from the telomere (UCC52), 0.26 of the cells were 5-FOA^R. The orientation of *TRP1* and *ADE2* had smaller, but detectable, effects on *URA3* expression. Thus further studies on the level of *URA3* expression as a function of distance from the telomere must take into account both the composition and orientation of the DNA sequences located between the telomere and *URA3*.

Internal Tracts of (TG_{1-3}) Do Not Cause Repression, but They Can Become Chromosome Ends and Consequently Cause Position Effect

Internal tracts of (TG_{1-3}) sequence occur naturally between the telomere-associated elements X and Y′ and between tandem Y′ elements (Chan and Tye, 1983a, 1983b). Internal (TG_{1-3}) tracts range from 50–130 bp (Walmsley et al., 1984). To determine whether these internal (TG_{1-3}) sequences might also exert a position effect, 81 bp of (TG_{1-3}) was introduced adjacent to the telomeric side of *URA3*, within the *ADH4* locus (DG26; Figure 2). RNA levels in these cells were somewhat higher than in cells with *URA3* at its normal locus or at the *ADH4* locus without (TG_{1-3}) (Figure 3A; DG26, DG27, and 1GA2). This elevated transcription was observed for both constitutive and induced *URA3* gene expression (Figure 3A; DG26 and 1GA2). These elevated mRNA levels are probably explained by an enhancer-like activity associated with (TG_{1-3}) repeat sequences when they are adjacent to a gene in a nontelomeric location (Runge and Zakian, 1990). Whatever the mechanism responsible for elevated expression, the internal tract of 81 bp of (TG_{1-3}) at the *ADH4* locus clearly does not cause repression of constitutive expression. These data demonstrate that (TG_{1-3}) sequences are not sufficient to cause position effect: the *URA3* gene must be positioned near a telomere (or alternatively, near a (TG_{1-3}) tract longer than 81 bp) for transcription to be repressed.

Consistent with the high level of *URA3* expression seen in the RNA analysis, the fraction of 5-FOA^R colonies from cells with *URA3* next to the internal tract of (TG_{1-3}) and grown in uracil was ~10^{-6} (Table 1, DG26 COMP). Although this value was low relative to cells with a telomere-linked copy of *URA3*, it is an order of magnitude greater than the fraction of 5-FOA^R colonies in cells with *URA3* at its normal locus (Table 1 and Figure 3B, strains 1GA2 and DG26). Replica plating of the 5-FOA^R colonies derived from DG26 cells revealed that they were all still Ura$^+$(in contrast to 5-FOA^R colonies arising from cells with *URA3* at its normal locus, which were typically Ura$^-$). This phenotype is identical to that seen for the cells with *URA3* at the telomere (i.e., DG28; Table 1), suggesting that the internal (TG_{1-3}) sequences might have become telomeric in those cells able to form colonies on 5-FOA.

This hypothesis was confirmed by Southern analysis. In four out of four independent isolates in which DG26 cells gave rise to 5-FOA^R colonies, the *URA3* sequences were on a restriction fragment of the size expected for a telomeric location (Figure 7a, lanes A–D). In addition, Southern hybridization demonstrated that sequences immedi-

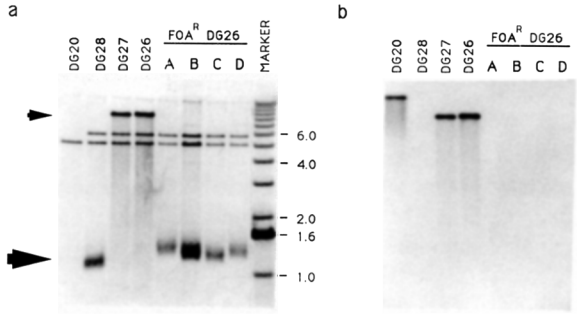

Figure 7. Internal Tracts of (TG_{1-3}) Repeats Can Become Telomeric

Total yeast DNA was isolated from cells of DG20, DG26, DG28, and four independent isolates of FOA^R colonies that arose spontaneously from DG26 (lanes A–D). The DNA was cleaved with PstI, and subjected to gel electrophoresis and Southern blot analysis.

