RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae*

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To investigate the role of the yeast telomere-, silencing-, and UAS-binding protein RAP1 in telomere position effects, we have characterized two sets of mutant cells: (1) a set of *rap1* alleles (termed the *rap1* alleles) that produce truncated RAP1 proteins missing the carboxy-terminal 144–165 amino acids; and (2) null mutants of the *RIF1* gene, encoding a protein capable of interaction with the carboxyl terminus of RAP1. The data presented here indicate that loss of the carboxyl terminus of RAP1 abolishes position effects at yeast telomeres and diminishes silencing at the *HML* locus. Elimination of position effects in these cells is associated with increased accessibility to the *Escherichia coli dam* methylase in vivo. Thus, the carboxy-terminal domain of RAP1 is required for telomere position effects. In contrast, *rif1* deletion alleles increase the frequency of repressed cells. Using the *rap1* alleles to generate wild-type cells differing only in telomere tract lengths, we also show that telomere position effects are highly sensitive to changes in the size (or structure) of the telomeric tract. Longer poly(G1−3T) tracts can increase the frequency of transcriptional repression at the telomere, suggesting that telomeric poly(G1−3T) tracts play an active role in the formation or stability of subtelomeric transcriptional states.

[Key Words: RAP1; telomere position effects; *dam* methylase; *HML* silencing, *RIF1*]

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Telomeres, the unique protein–DNA structures present at the termini of linear eukaryotic chromosomes, confer metastable position effects on the transcription of neighboring genes (Levis et al. 1985; Gottschling et al. 1990). In the yeast *Saccharomyces cerevisiae*, genes positioned adjacent to the ends of chromosomes undergo repetitive cycles of activation and repression, with each state maintained for multiple generations (Gottschling et al. 1990). Interestingly, genes subject to these telomere position effects are only weakly accessible to methylation in vivo by exogenously introduced *Escherichia coli dam* methylase, leading to the suggestion that changes in chromatin structure may be associated with these repressive effects (Gottschling 1992). Although telomere position effects appear to require a physical terminus (Gottschling et al. 1990), the involvement of telomeric poly(G1−3T) tracts in this process has remained unknown.

Telomere position effects share trans-acting requirements with the silencing of the cryptic mating-type information present at *HMLα* and *HMRα* located 12 and 30 kb from the left and right telomeres of chromosome III, respectively (Aparicio et al. 1991; Laurenson and Rine 1992). The repressed state at *HMLα* and *HMRα* is dependent on the *SIR1*, *SIR2*, *SIR3*, and *SIR4* gene products. Mutations in any one of these four genes lead to derepression of mating-type information. In addition, mutations in the two genes encoding the subunits of the amino-terminal acetyltransferase (NAT1 and ARD1) and amino-terminal deletions of histone H4 result in partial or complete derepression of *HMLα* and *HMRα*. With the exception of *sir1*, mutations in all of these gene products relieve the repression of telomere-adjacent genes (Aparicio et al. 1991) and, where tested, lead to a deprotection against *dam* methylation in vivo (Gottschling 1992). The common dependence of telomere position effects and HM silencing on these gene products suggests that these two processes may be mechanistically linked. Although telomeric position is not required for HM silencing, it may nonetheless influence this process, as the cis-acting requirements for the silencing of *HMLα* on a circular plasmid and at its normal subtelomeric chromosome position are substantially different (Mahoney and Broach 1989; Mahoney et al. 1991).

The UAS-, silencer-, and telomere-binding protein RAP1 has been implicated in the control of HM silencing. Binding sites for this protein are located in the silencer elements present at both *HMLα* and *HMRα* (Shore et al. 1987). At *HMRα*, sites for the binding of three activities ABF1, ORC [the activity thought to bind to the
consensus autonomously replicating sequence (ARS) element, and RAP1 are present (Shore et al. 1987; Bell and Stillman 1992, for review, see Laurenson and Rine 1992). Although deletions of any one of these sites do not have a substantial effect on silencing, elimination of any two sites results in the total derepression of this locus (Brand et al. 1987). Direct evidence for a role of RAP1 in silencing at HMRα has been provided by the identification of carboxy-terminal missense mutations of rap1 [the rap1Δ alleles] that, in strains deleted for the consensus ARS element at HMR [HMRΔA], cause a loss of HMR silencing (Sussel and Shore 1991). Overproduction of the carboxy terminus of RAP1 similarly results in a loss of silencing of the HMRΔA silencer, suggesting that a protein interacting with the carboxy terminus may be titrated in these strains (Hardy et al. 1992a). One such protein, RIF1, has recently been identified (Hardy et al. 1992b). Null mutations in RIF1 confer rap1Δ-like phenotypes. The observations that the Rap1p proteins are defective in their interaction with wild-type RIF1, and that a specific missense allele of rif1 can suppress this defect, argue for a direct physical association between the carboxy terminus of RAP1 and RIF1. At the HMLα locus, two functionally redundant silencer elements, E and I, are present (Hofmann et al. 1989; Mahoney and Broach 1989; Mahoney et al. 1991; Laurenson and Rine 1992). The E element contains a RAP1-binding site which, when deleted, results in partial derepression of HML in strains lacking the I element (Mahoney et al. 1991). Although these data suggest that RAP1 is involved in HML silencing, rap1 mutations affecting HML silencing have not yet been identified (D. Shore, pers. comm.).

High-affinity RAP1-binding sites are also present within the poly(GI₃ T) tract of the yeast telomere (Buchman et al. 1988; Longtine et al. 1989). Recent studies have demonstrated that RAP1 binds to the yeast telomere in vivo, where it plays a role in the maintenance of telomere size and stability (Conrad et al. 1990; Lustig et al. 1990; Klein et al. 1992; Kyriyan et al. 1992). The role, if any, of RAP1 in telomere position effects has, however, remained unknown. While the rap1Δ alleles have substantial effects on HMR silencing, these alleles do not influence telomere position effects (E. Wiley and V. Zakian, pers. comm.).

We have recently identified a set of alleles (termed the rap1Δ alleles) containing nonsense codons that result in a truncation of the carboxy-terminal 144–165 amino acids of the 827-amino-acid RAP1 protein (Kyriyan et al. 1992). Whereas these truncated Rap1p proteins are capable of specific DNA binding, they have numerous effects on telomere length and stability in vivo. First, the rap1Δ mutants display promiscuous telomere elongation, with poly(GI₃ T) tracts increasing in size from 300 bp present in wild type to > 4 kb. Second, rap1Δ telomeres are highly unstable and are capable of undergoing rapid deletion of up to 3 kb of tract in a single generation. Third, both chromosome loss and nondisjunction are elevated in rap1Δ alleles.

Because the carboxy terminus has been implicated in the silencing function of RAP1, we sought to test whether the terminally truncated rap1Δ alleles influence either telomere position effects or HMLα silencing. Our results demonstrate that a carboxy-terminal function of RAP1 is an absolute requirement for telomere position effects. We also demonstrate that the structure of the telomere tract itself can influence the efficiency of telomere position effects.