(a) The 1.1 kb HindIII fragment of *URA3* was used as a probe; the resulting autoradiogram is presented. The small arrow points to the band that contains the *URA3* gene at the internal *ADH4* locus (see DG26; Figure 2). The large arrow points to the heterogeneous band indicative of the *URA3* gene adjacent to the telomere (see DG28; Figure 2). The size (in kb) of some of the molecular weight markers (1 kb ladder from BRL) is displayed on the right. The 5 kb band present in DG20 and all other DNA samples is due to hybridization of the probe to the noncoding sequences of the *URA3* gene still present at its normal chromosomal locus. The band at 5.8 kb is hybridization to a fragment containing 200 bp of *URA3* sequence and sequences centromere proximal to it at the *ADH4* locus.

(b) The probe used in (a) was removed from the Southern blot, and the blot was reprobed with the distal fragment of the *ADH4* locus ("b" in Figure 2). Note that DNA from DG28 or any of the 5-FOA^R DG26 cells does not hybridize to this probe.

ately distal to the internal (TG_{1-3}) tract were no longer detectable in the 5-FOA^R cells (Figure 7b, lanes A–D). These results show that internal tracts of (TG_{1-3}) sequence can spontaneously become chromosomal ends by a mechanism that results in the deletion of sequences distal to the internal (TG_{1-3}) tract. In addition, the results provide independent evidence that the repressed expression of *URA3* at the telomere is neither an artifact of transformation nor a mutation within the *URA3* gene or one of its *trans*-activating factors.

Discussion

Position Effect at Yeast Telomeres

A position effect was demonstrated at the telomeres of S. cerevisiae chromosomes. The effect resulted in reduced gene expression of telomere-linked genes as assayed both by amount of stable mRNA and by phenotype. For instance, cells with a telomere-linked *URA3* gene were able to grow in the presence of 5-FOA, behavior consistent with a *ura3*$^-$ phenotype. When *ADE2* was telomere linked, many cells produced predominantly red colonies, as is characteristic of *ade2*$^-$ cells. The position effect altered the expression of four out of four Pol II genes: *ADE2, HIS3, TRP1,* and *URA3*. In addition, the effect was observed at four out of four telomeres, including an artificial linear chromosome. Therefore we conclude that the position effect is a general phenomenon of S. cerevisiae telomeres.

The position effect acted upon the *URA3* promoter at distances of at least ~4.9 kb from the telomere, but at ~20 kb from the left end of chromosome VII the effect was not observed. In addition, the influence of distance on position effect strongly depended upon the specific DNA sequences located between *URA3* and the telomere and probably other factors that are not yet well understood. For example, the transcriptional activity of *ADE2*, *HIS3*, and *URA3* was dependent upon the gene's orientation with respect to the telomere (see Results and D. E. G., unpublished data). In the process of generating artificially fragmented linear chromosomes, Hegemann et al. (1988) report a "leaky" 5-FOA^R phenotype for a *URA3* gene located 6–8 kb from the telomere. In these constructs, most of the 6–8 kb was the subtelomeric middle repetitive element Y′. We postulate that the reported "leaky" phenotype is due to a telomeric position effect and suggest that telomeric repression can act at a distance of at least 6 kb and through a Y′ element.

The telomeric position effect appears to be a result of proximity to the end of the chromosome and is not simply due to the telomeric DNA sequence (TG_{1-3}). Eighty-one base pairs of (TG_{1-3}) sequence ~20 kb from the telomere did not repress *URA3* expression. While longer lengths of (TG_{1-3}) were not tested at internal loci, one of several strains that were tested for telomeric position effect contained the *tel1* mutation (D. E. G., unpublished data). In the *tel1* strain, the telomere adjacent to *URA3* had a (TG_{1-3}) tract of 95–120 bp, yet the level of 5-FOA^R in this strain was similar to that for all other strains tested. Taken together, these results argue that the telomere itself, not simply (TG_{1-3}) repeats, is responsible for telomeric position effect in S. cerevisiae.

The repressed state conferred by the telomere was mitotically inherited over a number of generations, but the genes could escape from repression and switch to a state of active transcription. This reversibility was visually demonstrated by the red and white sectored colonies of cells with *ADE2* near the telomere (UCC41) and was also supported by the single-cell analysis of DG28 cells on 5-FOA. The transcriptional state of a gene, whether repressed or active, appeared to be stable over many generations.

The switching between active and repressed transcriptional states for genes at telomeres is not due to genetic alteration, but rather to an epigenetic switch. Several lines of evidence support this interpretation: First, the repression was readily reversible, in the presence or absence of selection. Second, there were no differences in DNA structure or copy number of the telomeric genes, as judged by Southern analysis, regardless of whether these haploid cells were grown under conditions that were nonselective or that selected for expression or repression of the genes (D. E. G. and B. L. B., unpublished data). Third, the telomeric position effect was gene nonspecific.

Epigenetic variation of gene expression has been observed in plants, insects, mammals, and S. cerevisiae. In Drosophila, position-effect variegation is observed when a euchromatic gene is moved within or near a heterochromatic region of the chromosome (Eissenberg, 1989; Spofford, 1976). Heterochromatin is a portion of the chro-

mosome that remains visibly condensed throughout interphase of the cell cycle. In contrast, euchromatin decondenses after telophase and appears diffuse during interphase. When the *white* gene is located near some types of heterochromatin, a salt-and-pepper mosaicism in eye color is observed (Spofford, 1976). This mosaicism is visually analogous to the sectored colonies produced by cells with *ADE2* at the telomere (Figure 5), and it is tempting to infer that similar mechanisms are at work in the two organisms. In S. cerevisiae, epigenetic switching has been reported at the silent mating type locus *HML* (Pillus and Rine, 1989). In a wild-type cell *HMLα* is not expressed. However, in a *sir1* strain *HMLα* switches between repressed and expressed states. Current models for the *HML* switch favor a change in chromatin conformation between the two phenotypic states. Besides changes in chromatin structure, postulated mechanisms of epigenetic variation in plants and mammals include changes in DNA methylation, topology, and nuclear locale (Fedoroff et al., 1989; Holliday, 1987; Monk, 1990; Weintraub, 1985).

Cytological observations in plants, insects, and mammals indicate that telomeres occupy specific regions within the nucleus and are frequently associated with the nuclear envelope (Lima-de-Faria, 1983a; White, 1973). In addition, telomeres are usually packaged as heterochromatin (Fussell, 1975; Traverse and Pardue, 1989). In the single-celled eukaryotes Oxytricha, Dictyostelium, and Tetrahymena, the DNA adjacent to the chromosome termini is packaged in an orderly array of phased nucleosomes, which is consistent with the presence of a highly ordered chromatin structure (Budarf and Blackburn, 1986; Edwards and Firtel, 1984; Gottschling and Cech, 1984). In Drosophila, P element-mediated transposition of the *white* gene near the 3R telomere results in mosaic expression of the gene, indicative of a position effect caused by proximity to the heterochromatin observed at this telomere (Hazelrigg et al., 1984; James et al., 1989; Levis et al., 1985). We note that S. cerevisiae telomeres have two of the classic features of heterochromatin: telomeres replicate late in S phase (McCarroll and Fangman, 1988) and, as shown here, they exert position effect on the expression of nearby genes. We propose that the phenotypic switching of telomere-linked genes in yeast is the result of a competition between the formation of a stable active transcriptional complex and the normal telomeric chromatin structure that prevents gene expression. Such a chromatin structure must originate from the end of the chromosome. In Oxytricha the molecular ends of macronuclear minichromosomes are recognized by a heterodimeric protein complex (Gottschling and Zakian, 1986; Price and Cech, 1989). Similar proteins in yeast may form a telomeric structure that is important in establishing the position effect.

The semistable, reversible repression (or expression) at yeast telomeres may be analogous to a primitive developmental switch. When cells with a telomere-linked copy of *ADE2* were pregrown under selection for *ADE2* expression, most (~80%) subsequently gave rise to colonies of primarily white (transcriptionally active) cells under nonselective growth conditions (Figure 6B). The active transcriptional state of *ADE2* can be inherited for at least 15–20 generations after removal of selection. This primitive control mechanism for gene expression may be exploited by some organisms to allow developmentally controlled expression of telomere-linked genes. In Trypanosomes, telomeres are the exclusive genomic expression sites for surface antigen genes (reviewed in Pays and Steinert, 1988). Many telomeres within a cell can carry transcriptionally competent genes, yet only one such gene is expressed at a time. Perhaps the other telomere-linked genes are kept repressed, albeit reversibly, by telomeric position effect.