**Results**

Deletion of the carboxyl terminus of RAP1 results in loss of telomere position effects

To investigate the role of RAP1 in telomere position effects, we tested the ability of cells containing a mutation in one of the rap1Δ alleles, rap1-17, to repress the URA3 gene when introduced adjacent to the telomeric tract on either the right arm of chromosome V (VR) or the left arm of chromosome VII (VIL). The rap1-17 gene encodes a protein that, while able to bind specifically to its cognate site, is missing the terminal 165 amino acids (Kyriyan et al. 1992). The frequency of ura3Δ cells was assayed by determining the fraction of cells capable of growth on medium containing 5-fluoro-orotic acid (5-FOA), a uracil analog that allows the growth of ura3Δ cells (Fig. 1; Table 1). In this genetic background, wild-type RAP1 cells containing URA3-marked VR and VIIΔ telomeres give rise to FOA-resistant colonies at median frequencies of 1% and 52%, respectively (Table 1; Aparicio et al. 1991). We have observed no differences from wild-type expression when either the right arm of chromosome V (VR) or the left arm of chromosome VII (VIL) is missing the terminal 165 amino acids (Kyrion et al. 1992). Null mutations in URA3 confer ura3Δ-phenotypes at the frequency of 8 × 10⁻⁶. The magnitude of URA3 derepression observed in rap1-17 and rap1-18 cells is similar to that observed for mutations in the SIR2 gene (Fig. 1; Table 1; Aparicio et al. 1991). We have observed no differences from wild-type expression when URA3 is placed at internal positions in these strains (Fig. 1). Derepression of telomeric genes is recessive. Diploids heterozygous for rap1-17 display wild-type frequencies of FOAΔ colonies (Table 1, bottom).

Three lines of evidence indicate that the observed derepression of URA3 is the consequence of the rap1-17 mutation. First, loss of telomere position effects is tightly linked genetically to RAP1: Following crosses between rap1Δ and wild-type cells, only rap1Δ spore colonies containing the marked telomere are fully sensitive to FOA (data not shown). Second, replacement of a wild-type copy of RAP1 with the rap1-17 gene by a plasmid shuffle (Kyriyan et al. 1992) is sufficient to confer URA3 derepression (Table 1). While strains carrying the wild-

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**Table 1**

| Strain | Type | FOA resistance frequency |
|--------|------|--------------------------|
| rap1-17 | VR  | <1 × 10⁻⁷ |
| rap1-17 | VIL | <1 × 10⁻⁷ |
| rap1-17 | VR  | 1% |
| rap1-17 | VIL | 52% |
| rap1-17 ura3-1 | VR  | 1% |
| rap1-17 ura3-1 | VIL | 52% |

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type copy of RAP1 on a centromeric plasmid form colonies on FOA medium at a median frequency of 4 × 10^{-4}, replacement of the wild-type copy with the rap1-17 gene generates cells that are fully sensitive to FOA [Table 1, cf. RAP1 (plasmid) and rap1-17 (plasmid)]. Third, the presence of a wild-type RAP1 gene on a CEN plasmid fully complements the effects of an integrated copy of rap1-17 on gene expression at VII_L [Table 1, rap1-17/RAP1].

To confirm that the FOA assay measures differences in URA3 expression, we compared the levels of URA3 transcripts produced in wild-type and rap1-17 strains carrying both a URA3-marked telomere at VII_L and a deletion allele of URA3, ura3Δ1, at its normal locus on chromosome V (Fig. 2). The level of URA3 mRNA in logarithmically growing cells was determined by Northern analysis. Cells wild-type for RAP1 contained telomeric URA3 mRNA at levels below detection (Fig. 2, lane 1). A similarly high level of telomeric repression has been described previously (Aparicio et al. 1991) and is the likely consequence of a reduction in the abundance of transcripts in the fraction of cells that express URA3. In contrast, rap1-17 cells produced these transcripts at levels close to those found in strains carrying only the ura3-1 allele (Fig. 2, lanes 2,3). We estimate a difference of >10-fold between the level of telomeric transcripts in wild-type and rap1-17 cells, similar to the derepression observed in other mutants defective in telomere position effects (Aparicio et al. 1991). In contrast, mRNA levels from the ura3-1 locus were identical in both wild-type and rap1-17 strains (Fig. 2, lanes 4,5).

To test whether the effect of rap1t alleles on telomere position effects is specific to the URA3-marked telomeres, we introduced the ADE2 gene adjacent to the telomeric tract on chromosome VII_L (Fig. 3, top). The repressed and derepressed states were subsequently monitored visually through the production of red and white sectors, respectively (Gottschling et al. 1990). In this genetic background, ~10% of cells wild-type for RAP1 and containing a telomeric ADE2 gene produced white colonies with red sectors. A smaller fraction (~2%) formed red colonies with white sectors (Fig. 3, upper left; Fig. 6, below). In contrast, of >5000 rap1-17 colonies assayed, all but 1 produced a white colony color, and none of these were capable of forming red sectors (Fig. 3, upper right), indicating that these cells cannot repress ADE2 expression at the telomere.

Derepression of telomeric gene expression in rap1-17 cells is independent of poly(G13T) tract length

Recent studies have indicated that the strength of position effects at the telomere is inversely proportional to the distance of the gene from the telomere, with the frequency of repressed states decreasing with increasing distance (Renauld et al., this issue). This effect may be the consequence of an increased distance either between the gene and the beginning of the telomere tract or between the gene and the physical end of the chromosome. One possible explanation of our data, therefore, is that the extended telomere tract in rap1t cells eliminates telomere position effects by increasing the distance between the marker gene and the chromosomal terminus.

To test this hypothesis, we examined URA3 expression in wild-type spore colonies inheriting elongated rap1-17 telomeres from heterozygous diploids [see Materials and methods]. Wild-type strains inheriting V_R telomeres of ~1.4 kb nonetheless produced a high frequency of FOA+ colonies, indicating that increased telomere length alone is insufficient to explain the loss of position effects [Table 1, cf. RAP1 (Chr)-300 bp with RAP1 (Chr)-1370 bp]. A similar result was obtained when a series of plasmid shuffles was used to generate a set of isogenic wild-type and rap1-17 strains differing...
Table 1. FOA\(^{+}\) frequencies in rap1\(^{+}\) cells carrying URA3-marked telomeres

| URA3 locus | Genotype\(^{a}\) (site) | Tract size\(^{b}\) (bp) | Fraction FOA\(^{+}\) range of values; number of colonies\(^{c}\) |
|------------|--------------------------|------------------------|-------------------------------------------------|
| VR         | RAP1 [Chr]\(^{d}\)       | 300                    | 1.1 \(\times\) \(10^{-2}\) (0.03–6.5; 30)        |
|            | RAP1 [Chr]\(^{d,e}\)     | 1370                   | 3.2 \(\times\) \(10^{-2}\) (0.006–25; 24)       |
|            | RAP1 [plasmid]\(^{d}\)   | 350                    | 4.3 \(\times\) \(10^{-4}\) (0–63.6; 16)         |
|            | RAP1 [plasmid]\(^{d}\)   | 1100                   | 5.8 \(\times\) \(10^{-4}\) (3.3–35; 8)          |
|            | rap1-17 [Chr]            | 700–2000               | \(<1.0 \times\) \(10^{-7}\) (20)                |
|            | rap1-17 [plasmid]        | 650                    | \(<1.0 \times\) \(10^{-7}\) (8)                 |
|            | sir2::TRP1 [Chr]         | 300                    | \(<1.0 \times\) \(10^{-7}\) (16)                |
| VII\(_{l}\) haploids | RAP1 [Chr]       | 5.2 \(\times\) \(10^{-1}\) (1.1–4.2) |
|            | rap1-17 [Chr]            | \(<1.0 \times\) \(10^{-7}\) (26) |
|            | rap1-17/RAP1             | 4.0 \(\times\) \(10^{-1}\) (2.5–10) |
|            | [Chr/plasmid]            | \(<1.0 \times\) \(10^{-7}\) (10) |
|            | rap1-18 [Chr]            | 8.4 \(\times\) \(10^{-6}\) (0.006–25; 24)     |
|            | sir2::TRP1 [Chr]         | \(<1.5 \times\) \(10^{-7}\) (20)             |
| VII\(_{l}\) diploids | RAP1/RAP1           | 3.7 \(\times\) \(10^{-1}\) (3.5–5.6; 5)      |
|            | rap1-17/rap1-17          | 2.5 \(\times\) \(10^{-1}\) (2.3–4.8; 5)      |

\(^{a}\)Chr: Chromosomal rap1 allele; [plasmid] rap1 allele on a CEN plasmid in a strain containing a rap1::LEU2 disruption at its chromosomal locus.

\(^{b}\)Average poly(G\(_{1-3}\)) tract length is presented. In the case of rap1-17 strains, the average length varied in different strains and subculturings between 700 and 2000 bp.

\(^{c}\)Observed FOA\(^{+}\) frequencies are presented as median values, with the range of values and the number of colonies assayed in parentheses.

\(^{d}\)RAP1 [Chr]–300 and RAP1 [Chr]–1370 represent isogenic wild-type strains differing only in tract size.

\(^{e}\)Pooled data from strains 383-3c s0, 384-1c, and 384-4a presented in Table 2.

\(^{f}\)RAP1 [plasmid]–350 and RAP1 [plasmid]–1100 represent isogenic wild-type strains derived from a plasmid shuffle and differ only in telomere tract size.

\(^{g}\)Of the 15 colonies tested, 1 failed to yield any FOA\(^{+}\) cells in the population tested [0/1.3 \(\times\) \(10^{7}\)].

Only in the length of the URA3-marked VR telomere. Following a plasmid shuffle, wild-type cells inheriting elongated telomeres from rap1-17 cells displayed FOA\(^{+}\) rates close to the progenitor wild-type strain, despite their increased tract length [cf. Table 1, RAP1 [plasmid]–350 bp with RAP1 [plasmid]–1110 bp]. An additional indication that telomere tract size is not responsible for the effects of the rap1\(^{f}\) mutations on telomere position effects is the finding that rap1-17 cells are completely defective in conferring the repressed state regardless of telomeric length [Table 1].

On the basis of these results, we conclude that loss of the carboxyl terminus of RAP1 in rap1-17 and rap1-18 alleles results in the derepression of telomERICally located genes in a process that appears to be independent of either the identity of the telomere [VR or VII\(_{l}\)], the gene examined [URA3 or ADE2], or the length of the telomeric tract.

HML\(_{a}\) is partially derepressed in rap1-17 cells

Given the profound reduction of telomere position effects in rap1-17 alleles, we sought to determine whether HML silencing is also disrupted in these cells. To directly test for the derepression of HML\(_{a}\), we isolated RNA from wild-type, rap1-17, and sir2 cells and analyzed the expression of \(\alpha_{1}\), \(\alpha_{2}\), and \(\alpha_{2}\) transcripts in these cells by Northern analysis (Fig. 4). As expected, wild-type MAT\(_{a}\) cells expressed only \(\alpha_{1}\) and \(\alpha_{2}\) transcripts [Fig. 4, lanes 6, 7], whereas only MAT\(_{a}\) cells expressed \(\alpha_{2}\) message [Fig. 4, lanes 1–5]. In contrast, \(\alpha\) transcripts were detected for all rap1-17 MAT\(_{a}\) strains tested [Fig. 4, lanes 2–4] at levels close to those found in sir2 MAT\(_{a}\) cells [Fig. 4, lane 10], indicating that HML\(_{a}\) is transcriptionally derepressed in rap1-17 cells. The derepression of \(\alpha\) tran-

Figure 2. rap1\(^{+}\) alleles derepress transcription of URA3-marked telomeres. RNA was isolated from rap1-17 and wild-type strains containing either the ura3-1 or ura3\(_{Δ1}\) allele at its normal locus in the presence or absence of the URA3-marked VII\(_{l}\) telomere, and subjected to Northern analysis using a probe to the URA3 gene. The presence (+) or absence (−) of each of the alleles of URA3 and RAP1 is indicated at top. In addition to the 800-nucleotide URA3 transcript, indicated by the arrow, multiple species derived from transcription initiating within the ura3\(_{Δ1}\)::TRP1::ura3\(_{Δ1}\) duplication were also observed. To measure the relative levels of RNA in each lane, blots were stripped and rehybridized with a probe to actin RNA, and the hybridization signal was quantitated using the \(^{[}\)scope Beta-Gen. Control actin mRNA levels in each lane varied by less than twofold. Strains used [in order of lanes] are as follows: AJL401-9c, AJL406-3d, AJL406-4d, AJL401-5a, AJL406-7b, and AJL401-2c.
scripts is fully complemented by a plasmid-borne copy of the 
RAP1 gene (Fig. 4, lane 5). This derepression of α1 and α2 transcription is HML specific. Transcription of the MATα locus is not affected severely by the rap1-17 mutation, with transcript levels increasing by no more than twofold from expected values [Fig. 4, lanes 6–9]. The rap1-17 mutation does not significantly derepress HMRα. Both wild-type and rap1-17 cells do not express detectable levels of α2 message.

Consistent with the transcriptional derepression of HMLα, the mating efficiency of rap1-17 HMLα MATα HMRα strains is significantly decreased. Whereas wild-type cells repressed for HMLα expression display high levels of mating (62%), rap1-17 cells exhibit 2- to 200-fold lower mating efficiencies, with the extent of the decrease varying widely both among different isogenic rap1-17 strains and among different colonies derived from a common rap1-17 progenitor. In contrast, rap1-17 HMLα MATα HMRα strains mate at wild-type efficiencies [data not shown], indicating that derepression of HML α is required for the lowered mating efficiency. As expected, rap1-17 HMLα MATα HMRα cells mate at wild-type efficiencies. These data suggest that HMLα, but not HMRα, is partially derepressed in rap1-17 cells.

Increased accessibility of E. coli dam methylase to subtelomeric chromatin in rap1-17 cells

Recent studies have used the accessibility of chromosomal sequences to dam methylase as an in vivo assay for chromatin structure (Gottschling 1992, Singh and Klar 1992). Two GATC sites have been identified in the URA3 gene that display unique characteristics when placed adjacent to telomeric sequences in wild-type strains (Gottschling 1992, Wright et al. 1992). One site, at the junction between URA3 and poly(G1–3T) sequences (Fig. 5, site 1), displays hypersensitivity to methylation, whereas a second site within the coding region of URA3 (Fig. 5, site 2) is only weakly accessible to methylation. The accessibility of site 2 to methylation is regained in strains defective for telomere position effects (e.g., sir2 mutants), suggesting that the formation of a closed chromatin state may be mechanistically linked to the transcriptionally repressed state at the telomere (Gottschling 1992).