New Telomere Formation

We found that internal tracts of (TG_{1-3}) sequence can spontaneously become chromosomal ends. Since the DNA distal to the (TG_{1-3}) tract is deleted, it seems unlikely that telomere formation occurred by reciprocal recombination between the internal (TG_{1-3}) sequence and another telomere (Figure 7b). New telomere formation may have occurred through intrachromosomal recombination between the internal (TG_{1-3}) sequence and the telomere, with a resulting deletion of intervening sequences (as has been postulated for deletion of the subtelomeric repeat Y′ [Horowitz and Haber, 1985]), by unequal sister chromatid exchange or conversion, or by a distal chromosome break followed by telomere "healing" at the (TG_{1-3}) sequence.

New telomere formation in conjunction with deletion of all terminal sequences has been observed cytologically and has been an area of intense interest because of its implications for chromosome breakage at fragile sites and for the generation of chromosomal abnormalities in cancer cells (Le Beau, 1988; Sutherland and Hecht, 1985). Recently it has been postulated that a subclass of such sites might in fact be regions of the chromosome that contain internal stretches of telomeric DNA sequences (Hastie and Allshire, 1989). In this study we find that internal tracts of telomeric DNA do indeed spontaneously become chromosomal termini, albeit at a low frequency ($\sim 10^{-6}$). A molecular analysis of this chromosomal rearrangement and telomeric position effect will aid our understanding of the dynamic relationship between chromosome organization and cell function.

Experimental Procedures

Construction of Plasmids

Plasmid pVII-L URA3-TEL was constructed in two steps, beginning with the plasmid pYTCA-2. Plasmid pYTCA-2 has the 125 bp HaeIII–MnlI fragment from pYt103, which contains 81 bp of (TG_{1-3}) sequence derived from a yeast telomere in the SmaI site of pUC9 (Runge and Zakian, 1989; Shampay et al., 1984). The (TG_{1-3}) sequence is oriented such that digestion of pYTCA-2 with EcoRI will yield an end that is a substrate for telomere formation in yeast. Plasmid pYTCA-2 was digested with HindIII and HincII, and a 1.1 kb HindIII–SmaI DNA fragment that contains the *URA3* gene was ligated between these sites (Rose et al., 1984) to form pURA3-TEL. Plasmid pYA4–2, a gift from V. Williamson, contains the *ADH4* gene on an EcoRI–BglII fragment inserted within the EcoRI–BamHI sites of pUC8 (Walton et al., 1986; Williamson and Paquin, 1987). Plasmid pURA3-TEL was digested with HindIII, and the 1.2 kb HindIII fragment of pYA4–2 ("a" in Figures 1A and 1C) was ligated within, such that the SalI site of the inserted fragment was positioned away from the *URA3* gene. This results in plasmid pVII-L URA3-TEL (Figure 1A).

Plasmid padh4::URA3-TEL was also constructed in two steps. First, pVII-L URA3-TEL was digested with BamHI, the DNA ends were made blunt by treatment with T4 DNA polymerase and dNTPs, and the plasmid was recircularized. Next, this new plasmid, pVII-L URA3-TEL (-BamHI), was digested with EcoRI, the ends were made blunt as before, and ligated to the blunt-ended 1.8 kb HindIII–EcoRI fragment of YA4–2. Plasmids with the BamHI site furthest from the (TG_{1-3}) sequence have the correct orientation of the insert (see Figure 1A).

Plasmid padh4::URA3 was constructed by digesting pVII-L URA3-TEL with BamHI, making the ends blunt, then treating the plasmid with EcoRI; the 1.8 kb HindIII–EcoRI fragment of YA4–2, with only its HindIII end made blunt, was ligated into the plasmid.

Plasmid pV-R URA3-TEL was made by digesting pVII-L URA3-TEL with HindIII and replacing the *ADH4*-derived sequence with the 2.8 kb HindIII fragment of plasmid pB6–10H, such that the EcoRI site of the insert was furthest from the *URA3* gene (see Figure 1A). Plasmid pB6–10H, a gift from C. Newlon, contains ~19 kb of unique DNA sequence from the region adjacent to the subtelomeric Y′ repeat on the right arm of chromosome V (Chan and Tye, 1983b; McCarroll and Fangman, 1988). The 2.8 kb HindIII fragment from B6–10H used in this study is unique sequence ~5.5 kb from the Y′ repeat.

Plasmid pULA was constructed in a two step process. First, the 1.1 kb HindIII *URA3* fragment was inserted into the HindIII site of a pUC9 derivative in which the PstI site has been deleted. The resulting plasmid was digested with PstI and NsiI; the coding sequence of *URA3* was removed and replaced with a 4 kb PstI fragment containing *LEU2* isolated from YEP13 (Broach et al., 1979).