Because rap1-17 and sir2 cells display similar levels of URA3 derepression, we tested whether these strains also exhibit similar changes in accessibility to the dam methylase. To this end, a series of isogenic wild-type, rap1-17, and sir2 strains were constructed, each of which contained an integrated copy of the E. coli dam methylase gene and a URA3 gene adjacent to either the Vα or VIIL telomere. To determine the methylation state of site 2, DNA was digested first with HindIII and BamHI, releasing a 1.15-kb internal fragment containing the ura3-1 gene [fragment A] and a 1.10-kb telomeric URA3 containing fragment [fragment B], and subsequently cleaved with MboI, DpnI, or Sau3AI (Fig. 5). MboI and DpnI
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Figure 4. Transcription of HMLα is derepressed in rap1-17 cells. RNA was isolated from MATα and MATα derivatives of wild-type RAPI, rap1-17, and sir2::TRP1 cells, and the resulting blots were probed with a restriction fragment containing the MATα gene. This probe hybridizes to both the α1 and α2 transcripts (expressed at the MATα locus) as well as the partially homologous α2 transcript [expressed at the MATa locus]. The presence (+) or absence (−) of rap1 and sir2 alleles and the mating type of each strain is shown above each lane. [Top] The relative abundance of the 740-nucleotide α transcripts (α1/α2) and the 590-nucleotide α2 transcript. As a loading control, blots were stripped and hybridized with a probe to actin mRNA [bottom]. Signals were quantitated using the β-scope (Betagen). α1/α2 Levels were normalized to actin mRNA levels and expressed relative to the MATα wild-type control in lane 6. Relative values are as follows: (Lane 2) 0.23; (lane 3) 0.46; (lane 5) 0.42; (lane 6) 1.0; (lane 7) 1.4; (lane 8) 2.5; (lane 9) 1.8; (lane 10) 0.35. The relative abundance of the α2 transcript did not differ between wild-type and rap1-17 MATα cells, and the α2 transcript could not be detected in either wild-type or rap1-17 MATa cells. Taking into account the expected derepression of HMLα in rap1-17 MATa cells, we estimate that the expression of α1 and α2 at the MATα locus is increased by no more than twofold in rap1-17 cells. The mating efficiencies of the MATα strains shown, relative to W303a, are 0.55 [AJL274-4c], 0.12 [AJL278-4d], 0.03 [AJL369-4d], and 1.15 [AJL369-4d/RAPI]. Strains used are as follows [in order of lanes]: W303a, AJL274-4c, AJL278-4d, AJL369-4d, AJL369-4d/RAPI, W303a, AJL274-3c, AJL278-1a, AJL274-1c, AJL387-5Δsir2.

Loss of RIF1 enhances telomere position effects

One possible cause for the derepression of telomeric genes observed in rap1' cells is the absence of specific contacts between the carboxyl terminus of RAP1 and other proteins involved in this process. One candidate for such a factor is RIF1, identified in a genetic screen for carboxyl terminus-interacting proteins (Hardy et al. 1992b). Deletion of the RIF1 gene results in two phenotypes: derepression of silencing in strains containing HMRΔ silencers and elongation of the telomeric tract by 150–300 bp (Hardy et al. 1992b, see Fig. 6). Although phenotypically distinct from rap1' alleles with regard to telomere length and stability, the inability of RIF1 to interact with the Rap1' protein may nonetheless be partially or completely responsible for their effects on telomere position effects.

To test this possibility, we analyzed the red/white sorting patterns in a strain carrying a rif1::URA3 null allele and a subtelomeric ADE2 gene at chromosome VII. Strains carrying the rif1::URA3 allele sectored at high frequency [Fig. 3, lower left], with 31% of cells forming white colonies with red sectors. Nonetheless,
rif1::URA3 cells displayed a sectoring phenotype distinct from wild-type. Two classes only rarely found in wild-type cells, red colonies with white sectors and unsectored red colonies, were found at elevated frequencies (~10% each). Furthermore, the frequency of white colonies with red sectors was reproducibly two- to three-fold higher than in wild-type cells. In contrast, neither wild-type cells nor rif1::URA3 cells displayed red/white sectoring patterns when ADE2 was introduced at a chromosome-internally position at HIS4 (data not shown). Hence, elimination of RIF1 association with the carboxyl terminus of RAP1 cannot be responsible for the phenotypes observed in rap1-17 and rap1-18 cells. The hyper-repressed state in rif1 cells requires the carboxyl terminus of RAP1, because rif1::URA3 rap1-17 double mutant cells have a phenotype indistinguishable from rap1-17 single mutants (1 red sector of 2900 colonies assayed).

Telomere position effects are influenced by the structure of the telomeric tract

The ability to generate rap1-17 cells with telomeres of varying sizes allowed us to explore the effect of telomere tract size on telomere position effects in wild-type cells (Figs. 3 and 6; Table 2). For these studies, rap1-17 cells containing marked VII L or VR telomeres of differing sizes were crossed with wild-type cells. Wild-type spore colonies inheriting telomeres of differing sizes were then examined for their ability to repress the ADE2 gene at VII L or the URA3 gene at VR. This approach was made possible by the observation that, except for the rapid deletion events noted below, telomeres inherited by wild-type cells decrease in size only slowly (~2 bp per generation, data not shown). Interestingly, increased telomere tract size appears to enhance the frequency of repressed
Telomeres of approximately wild-type length (300–350 bp) from the same cross have sectoring frequencies close to wild-type (Fig. 6, 412-1c). Similarly, wild-type spore colonies containing tracts approximately wild-type in length derived from the reciprocal cross, between a wild-type strain containing the ADE2-marked telomere and an unmarked rap1-17 strain, also exhibited sectoring frequencies close to wild-type (Fig. 6, 394-2d). The hyper-repressed phenotype is never observed among progeny from crosses between marked and unmarked wild-type strains (data not shown).

An analogous effect was observed at the URA3-marked Vr telomere (Table 2, top). Increased telomere tract length at the Vr telomere enhances the frequency of FOA+ colonies, indicating an increase in repression of the subtelomeric URA3 gene. Telomeric tracts of >1.3

Table 2. Telomere position effects in wild-type cells inheriting elongated Vr telomeres

| Wild-type strain | Progenitor diploid genotype | Tract size (bp) | FOA+ colonies/10⁶ cells² (range of values, number of colonies) |
|------------------|-----------------------------|----------------|-------------------------------------------------------------|
| 383-3c s1        | RAPI/rap1-17                | 420            | 0.7 (0.6-18, 10)                                            |
| 392-2d           | RAPI/rap1-17                | 520            | 4.0 (1.5-119, 14)                                           |
| 392-6a           | RAPI/rap1-17                | 620            | 1.7 (0.78-112, 10)                                          |
| 384-3a           | RAPI/rap1-17                | 650            | 9.7 (0.78-112, 10)                                          |
| 392-1c           | RAPI/rap1-17                | 670            | 42.0 (1.4-820, 14)                                          |
| 383-3c s0        | RAPI/rap1-17                | 1320           | 320.0 (0.55-2448, 10)                                       |
| 384-1c           | RAPI/rap1-17                | 1420           | 118.0 (111-288, 4)                                          |
| 384-4a           | RAPI/rap1-17                | 1420           | 520.0 (18.9-2199, 10)                                       |
| KL4-2d, -3b, -5b | RAPI/rap1-17                | 300            | 110.0 (3-650, 30)                                           |

²FOA+ frequencies (per 10⁶ cells), presented as described in Table 1, were obtained from a population of cells having the indicated average telomere tract length.
³383-3c s1 is isogenic to 383-3c s0, and was derived from 383-3c s0 as a single colony after one round of subculturing (≈25 generations).
⁴For 392-6a, one colony also displayed a complete absence of FOA+ cells (0/23,000 cells). For 383-3c s1, 2 of the 10 colonies displayed a complete absence of FOA+ cells (0/63,000 cells in both cases).
⁵The data derived from these strains were also presented in Table 1, line 1.
⁶(s0-1, s0-3, s0-4, s0-5) Four of the 10 colonies (having the indicated FOA+ frequencies) used for the derivation of the median FOA+ frequency of 383-3c s0.

Figure 6. Increased telomere length enhances telomere position effects in wild-type cells. The telomeric fragment sizes of wild-type, rif1, and rap1-17 strains carrying the ADE2-marked Vr telomere are displayed together with quantitation of their sectoring patterns. [wt] The original wild-type strain (AJL275-2a–Vr–ADE) carrying the ADE2-marked Vr telomere. AJL394-2d is a wild-type spore colony derived from a cross between AJL275-2a–Vr–ADE and an unmarked rap1-17-containing strain. AJL412-1c, -2c, -3c, -4d, and -5a are wild-type spore colonies derived from a cross between an unmarked wild-type strain and a rap1-17 strain carrying elongated ADE2-marked telomeres. ADE2-marked rap1-17 (AJL394-1d) and rif1::URA3 (AJL395-1d) mutant cells are also shown at right. DNAs isolated from these strains were digested with Ndel, which distinguishes between the internal and telomeric ADE2 alleles (see Fig. 3), and the resulting blots hybridized to an ADE2 probe. The two high-molecular-mass species reflect subtelomeric and internal fragments of ADE2. The percentages of each population displaying white colonies with red sectors [%rw] and red colonies with white sectors [%rw] are shown below the autoradiograph. Sample sizes for these genotypically wild-type strains in lanes 1–7 are 3240, 985, 812, 793, 831, 690, and 1095, respectively. The data for the rap1-17 and rif1 lanes are derived from multiple strains (including the strain displayed) representing cumulative sample sizes of >5000 and 868, respectively. Size markers [in kb] are displayed at right.

Wild-type cells inheriting an ADE2-marked Vr telomere 800–900 bp in length exhibited an elevated level of both white colonies with red sectors and red colonies with white sectors [Fig. 3, lower right; Fig. 6, 412-2c, 412-3c, 412-4d, and 412-5a]. The vast majority of colonies are sectored in these strains. A similar effect is found in rif1 mutant cells inheriting elongated telomeres from rap1-17 cells, with the frequency of repression exceeding that found in rif1 cells [data not shown]. The hyper-repressed state is dependent on the presence of elongated telomeres. Wild-type spore colonies inheriting
paper, we demonstrate that the carboxy-terminal do-
mains of RAP1 also play a critical role in regulating
in regulating telomere and chromosome stability. In this
Discussion
terminal 165 or 144 amino acids of RAP1 in the
chromatin structure in yeast. This conclusion is based
telomere-specific position effects on transcription and
in vivo, these truncated forms of RAP1 result in telomere
shown). These data indicate that changes in telomere
exhibited wild-type sectoring frequencies (data not
creased tract size to between 400 and 700 bp decreases
FOA\(^\text{+}\) frequencies, with median values ranging from
0.02% to 0.4% in different segregants. In contrast to the
V\(_r\) telomeres, URA3-marked VII\(_r\) telomeres do not display
this variation in frequency, with wild-type values
ranging between 25% and 50% in all spore colonies, sug-
gesting that chromosomal context may influence the
susceptibility of termini to changes in telomere size.

A further indication of a relationship between telo-
more size and telomere position effects is based on ob-
servations of rapid deletion events acting on the elo-
gated telomeres inherited by wild-type cells. We have
found previously that single-step deletion events can, at
high frequency, eliminate substantial amounts of the
elongated telomeric poly\([\text{G}_{1-3}]\) tracts in rapl-17 cells
during mitotic growth [Kyrion et al. 1992]. We have re-
cently observed that wild-type cells inheriting elongated
telomeres from rap1-17 cells are also sometimes capable
of producing telomeres near wild-type length in a similar
single-step process (e.g., Fig. 6, 412-3c). Interestingly, the
loss of 1 kb of telomeric tract at the URA3-marked V\(_r\)
telomere is associated with a concomitant 500-fold de-
crease in the frequency of FOA\(^\text{+}\) colony formation, even
though these cells are otherwise isogenic (Table 2, cf.
383-3c s0 and 383-3c s1). The presence of truncated telo-
more helps to explain the broad distribution of FOA\(^\text{+}\)
frequencies observed in 383-3c s0, because colonies that
displayed reduced FOA\(^\text{+}\) frequencies (Table 2, s0-1 and
s0-3) also exhibited shortened telomeres (Table 2). A
similar effect was observed at ADE2-marked VII\(_r\) telo-
meres. Following rapid deletion at the VII\(_r\) telomere
(e.g., Fig. 6, 412-3c), wild type cells inheriting only the
deleted species lost the hyper-repressed phenotype and
exhibited wild-type sectoring frequencies (data not
shown). These data indicate that changes in telomere
size significantly influence telomere position effects in
yeast.

Discussion
Previously, we have demonstrated that rap1\(^{1}\) alleles pro-
duce truncated RAP1 molecules that, while capable of
binding specifically to DNA, are missing the carboxy-
terminal 144–165 amino acids [Kyrion et al. 1992]. In
vivo, these truncated forms of RAP1 result in telomere
elongation and instability, slow growth rate, and high
rates of chromosome loss and nondisjunction, suggesting
that the carboxyl terminus of RAP1 plays a critical role
in regulating telomere and chromosome stability. In this
paper, we demonstrate that the carboxy-terminal do-
mains of RAP1 also play a critical role in regulating
telomere-specific position effects on transcription and
chromatin structure in yeast. This conclusion is based
on two lines of evidence. First, truncation of the carboxy-
terminal 165 or 144 amino acids of RAP1 in the rap1-17
and rap1-18 alleles results in the inability of telomeres to
confer a transcriptionally repressed state onto adjacent
polymerase II-transcribed genes. This phenotype is not
the simple consequence of increased telomere tract
length, because cells wild-type for RAP1 that inherit
elongated telomeres regain high levels of repression.