Plasmids pADHIS3(+), pADHIS3(−), pADADE2(+), pADADE2(−), pADTRP1(+), and pADTRP1(−) were all constructed by inserting the wild-type *HIS3*, *ADE2*, or *TRP1* gene in either orientation, into the BamHI site in the vector pVII-L URA3-TEL. For *HIS3*, a 1.85 kb BamHI fragment from plasmid pHIS3 (Struhl, 1985; a gift from K. Runge) was inserted into the BamHI site of pVII-L URA3-TEL. Two plasmids are formed: pADHIS3(+), in which the *HIS3* gene is in the same transcriptional orientation as the *URA3* gene, and pADHIS3(−), which has the *HIS3* gene in the opposite orientation.

For *ADE2*, a 3.6 kb BamHI fragment in plasmid pL909 (a gift from R. Keil), was inserted into the BamHI site of the vector VII-L URA3-TEL. The resulting plasmids were designated pADADE2(+), indicating *ADE2* transcription in the same direction as the adjacent *URA3* gene, or pADADE2(−) for *ADE2* transcription in the opposite direction.

For *TRP1*, a 0.85 kb EcoRI–BglII fragment from plasmid pYRp7 (Struhl et al., 1979) was blunt ended with T4 DNA polymerase and inserted into the BamHI site in pVII-L URA3-TEL, which also had the BamHI ends filled in with T4 DNA polymerase. The plasmid with the *TRP1* gene in the same transcriptional orientation as *URA3* was denoted pADTRP1(+), while the plasmid in which *TRP1* transcription was in the opposite direction as *URA3* transcription was pADTRP1(−).

Plasmid pTRP1/RS306 was made by inserting the EcoRI–BglII fragment of *TRP1* into the EcoRI–BamHI site of pRS306 (Sikorski and Hieter, 1989).

E. coli strain MC1066 (*r⁻ m⁻ trpC9830 leub600 pyrF::Tn5 lacΔX74 strA galU galK*) was used as a host for all plasmids (Casadaban et al., 1983). LB medium with ampicillin (100 μg/ml) and M9 medium supplemented with the appropriate amino acids were prepared as described by Maniatis et al. (1982). Complementation of MC1066 mutations by the homologous yeast genes was used when applicable.

Yeast Strains and Methods

Media used for the growth of S. cerevisiae were based on synthetic complete media as described by Sherman et al. (1986) to which uracil (35 mg/liter), tyrosine and lysine (60 mg/liter), and leucine and isoleucine (80 mg/liter) had been added. One gram of 5-FOA per liter of medium was added to determine resistance to 5-FOA. Medium for *ADE2* red/white sectored colony growth was as described (Klapholz and Esposito, 1982) except arginine was 50 mg/liter and threonine was 100 mg/liter. Colonies were grown for 3 days at 30°C, then incubated for 1–2 weeks at 4°C for full color development. S. cerevisiae transformation was performed using the lithium acetate procedure (Ito et al., 1983).

To delete the *URA3* gene, strain 1GA2 (*MATα ade2 ade5 leu2-3,112 lys5 cyh2ʳ can1ʳ*; made in this study) was transformed with HindIII-digested pULA (see above), and Leu⁺ colonies were isolated. The structure of the chromosome from which *URA3* was deleted was checked by Southern analysis in Leu⁺ isolates, which also tested Ura⁻. DG20 is the *ura3Δ::LEU2* derivative of 1GA2. Strains DG26, DG27, DG28, and DG30 were constructed by transforming DG20 with different DNA fragments: DG26 with plasmid padh4::URA3-TEL cleaved by BamHI and SalI, DG27 with plasmid padh4::URA3 cleaved by BamHI and SalI, DG28 with plasmid pVII-L URA3-TEL cleaved by SalI and EcoRI, and DG30 with plasmid pV-R URA3-TEL cleaved by EcoRI. All transformants were selected as being both Ura⁺ and Leu⁺. The expected structure for each transformant (Figure 2) was verified by Southern analysis. In each case, total genomic DNA was cleaved twice, once by BglII and once by PstI. The Southern blots of DG26, DG27, and DG28 were hybridized with a series of DNA probes that included: the 1.1 kb HindIII *URA3* gene, the proximal *ADH4* probe labeled "a" in Figure 2, and the distal *ADH4* probe labeled "b" in Figure 2. The structure of DG33 was verified in a similar manner using probes from plasmid pB6–10H.