The second observation that argues for a critical role
of the carboxyl terminus of RAP1 in telomeric suppression
is the increased accessibility of subtelomeric chromatin
to dam methylase in rap1-17 alleles. The level of site 2
methylation at telomeric URA3 genes is reduced signifi-
cantly relative to wild-type. In this regard, the pheno-
type of this rap1 mutant is similar to strains containing
the sir2 and sir4 mutations, which display a complete
loss of telomere-specific protection (Fig. 5; Gottschling
1992). However, unlike sir2 mutants, elements of wild-
type chromatin are still maintained in at least a fraction
of rap1-17 cells at both V\(_r\) and VII\(_r\) telomeres. Both
strands appear to be protected against methylation in
both wild-type and rap1-17 cells (data not shown). One
explanation for these results is that the fraction of DNA
molecules that are methylated directly reflects the pro-
portion of cells retaining protection against methylation.
If this were true, protection against methylation could
not be a sufficient criterion for forming the repressed
state, because < 1 x 10\(^{-7}\) cells are phenotypically Ura\(^{-}\).
Alternatively, all cells in the population may be equally
affected by the rap1-17 mutation, but the repressed state
is transient and unstable, possibly as a consequence of
rapid switching between chromatin conformations.

The majority of the cells assayed in wild-type, rap1-17,
and sir2 cells are in the repressed state at the V\(_r\)
telomere. Curiously, the nature of the repressed state
appears to be qualitatively different in sir2 cells than in
either wild-type or rap1-17 cells, both of which have lev-
els of protection against dam methylase greater than ex-
pected on the basis of their FOA\(^\text{+}\) frequencies. While con-
firming that the structure conferring protection against
methylation is insufficient for the formation of a stable
repressed state, these data raise the possibility that both
wild-type and rap1-17 derepressed cells retain elements
of the closed chromatin state. In contrast, the effects of
the sir2 mutations appear to be more global in nature,
eliminating all vestiges of the wild-type state.

Our data indicate that HMLA, but not HMR\(_A\), is par-
tially derepressed in rap1-17 cells. These results provide
the first direct evidence that RAP1 has a function in
HML silencing, a possibility inferred previously from the
functional importance of RAP1-binding sites within the
HML silencer [Mahoney et al. 1991]. We note that differ-
ent cells in a population vary widely in mating effi-
ciency. This phenotype is somewhat similar to the be-
havior of sir1 mutants [Pillus and Rine 1989]. The SIR1
gene product has been shown to be important for the
establishment, but not maintenance, of the repressed
state at HML\(_A\). Whether RAP1 plays a similar role re-
mains to be determined.

It is noteworthy that rap1-17 mutations affect HMLA
despite the functional redundancy present within the
HML silencer elements. Deletion of either the E or I
silencer element has no effect on silencing (Mahoney and Broach 1989). Furthermore, deletion of the RAP1-binding site in the E box reduces mating efficiency only in the absence of the I silencer (Mahoney et al. 1991). One possible explanation for this paradox is that the weak RAP1-binding site found previously in the I element (Hofmann et al. 1989) may act as a redundant element in HML silencing, so that occupation of the RAP1-binding sites of both E and I elements by the terminally truncated RAP1 protein results in a loss of silencing. Alternatively, aberrant associations of RAP1 with other factors involved in the function of both E and I silencer elements (e.g., the consensus ARS element-binding complex ORC) may attenuate transcriptional repression. A third possibility is that interaction of the E and I silencers with telomeric RAP1-binding sites normally plays a role in stabilizing HML repression in wild-type cells. Disruption of this association in the rap1-17 allele might then result in partial loss of E and I redundancy. In this regard, it is interesting that the I element is sufficient for repression when located at its genomic locus, but not when present on a circular plasmid, possibly reflecting an interaction between the I element and the telomere (Feldman et al. 1984; Mahoney and Broach 1989). Whether the participation of RAP1 in HML silencing is related to its role in telomere position effects remains unknown.

The phenotypes of the rap1 allele on telomere position effects are most consistent with a direct involvement of RAP1 mediated through binding to the telomere. However, some indirect models for the effect of the rap1-17 mutation need to be considered. First, the rap1-17 allele may indirectly influence the expression of another gene important for this process (or, alternatively, hyperactivate the telomERICally located gene). However, the fact that transcription of the MATa gene, which is regulated by RAP1 (Giesman et al. 1991; Kurtz and Shore 1991), is only slightly influenced by the rap1-17 allele makes this possibility unlikely. Second, the slow growth rate of rap1 cells could contribute to the phenotypes observed here. Although we cannot fully exclude this possibility, we note that wild-type cells grown on minimal and complete media, which vary twofold in growth rate, do not differ in position effects. In addition, we have recently identified missense alleles of rap1 that completely derepress telomeric gene expression while not affecting growth rate (C. Liu and A. Lustig, unpubl.). Finally, it is conceivable that a lowered abundance of the Rap1-17 protein influences position effects. This possibility is unlikely, however, as the levels of DNA-binding (per microgram of extract) are identical in wild-type and rap1-17 extracts over a broad range of substrate concentrations (G. Kyrian and A. Lustig, unpubl.).

The requirement of the carboxyl-terminal region of RAP1 in telomere position effects is likely to reflect critical associations with other position–effect-related factors. Consistent with this proposal is that overproduction of the carboxyl terminus of RAP1 also results in a loss of position effects at the telomere, arguing that a limiting factor important for this process is titrated by the plasmid (E. Wiley and V. Zakian, pers. comm.). One candidate for such a factor is the RAP1-interacting protein RIF1, which plays a role in HMRa silencing (Hardy et al. 1992b). However, our studies indicate that loss of RIF1 function actually increases the efficiency of repression. This effect is likely to be a general phenomenon, as an independent study analyzing the effect of rif1 null alleles on telomere position effects at a URA3-marked VIIg telomere produced similar results (E. Wiley and V. Zakian, pers. comm.). Two models may explain these data. First, RIF1 may normally act to antagonize the formation or stabilization of the repressed state, possibly competing with a second factor (e.g., one of the SIR gene products) for association with the carboxyl terminus. Such a competition between factors may help to explain the metastable nature of telomere position effects. Second, the longer telomeres present in rif1 cells (Hardy et al. 1992b, see Fig. 6) may enhance position effects analogous to the behavior of wild-type cells inheriting elongated telomeres from rap1-17 cells.

The results presented here provide the first evidence that telomere position effects are sensitive to the structure of the telomere itself. Two overlapping processes appear to influence telomere position effects in wild-type cells. First, position effects at both VIIg and Vr telomeres in wild-type cells are exquisitely sensitive to the size of the inherited marked telomere. Longer telomeres increase the frequency of repression. This size dependency is in striking contrast to the decreasing efficiency of position effects with increasing distance from the telomere (Renaud et al., this issue). These data suggest that the efficiency of telomere position effects may be governed by both the distance of the gene from the telomere tract and the length of that tract. Conceivably, telomere size may promote position effects by creating additional binding sites for either RAP1 or other telomere-binding proteins necessary for this process. An alternative, but not mutually exclusive, possibility is that longer telomeres are more capable of forming higher order structures (e.g., DNA loops) that may be important for telomere position effects.

The size dependency of position effects reopens the issue as to whether position effects may be exerted by poly$(G_{1-3}T)$ tracts at internal positions. A previous study (Gottschling et al. 1990) demonstrated that 80 bp of poly$(G_{1-3}T)$, while able to repress telomeric genes, did not influence gene expression at an internal site. Given the profound effect of telomere size on position effects, the possibility that longer tracts may repress transcription even at internal sites needs to be reexamined.

Telomere position effects also appear to be influenced by other structural changes occurring in rap1-17 cells, unrelated to telomere size. Wild-type cells inheriting rap1-17 Vg telomeres of near-wild-type size display a reduced efficiency of URA3 repression relative to the original wild-type strains. The impact of these changes appears to be telomere specific, with the URA3 gene at Vg being far more sensitive to these effects than either the ADE2 or URA3 gene at VIIg. Although the basis of this effect remains unknown, both its reversibility by in-
increased tract size and its genetic linkage to the V_R telomere [data not shown] suggest that a permanent change in telomere structure or sequence, rather than a mutation in a second gene, is responsible for this reduced efficiency. Such effects occurring in rap1-17 cells may help to explain the observation that in some cases, the efficiency of telomere position effects varies even for tracts of similar size [e.g., Table 2, cf. 392-6a with 392-2d]. Given the effects of both telomere size and structure on telomere position effects, it is likely that the protein–DNA interactions present at the telomere are actively involved in establishing or maintaining the transcriptionally repressed state and, consequently, in governing the chromatin structure of subtelomeric domains.

Permanent changes in telomere structure or sequence, such as those noted above, may contribute to the derepressed state observed in rap1-17 cells. However, it seems unlikely that such effects fully account for the rap1-17 phenotypes, as a fully derepressed state is observed in rap1-17 cells immediately upon inheriting either a V_R or V_II wild-type telomere and does not change regardless of the degree of subculturing or telomere size. We suggest, therefore, that RAPI participates directly in conferring telomere position effects, possibly through facilitating the formation of telomeric chromatin structures essential for this process.

Materials and methods

Plasmids

Plasmids pVII-L–URA3–TEL, pV-R–URA3–TEL, padh4::URA3, and PADADE2[+], used for the construction of telomeric and internal copies of URA3 and ADE2, have been described previously [Gottschling et al. 1990]. pDP6–dam is a LYS2-containing integrating plasmid carrying the E. coli dam methylase gene [Gottschling 1992]. pKL1 was derived from pDP6–dam by insertion of the HIS3 gene into the BglII site of the LYS2 gene. pEF1332, a TRPI-containing integrating plasmid carrying the ura3Δ1 deletion allele, has been described previously [Boeke et al. 1987].

Plasmid pRS313/RAP1 was derived by cloning an EcoRI–Xbal fragment carrying the RAPI gene into the HIS3-containing centromeric plasmid pRS313 [Sikorski and Hieter 1989]; pRS313/rap1-17 was derived in a similar fashion using the EcoRI–Xbal fragment of pRS316/rap1-17 [Kyrion et al. 1992]. Plasmid pRS306/his4/ADE2 was constructed by cloning a SalI–BamHI fragment of HIS4 into the polylinker of the URA3-containing plasmid pRS306 [Sikorski and Hieter 1989]. The 3.6-kb BamHI fragment containing the ADE2 gene was then cloned into the polylinker of pRS306/his4/ADE2, creating the plasmid pRS306/his4/ADE2.

Yeast strains and growth

Methods for yeast growth and manipulations were performed as described [Sherman et al. 1986]. The yeast strains used for this study are shown in Table 3. Unless otherwise indicated, all strains are isogenic to the wild-type strain W303. The structure of the strains described below was confirmed by Southern and [where appropriate] genetic analyses. To construct strains containing the URA3-marked V_R telomere, and the URA3- and ADE2-marked V_II telomeres, the wild-type strain AJL275-2a, isogenic to W303, was transformed with linearized pV-R–URA3–TEL, pVII-L–URA3–TEL, and PADADE2[+] as described [Gottschling et al. 1990], giving rise to strains AJL275-2a–V_R, AJL275-2a–VII–URA, and AJL275-2a–VII–ADE, respectively. Isogenic strains containing an adh4::URA3 disruption on V_II [AJL275-2a–adh4::URA] were constructed as described [Gottschling et al. 1990].

The rap1-17 and rap1-18 mutations were introduced into strains containing a URA3- or ADE2-marked telomere or a URA3-marked adh4 locus by appropriate crosses with AJL275-2a–V_R, AJL275-2a–VII–URA, AJL275-2a–VII–ADE, and AJL275-2a–adh4::URA. AJL369-4d/RAP1 was constructed by transforming the rap1-17-containing spore colony AJL369-4d with pRS313/RAP1.

To generate strains carrying the ura3Δ1 allele, KL5-5a was transformed with Stul-digested pJEF1332. A transformant, carrying tandem copies of the ura3Δ1 gene [generated by gene conversion during integration], was backcrossed to generate a wild-type spore colony [AJL398-5a] containing the ura3Δ1 allele. This strain was used to generate wild-type and rap1-17 strains carrying both the URA3-marked V_II telomere and the ura3Δ1 allele.

A sir2::TRPI null allele was introduced into strains KL4-5b and AJL387-5a as described [Shore et al. 1984]. The rifl::URA3 allele was introduced into W303a as described [Hardy et al. 1992], and subsequently crossed into wild-type and rap1-17 strains containing the ADE2-marked V_II telomere. Strains containing both the rifl::URA3 allele and the ADE2 gene inserted at the HIS4 locus were identified after sporation of a diploid derived from a cross between a rifl::URA3-containing strain and strain CK30. CK30, containing an integrated copy of ADE2 at the HIS4 locus, was generated by transformation of Nhel-digested pRS306/his4/ADE2 into W303a.

To generate rap1-17 HMLA MATα HMRα strains, a rap1-17-containing strain was crossed to the nonisogenic HMLA MATα HMRα HO strain K828. Tetrad from this diploid segregated 2 : 2 for slow growth rate, indicating that rap1-17 is penetrant in these backgrounds. Slow-growing colonies that were capable of mating only with an α-mating tester strain were identified. Southern analysis of DNA isolated from these candidates was used to identify strains carrying either the HMLα or HMLα alleles.

To introduce the E. coli dam methylase gene, pKL1 was linearized by digestion with Stul, which cleaves uniquely within the LYS2 gene, and the DNA was transformed into the rad1-containing strain W839-6b. His- transformants having the dam methylase gene integrated into the LYS2 locus [W839-6b–dam] were identified and crossed to appropriate strains to generate wild-type and rap1-17 strains containing a URA3-marked V_R or V_II telomere and the methylase gene. The introduction of the dam methylase and rad1 mutations into these strains had no effect on the expression of the telomeric URA3 genes.