Strains UCC41, UCC42, UCC45, UCC61, UCC62, UCC63, UCC81, UCC82, and UCC83 were derived from strain 4–1 (*MATα lys2 his4 trp1Δ ade2 leu2–3,112 ura3–52*; made in this study) by transforming strain 4–1 with different DNA fragments and selecting for Ura⁺ colonies: UCC41 with pADADE2(+) cut with SalI and NotI, UCC42 with pADADE2(-) cut with SalI and NotI, UCC45 with pL909 cut with BamHI, UCC61 by pADTRP1(+) cut with SalI and EcoRI, UCC62 with pADTRP1(−) cut with SalI and EcoRI, UCC81 with pVII-L URA3-TEL cut with SalI and EcoRI, UCC82 with padh4::URA3 cut with BamHI and SalI, UCC83 with pUCU (contains the 1.1 kb HindIII fragment containing the *URA3* gene in pUC9) cut with HindIII, and UCC63 with pTRP1/RS306 digested with NdeI.

Strains UCC51, UCC52, UCC53, UCC74, UCC75, and UCC76 were derived from strain 3482–16–2 (*MATa met2 his3Δ-1 leu2–3,112 trp1–289 ura3–52*; a gift from L. Hartwell) by transforming strain 3482–16–2 with different DNA fragments, again selecting for Ura⁺ colonies: UCC51 by pADHIS3(+) cut with SalI and EcoRI, UCC52 with pADHIS3(−) cut with SalI and EcoRI, UCC53 with pHIS3 cut with BamHI, UCC74 with pVII-L URA3-TEL cut with SalI and EcoRI, UCC75 with padh4::URA3 cut with BamHI and SalI, and UCC76 with pUCU cut with HindIII. The expected chromosome structure of each transformant was verified by Southern analysis.

Selection for 5-FOA^R Colonies

Cells were grown into colonies for 2–3 days at 30°C on YC plates or plates that lacked uracil. Colonies were picked and resuspended in 1.0 ml of H_2O, serial dilutions were made, and an appropriate amount of cell suspension was spread to produce ~200 colonies per plate. Cells were spread onto 5-FOA plates for selection, and YEPD or synthetic complete medium plates to determine the total number of colony-forming cells. The number of colonies on a plate was determined after 3–4 days of growth at 30°C.

Analysis of Nucleic Acids from S. cerevisiae

S. cerevisiae cells were grown in 5 ml of YEPD to stationary phase, and total genomic DNA was isolated by disrupting cells with glass beads as described (Runge and Zakian, 1989). Methods for cleavage of total genomic DNA with restriction enzymes, gel electrophoresis, and Southern hybridizations have been previously described (Gottschling and Cech, 1984; Runge and Zakian, 1989). For rehybridization experiments, probes were removed from blots with boiling water.

Cells were grown to a density of 0.5–2 × 10^7/ml, and total RNA was isolated as described (Sherman et al., 1986), except that the nucleic acids were precipitated with 2 vol of ethanol and resuspended in water at a concentration of 1–10 mg/ml. RNA concentration was determined by UV spectroscopy. Ten or 20 μg of total RNA was separated by electrophoresis on a 1.5% agarose–formaldehyde–MOPS gel and transferred to nitrocellulose or nylon membrane as described (Ogden and Adams, 1987; Wahl et al., 1987). Strand-specific RNA probes were made by in vitro transcription of linearized plasmids with T7 polymerase in the presence of [α-^{32}P]CTP (~600 Ci/mmol) (Wahl et al., 1987). Plasmids used for transcription were derivatives of pVZ1 (Henikoff and Eghtedarzadeh, 1987); the *URA3* probe contained the PstI–NcoI fragment of the gene, the *HIS3* probe contained the BamHI–KpnI fragment of the gene, and the *TRP1* probe contained the HindIII–BglII fragment of the gene. Northern hybridization was performed as described (Wahl

et al., 1987). Multiple exposures of autoradiograms were scanned with an LKB Ultroscan XL densitometer to determine the relative levels of *URA3* or *HIS3* mRNA. The values reported in Figure 3 were based on densitometric readings in the linear range of the film.

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