To generate wild-type strains having ADE2-marked V_II telomeres 800–900 bp in length, a rap1-17 strain containing a V_II telomere of ~1000 bp was crossed to W303a, and the resulting diploid [AJL412] was sporulated. To generate wild-type strains having URA3-marked V_R telomeres 500–1500 bp in length, a rap1-17 strain containing a V_R telomere of ~750 bp was crossed to W303a, and the resulting diploid [KL3] was sporulated. The rap1-17 spore colony KL3-1b was subcultured on solid media for four rounds [50–64], with each round consisting of ~25 generations of growth [Kyrion et al. 1992]. The average telomere tract lengths of KL3-1b s0, KL3-1b s1, KL3-1b s2, and KL3-1b s4 were 0.85, 1.1, 1.35, 1.45, and 1.85 kb, respectively. Each strain was crossed to W303a, the resulting diploids [AJL392, AJL383,
### Table 3. Yeast strains

| Strain      | Genotype                                                                 |
|-------------|---------------------------------------------------------------------------|
| W303a       | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 HIS3                               |
| W303a       | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3                              |
| AJL75-2a-VR | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3; VR::URA3                    |
| AJL75-2a-VILL-URA | RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 VIII; URA3                      |
| AJL75-2a-VILL-ADE | RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 VIII; URA3/ADE2                  |
| AJL75-2a-ade1:URA | RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 ade1:URA3                     |
| AJL369-4d   | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 his3 VIII; URA3                |
| AJL369-4d/RAP1 | MATa rap1-17 RAP1/CEN/HIS3 leu2-3,112 trp1 ade2-1 ura3-1 his3 VIII; URA3 |
| AJL369-5b   | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 his3 VIII; URA3                |
| AJL369-4b   | MATa rap1-18 leu2-3,112 trp1 ade2-1 ura3-1 his3 VIII; URA3                |
| AJL364-1c   | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 his3 ade2:URA3                |
| W398-6b-dam | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3:lys2 |
| KL4-5b      | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3:lys2 VR::URA3 |
| KL5-5a      | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3:lys2 VR::URA3 |
| KL4-5b sir2 | MATa RAP1 sir2::TRP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3::lys2 VR::URA3 |
| AJL387-5a   | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3::lys2 VR::URA3 |
| AJL391-1c   | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |
| AJL387-5a sir2 | MATa RAP1 sir2::TRP1 leu2-3,112 trp1 ade2-1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |
| GK23-1b    | MATa RAP1/CEN/TRP1 rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 his3 VR::URA3 |
| P17 s4     | MATa rap1-17 CEN/HIS3 rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 his3 VR::URA3 |
| P17 s4D-P  | MATa RAP1/CEN/TRP1 rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 his3 VR::URA3 |
| AJL398-5a  | MATa rap1-17 leu2-3,112 trp1 ade2-1 ura3-1 TRP1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3::lys2 |
| AJL401     | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |
| AJL406     | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |
| EMPY75c    | MATa rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |
| EMPY76c    | MATa rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |
| AJL74-4c, AJL78-4d | MATa rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 |
| AJL74-4d   | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 |
| AJL74-1c, AJL78-1a | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 |
| K828e      | MATa rap1-17 LEU2 leu2-3,112 trp1 ade2-1 ura3-1 HIS3 |
| AJL395, AJL396 | MATa RAP1 leu2-3,112 trp1 ade2-1 ura3-1 TRP1 ura3-1 his3 rad1::LEU2 LYS2::dam/HIS3::lys2 VIIIL::URA3 |

AJL384, and AJL386, respectively) were sporulated, and wild-type spore colonies, containing \( V_k \) telomeres of differing sizes, were identified.

### Telomere tract length determination

The telomere tract length of \( URA3 \)-marked \( V_k \) and \( VII_L \) telomeres were determined as described [Kyrion et al. 1992]. The tract lengths of \( ADE2 \)-marked \( VII_L \) telomeres were determined by probing Southern blots of \( NdeI \)-digested DNA with a 3.6-kb \( BamHI \) fragment carrying the \( ADE2 \) gene. \( NdeI \) cleaves \( \sim 750 \) bp from the \( ADE2/poly(G\_1\_T) \) junction.

### Plasmid shuffles

Following transformation of GK23-1b with \( pRS313/rap1-17 \), strains were identified that retained only the \( pRS313/rap1-17 \) plasmid.
plasmid. One such strain [P17] was subcultured and cells containing a V<sub>T</sub> telomere tract length of 1.5 kb [P17s4] were transformed with pDI30, containing a wild-type copy of RAP1 on a TRP1 centromeric plasmid [Kurtz and Shore 1991]. Following nonselective growth, strains were identified that contain only the wild-type copy of RAP1 [P17s4D-P] while retaining the elongated telomeres from tap1-17. We note that the lower level of repression observed when a wild-type RAP1 gene is located in a plasmid, rather than chromosomal, context is the probable consequence of altered expression of the plasmid-encoded product.

Assays for transcriptional repression

**FOA assay** To assay wild-type Ura<sup>-</sup> frequencies, 4 to 10 colonies were grown on YPD media at 25°C to a diameter of 1 mm, and the entire colony was suspended and diluted in SC media. Following appropriate dilutions, cells were spread onto FOA plates and incubated at 30°C for 3–5 days. To assay Ura<sup>-</sup> frequencies in tap1-17, rap1-18 and sir2:::TRP1 cells, 10 colonies were grown to a diameter of 2 mm and the entire colony containing 1 x 10<sup>7</sup> to 4 x 10<sup>7</sup> cells, following suspension, was plated onto two FOA plates. Plates were incubated at 30°C for 5–7 days. In each case, total cell counts were determined after plating appropriate dilutions onto YPD media. The data are presented as median values together with the range of values observed. The repressed state present in both wild-type and rap1-18 cells is reversible. FOA<sup>-</sup> cells switch to the Ura<sup>+</sup> phenotype at high frequencies during growth on nonselective media.

**ADE2 visual assay** Wild-type, tap1-17, rif1::URA3, and tap1-17 rif1::URA3 strains carrying the VII, telomere [or his4 locus] marked by the ADE2<sup>-</sup> gene were grown at 30°C for 2 days. Cells were suspended in SC media, diluted, and plated onto SC media containing limiting concentrations of adenine [Hieter et al. 1988]. Wild-type and rif1::URA3 strains were grown at 30°C for 3 days and then shifted to 25°C for full color development. rap1-17 and tap1-17 rif1::URA3 strains were grown at 30°C for 5 days before being shifted to 25°C.

**Transcriptional analysis** RNA was isolated by standard methods after growth in YPD at 25°C. Following electrophoresis of RNA on a 1.5% formaldehyde–agarose gel, Northern analysis was carried out using either a Psrl–Smal fragment derived from the URA3 gene or a 4.0-kb HindIII fragment carrying the MATa<sup>-</sup> gene as a probe.

**dam methylation analysis**

DNA was isolated from dam<sup>-</sup> methylase-containing wild-type, rap1-17, and sir2<sup>-</sup> cells grown in rich (YPD) media at 25°C, and digested as described in the text, and the resulting blots were probed with the Psrl–Smal fragment of URA3. This fragment has identical homology to both the telomeric and internal copies of URA3 [see Fig. 5]. The relative hybridization signals of the fragments were estimated using the β-scope [βetagen]. Under some conditions of growth, yeast strains carrying the dam methylase exhibited a generalized decrease in methylation, leading to an overall increase in the abundance of DpnI-resistant fragments. We therefore used both the DpnI digestion pattern of yeast DNA and the ability of the internal fragment A to be fully cleaved by DpnI as internal controls for cellular dam methylase activity.

**Quantitative mating assays**

Quantitative mating assays were carried out as described previously [Dutcher and Hartwell 1982], using MATa and MATa<sup>+</sup> derivatives of wild-type and tap1-17-containing strains, and the tester strains EMPI 75 and EMPI 76. The wild-type strain W303a was included in each trial as a control. As an additional control, six HMLa MATa HMRa and four HMLa MATa HMRa spore colonies derived from the same diploid strain were also tested. Whereas HMLa strains showed significant decreases in mating, HMLa derivatives always exhibited wild-type mating efficiencies.

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